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ARTICLE

# Analytical Performance Evaluation of the Cardiac Troponin T High Sensitivity Gen 6 Assay

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**Background:** High-sensitivity cardiac troponin (hs-cTn) assays are recommended for the diagnosis of acute myocardial infarction. Here, we characterize the analytical performance of a next-generation hs-cTn assay, Elecsys® Troponin T hs Gen 6 (Roche Diagnostics International).

**Methods:** Surplus lithium-heparin plasma or serum samples from patients or healthy volunteers were run on

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Previous presentation: Poster presentation (P0380) at EuroMedLab 2025, Brussels, Belgium.

Received November 21, 2025; accepted February 9, 2026.

https://doi.org/10.1093/jalm/jfag031

Cobas® e 801, e 402, and Pro analyzers. Limits of blank (LoB), limits of detection (LoD), and limits of quantitation (LoQ) were determined according to CLSI EP17-A2, with target values of 1.0 and 1.5 ng/L for LoB/LoD and 3.0 ng/L (10% CV) and 1.5 ng/L (20% CV) for LoQ, respectively. Precision was measured, per CLSI EP17-A2, using 3 QC samples (approximately 4, 30, and 220 ng/L), 12 native samples, and 3 reagent lots. Linearity, per CLSI EP06-Ed2, was determined by diluting samples with cardiac troponin T (cTnT) concentration above the measuring range with a low/blank sample. Interference (per Glick) with endogenous and assay components at 5 cTnT concentrations was assessed.

**Results:** Measured values for LoB, LoD, and LoQ at 10% and 20% CV were 0.1 to 0.7 ng/L, 0.3 to 1.4 ng/L, 1.0 to 2.9 ng/L, and 0.4 to 1.2 ng/L, respectively. Repeatability CVs were 1.0 to 5.8% for mean cTnT concentrations of 2.6 to 9230 ng/L in lithium-heparin plasma. High precision was shown across lots, and linearity was observed across the measuring range (1.5 to 9500 ng/L, all Pearson's $r = 1.00$). No interferences were observed, specified up to ≤1000 mg/dL hemoglobin, ≤50 mg/dL [≤855 µmol/L] icterus/bilirubin, and ≤1200 ng/mL biotin.

**Conclusions:** The analytical performance characterization of the assay demonstrated high sensitivity, high precision at the low end and across the measuring range, and resistance to interference.

## IMPACT STATEMENT

hs-cTn assays are recommended for the diagnosis of acute myocardial infarction. Here, we characterize the analytical performance of a next-generation hs-cTn assay, Elecsys Troponin T hs Gen 6. The assay demonstrated high sensitivity, repeatability and high precision at the low end of the measuring range, high correlation with the previous assay generation, and increased resistance to interference from various endogenous components. This study sets the foundations for a large, global, multicenter reference range study to determine new 99$^{th}$ percentile upper reference limits and the corresponding clinical performance study.

## INTRODUCTION

Cardiac troponin (cTn) is the preferred biomarker for the evaluation of myocardial injury and the diagnosis of acute myocardial infarction (AMI). Clinical practice guidelines worldwide recommend high-sensitivity (hs)-cTn assays over conventional cTn assays due to superior sensitivity and precision at low concentrations (1–3). AMI diagnosis requires a rise and/or fall in cTn concentrations, with ≥1 value above the sex-specific 99$^{th}$ percentile upper reference limit (URL), along with clinical evidence of ischemia (e.g., symptoms, electrocardiographic changes, imaging, or angiography) (1).

To define a cTn assay as "high-sensitivity," the IFCC recommends that hs-cTn assays measure cTn concentrations at or above the assay's limit of detection (LoD) in ≥50% of healthy females and males, with ≤10% CV at the sex-specific 99$^{th}$ percentile URL (4). Recent advances have improved both the sensitivity and precision of hs-cTn assays at low concentrations (5, 6), which may facilitate more accurate and efficient exclusion of AMI and may further improve cardiovascular risk prediction in the general population where overt cTn elevations are less common (7–9). Regulatory authorities have established standardization procedures to ensure metrological traceability of values assigned to calibrators, trueness control materials, and human samples for quantities measured by in vitro diagnostic medical devices (10).

The earliest hs-cTn assay, developed by Roche Diagnostics in 2009 (11, 12), was also the first hs-cTn assay to incorporate a rapid rule-out claim for early-discharge and outpatient management for patients with suspected acute coronary syndrome (13). An updated version of the assay to reduce biotin interference was subsequently introduced in 2019 (14). The assay has continued to evolve to improve its sensitivity and standardization, with the recent development of an updated Elecsys® Troponin T hs Gen 6 assay (Roche Diagnostics International). This assay was designed to improve analytical sensitivity and reduce lot-to-lot and platform-to-platform variation, compared with the previous generation assay (15). In addition, a new 3-level QC system compared with the previous assay generation's 2-level QC was developed to comply with IFCC recommendations (16).

Although hs-cTn assays are optimized to reduce analytical confounders, increases in analytical sensitivity may increase susceptibility to analytical interference (11, 17). This can, in turn, lead to falsely elevated cTn concentrations and unnecessary investigations for patients (18). Hemolysis is one of the most common preanalytical interferences in clinical laboratories leading to unreliable cTn results; therefore, assays should minimize the impact of hemolysis and other common interferences, such as bilirubin, turbidity, and biotin (19, 20).

We evaluated the analytical performance of the Troponin T hs Gen 6 assay to determine the analytical sensitivity; precision at the low end of the measuring range; and resistance to hemolysis, bilirubin, and biotin interference.

## MATERIALS AND METHODS

### Samples

Samples were obtained from biospecimen repositories specialized in providing samples for validation of medical devices, such as Milan Analytica AG, Biomex GmbH, Logical Biological Ltd., and Ino Specimens Biobank, and stored at −20°C or colder for up to 18 months before analysis. Surplus lithium-heparin (LiHep) plasma or serum samples were used, with approval by country-specific regulated institutional review boards (or equivalent committees). Vendors ensured compliance with the respective country-specific regulations and good laboratory practice was used for all purchased samples. Samples were pre-screened in batches using the Troponin T hs Gen 6 assay on the Cobas® Pro analyzer to determine cardiac troponin T (cTnT) concentration and allocated accordingly to experiments.

### The Troponin T hs Gen 6 Assay and Analyzers

The Troponin T hs Gen 6 assay was run on the Cobase 801, e 402, and Pro analyzers (Roche Diagnostics International) using the STAT (Short Turn Around Time) application at a single site (Roche Diagnostics GmbH). The Cobas e 801 and e 402 analyzers were operated as standalone systems; Cobas Pro is an integrated solution that can incorporate multiple analytical units into one workflow. The assay was developed to be used on the most recent configurations available on the market. The assay uses 2 high-affinity monoclonal antibodies (biotin- and ruthenium-labeled) directed against human cTnT to form a sandwich complex enabling detection of all circulating cTnT forms (21). The M11.7 antibody was improved by affinity maturation of the monoclonal antibody used in the previous assay version [fifth generation Elecsys Troponin T hs Biotin update (cTnT hs)] using ribosomal display (22). The antibodies used in the Troponin T hs Gen 6 assay recognize the same epitopes (amino acid positions 125–131 and 136–147 located in the stable center region of the cTnT protein) as the fifth generation cTnT hs assay (21), with improved affinity compared with the previous assay version. After the immunocomplexes are formed, the sandwich complexes are immobilized onto the surface of

streptavidin-coated paramagnetic micro-particles via biotin-streptavidin binding. The reaction mixture is then aspirated into the measuring cell of the Cobas instrument, where the streptavidin-coated paramagnetic micro-particles are drawn into the measuring cell and separation takes place. Further, the ruthenium complex and tripropylamine are involved in reactions that result in an electrochemiluminescent emission, which is measured by a photomultiplier; the electrochemiluminescence signal is proportional to the amount of cTnT in the sample.

To further improve the performance of the Troponin T hs Gen 6 assay compared with the previous assay generation, the assay formulation was optimized by including the assessment of various biotin- and ruthenium-label chemistry formats and improvements to the buffer composition.

### Analytical Performance at the Lower End of the Measuring Range (Limit of Blank, LoD, Limit of Quantitation) and Linearity

The limit of blank (LoB), LoD, and limit of quantitation (LoQ) were determined according to the CLSI EP17-A2 (23) guideline using LiHep plasma samples in up to 4 reagent lots on the Cobas e 801 and e 402 analyzers. Samples in serum matrix were additionally measured for selected reagent lots and analyzers. The LoB (95$^{th}$ percentile of analyte-free samples) was determined using one immunodepleted sample measured with 10 replicates per run across 6 runs over 3 days per lot/analyzer. The target LoB for the Troponin T hs Gen 6 assay was specified as 1.0 ng/L.

The LoD was determined using 5 pooled LiHep plasma samples with low cTnT content measured in duplicate across 6 runs over 3 days per lot/analyzer and calculated per EP17-A2 (23). The target LoD for the Troponin T hs Gen 6 assay was specified as 1.5 ng/L. The LoQ (concentrations achieving ≤20% and ≤10% within-laboratory CV) was derived from model fitting using 10 low-range samples measured in duplicate across 42 runs over 21 days for each of 3 selected reagent lots/analyzer. Linearity was assessed per CLSI EP06-Ed2 (24) by serial dilution of a high-concentration sample with a low/blank sample across 3 lots.

### Reference Standardization

To comply with International Organization for Standardization (ISO) 17511:2020 requirements for metrological traceability (10), the Troponin T hs Gen 6 assay was restandardized on Cobas e 801 and e 402 analyzers. The traceability chain to standard international units is presented in Supplemental Fig. 1. As no NIST reference material exists for cTnT, a certified primary reference material and a secondary calibrator panel were used to establish traceability to standard international units. The certified primary reference material, SIGMA-ALDRICH TraceCERT Amino Acids BCBZ8336 (produced according to ISO 17025:2017 and 17034:2016) (25, 26), is traceable to NIST SRM350b and SRM84 (27, 28). The secondary calibrator reference material, produced in accordance with CLSI EP32-Ed2 and ISO 17511:2020 (10, 29), was a sample panel prepared by spiking recombinant cTnT into cTnT-negative human serum matrix. The amino acid composition of the recombinant cTnT was determined using amino acid analysis, establishing metrological traceability to the standard international unit for mass concentration, and ensuring traceability to the certified reference material and therefore NIST reference materials. Commutability testing was performed with 3 validation lots, supporting the use of the serum panel as the master system for standardization. Measurement uncertainty is provided in Supplemental Table 1.

Because the Troponin T hs Gen 6 assay was restandardized and not calibrated to be numerically equivalent to the prior fifth generation cTnT hs assay, method comparisons between generations are descriptive only.

### Repeatability/Precision

Assay precision was determined according to CLSI protocol EP05-A2 (30) using 3 QC samples of known cTnT concentration (PreciControl® Troponin T hs Gen 6; Roche Diagnostics GmbH), 12 native plasma samples across the measuring range, and 3 reagent lots (reagent lots 1, 2, and 3) were run on Cobas e 801, e 402, and Pro analyzers. One reagent lot comprising 11 native serum samples and 3 precision control samples was run on the Cobas e 801 analyzer. Each sample was measured in duplicate in 42 separate runs over 21 days for each reagent lot. PreciControl Troponin T hs Gen 6 is a lyophilized control serum based on human serum, with recombinant human cTnT added at 3 concentration ranges. Level 1 has a cTnT concentration between the LoQ and the lowest sex-specific 99$^{th}$ percentile (approximately 4 ng/L), level 2 has a concentration around the highest sex-specific 99$^{th}$ percentile URL (approximately 30 ng/L), and level 3 has a concentration range that is multiples of the 99$^{th}$ percentile URL as recommended by the IFCC (approximately 220 ng/L). Within-lab precision was estimated for each reagent lot separately. The relationship between mean concentration in each sample and the percent CV was described using a corresponding power function, resulting in a precision profile.

### Lot-to-Lot and Matrix Comparisons

The lot-to-lot comparison was undertaken using 4 reagent lots, with reagent lot 1 compared with reagent lots 2, 3, and 4, respectively. For the comparison, 154 native LiHep plasma samples across the measuring range were analyzed in single determination. Passing Bablok and linear regression analyses were performed to compare lots. For the matrix comparison, 54 LiHep plasma and serum samples across the measuring range were analyzed in single determination on the Cobas e 801 analyzer and compared using Passing Bablok analysis.

### Interference

Interference from various endogenous and assay components [including freshly prepared hemolysate (concentration range: 0–1571 mg/dL), icterus/bilirubin (0–66 mg/dL; 0–1129 µmol/L), anti-Streptavidin antibodies (up to 100 µg/mL), human anti-mouse antibodies (805 µg/L), Intralipid® (0–2000 mg/dL final concentration), cholesterol (0–310 mg/dL; 0–8.0 mmol/L), rheumatoid factor (0–1200 IU/mL), and biotin (0–3600 ng/mL)] at 5 different cTnT concentrations across the measuring range from low (5, 34, and 100 ng/L) to medium and high (2600 ng/L and 8200–8600 ng/L, respectively) was determined according to the Glick model (31). Endogenous components were obtained internally from Roche Diagnostics. All interference testing was performed in LiHep samples using 3 reagent lots, which were measured in a single assessment on one analyzer. Interference was determined as the recovery of the measured cTnT concentration of the sample spiked with a given concentration of interference component to that measured without. The recovery criterion for no interference was within ±10% of that of the native sample (not spiked), except for the lowest sample where it was taken as absolute values within ±2.4 ng/L as changes in the lower ranges equate to higher percentage changes. The target interference measures for the Troponin T hs Gen 6 assay were specified as ≤1000 mg/dL for hemolysis, ≤50 mg/dL (≤855 µmol/L) for bilirubin, and ≤1200 mg/dL for biotin.

### Method Comparison: Fifth Generation cTnT hs vs Troponin T hs Gen 6

The Troponin T hs Gen 6 assay was compared against the fifth generation cTnT hs assay using 147 LiHep samples across the measuring range analyzed in single determination. In clinical practice, values below the LoD are not readable for the fifth generation cTnT assay (<3 ng/L). To determine analytical sensitivity below the reportable range for the

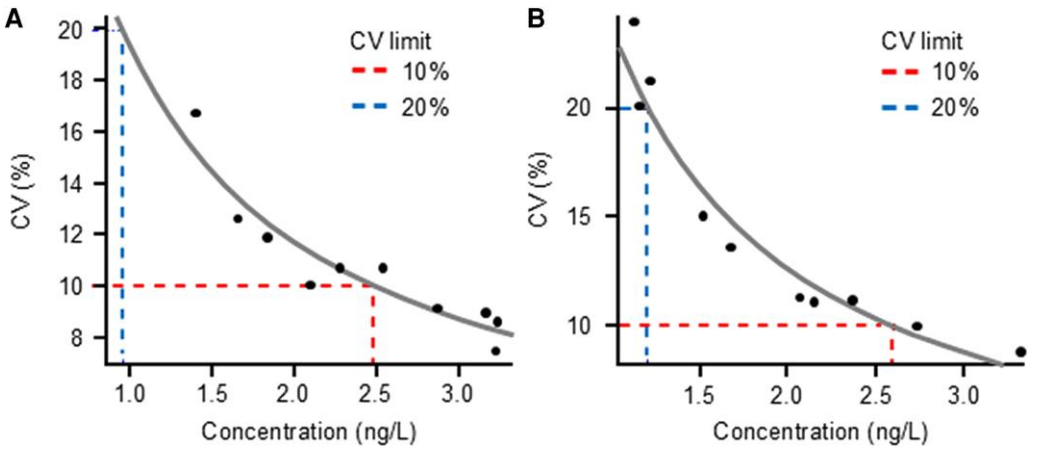


**Fig. 1. Examples of graphs used to estimate the functional LoQ. Functional LoQ for Reagent Lot 2 on the e 801 analyzer and LiHep matrix (A) and Reagent Lot 3 on the e 801 analyzer and serum matrix (B). Both graphs were selected since these demonstrated the highest ("worst") LoQ (ng/L) estimated at 20% and 10% CV in LiHep matrix. Abbreviations: LiHep, lithium-heparin; LoQ, limit of quantitation.**

fifth generation cTnT hs assay, a comparison at the low end of the measuring range (≤5 ng/L) was performed with LiHep samples using a proprietary research and development application that allows for detection and conversion of signals from concentrations below the LoD to ng/L.

## RESULTS

### Limits and Ranges

The measured LoB and LoD data were well within or below the prespecified targets (0.1–0.7 ng/L; 0.3–1.4 ng/L, respectively; Supplemental Table 2). At the 10% and 20% CV analytical precision levels, the LoQ was determined as 3.0 and 1.5 ng/L, respectively (Supplemental Table 3). Representative plots demonstrating highest (i.e., "worst") LoQ (ng/L) estimated at 10% and 20% CV in LiHep matrix samples are shown in Fig. 1; further reagent lots are included in Supplemental Fig. 2. The measuring range was identified as 1.5 to 9500 ng/L, and excellent linearity was demonstrated across 3 reagent lots (Fig. 2). Reagent lots 1, 2, and 3 demonstrated a linear measuring range of 0.9 to 10 615 ng/L, 1.3 to 10 708 ng/L, and 1.4 to 10 674 ng/L, respectively (all Pearson's $r = 1.00$ between expected and measured concentration).

### Repeatability/Precision

The precision profile from 3 reagent lots focusing on the low cTnT concentration end (≤32 ng/L) on the Cobas e 801 analyzer is shown in Fig. 3 and Table 1. The Troponin T hs Gen 6 assay demonstrated high precision across the Cobas Pro, e 801, and e 402 platforms and reagent lots (Supplemental Fig. 3). On the Cobas e 801 analyzer across 3 reagent lots, repeatability CVs were 1.0% to 5.8% and intermediate precision CVs were 2.0% to 9.0% for mean cTnT concentrations of 2.6 to 9230 ng/L in LiHep plasma samples. On the Cobas e 402 analyzer across 3 reagent lots, repeatability CVs were 0.8% to 3.5% and intermediate precision CVs were 1.6% to 7.2% for mean cTnT concentrations of 3.0 to 9479 ng/L in LiHep plasma samples. Using one reagent lot measured on the Cobas e 801 analyzer, repeatability CVs were 0.7% to 7.3% and intermediate precision

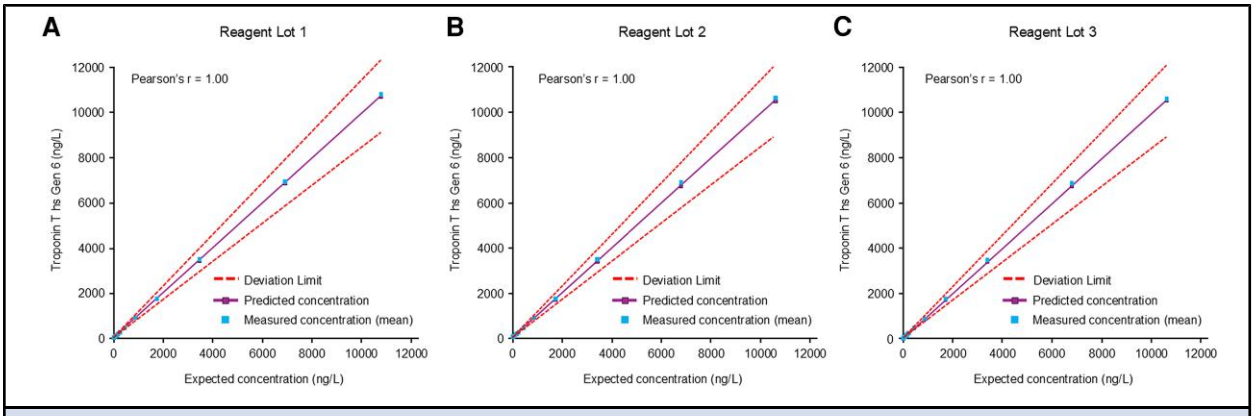


**Fig. 2. Linearity across 3 reagent lots shown in Reagent Lot 1 (A),Reagent Lot 2 (B), and Reagent Lot 3 (C).**

CVs were 2.9% to 9.0% for mean cTnT concentrations of 2.5 to 9338 ng/L in serum samples (Supplemental Table 4).

### Lot-to-Lot and Matrix Variation and Interferences

High lot-to-lot comparability was observed between all 4 lots assessed, with $\tau = 0.99$ and $r = 1.00$ for all comparisons (Supplemental Fig. 4). LiHep plasma and serum samples were highly comparable, with $r = 0.998$ (Supplemental Fig. 5). No interference was observed in LiHep samples when tested up to the highest spike concentrations of endogenous and assay components assessed (one representative reagent lot is shown in Supplemental Table 5). The measured interference data met the prespecified interference targets. As an example, Fig. 4 shows the interference of hemoglobin with assay measurement in samples with low (5, 34, and 100 ng/L), medium (2600 ng/L), and high (8200–8600 ng/L) cTnT concentrations.

### Method Comparison: Fifth Generation cTnT hs vs Troponin T hs Gen 6

A positive, monotonic, nonlinear relationship was observed between the fifth generation cTnT hs and Troponin T hs Gen 6 assays across the measuring range (3 to 10 000 ng/L) and at the low end of the measuring range (3 to 25 ng/L) of the fifth generation cTnT hs assay (Fig. 5A and B, respectively). In general, Troponin T hs Gen 6 values were higher than those for the fifth generation cTnT hs assay for a given sample (Fig. 5C). At the very low end of the measuring range (1.5 to 5 ng/L), values for the fifth generation cTnT hs and Troponin T hs Gen 6 assays are comparable and demonstrate a somewhat linear behavior (Fig. 5D). As values <3 ng/L are measurable with the Troponin T hs Gen 6 assay and would not be measurable in clinical practice using the fifth generation cTnT hs assay, the Troponin T hs Gen 6 assay has higher analytical sensitivity than the fifth generation cTnT hs assay, with 10/11 (90.9%) samples that are below the LoD of the fifth generation cTnT hs assay detectable with the Troponin T hs Gen 6 assay (Fig. 5D). A Bland-Altman plot demonstrated that although the assays correlate, the mean relative difference was −0.25 ng/L for values measured with the fifth generation cTnT hs compared with the Troponin T hs Gen 6 assay (Fig. 5E). Overall, the Troponin T hs Gen 6 assay yields a higher dynamic range, including values at the low end of the measuring range.

## DISCUSSION

The analytical performance evaluation of the novel Troponin T hs Gen 6 assay demonstrated high

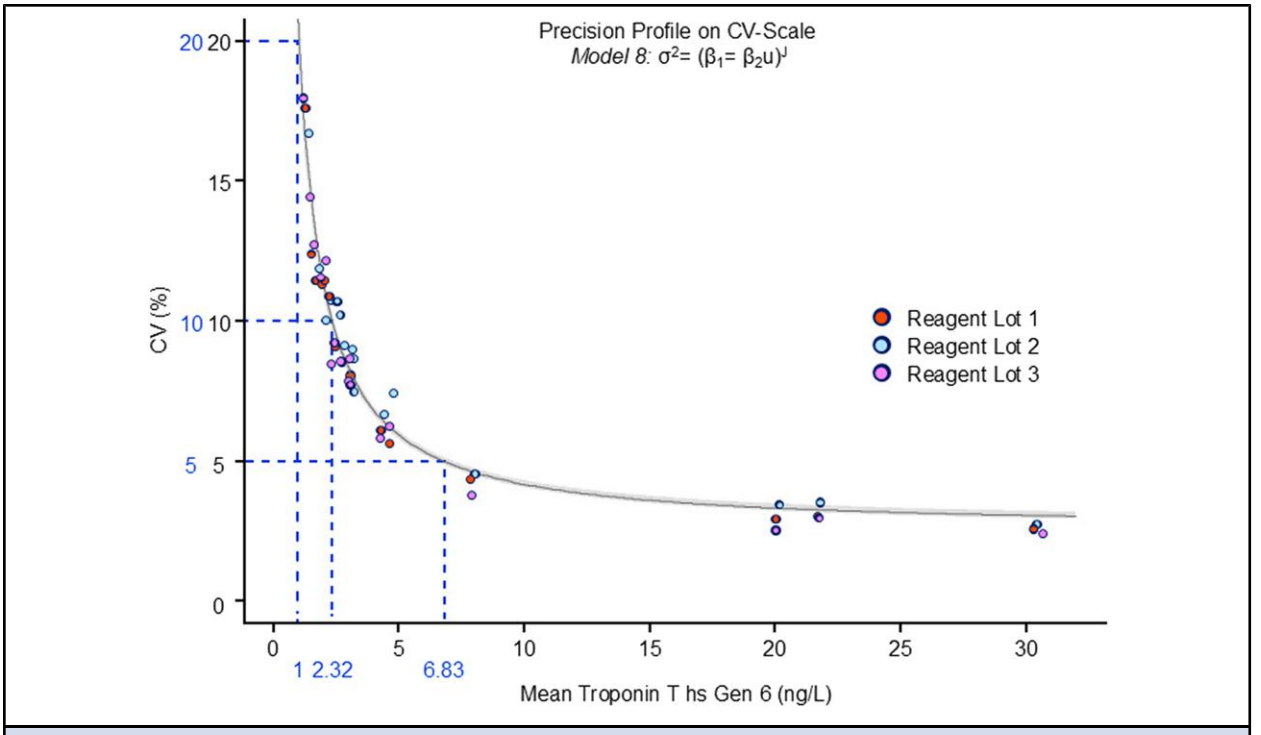


**Fig. 3. The Troponin T hs Gen 6 assay composite prediction data at the lower cTnT concentration end on the Cobas e 801 analyzer. Three reagent lots were measured over 21 days to show precision and accuracy according to CLSI guidelines (serum;1200000698885). CV at 20% and 10% is indicated by the dotted line. Abbreviations: cTnT, cardiac troponin T; hs, high sensitivity.**

sensitivity and high precision at the low end of the measuring range in serum and LiHep plasma samples on the Cobas e 801, e 402, and Pro analyzers and showed resistance to interference in LiHep samples. The prespecified LoB and LoD targets (1.0 and 1.5 ng/L, respectively) were met; the target LoQ was also met at 20% and 10% CV (1.5 and 3 ng/L, respectively), and linearity was demonstrated across the measuring range. The new assay formulation also demonstrated increased resistance against interference with hemoglobin (≤1000 mg/dL) and bilirubin (≤50 mg/dL; ≤855 µmol/L), a 10-fold and 2-fold improvement, respectively, compared with the previous assay generation, as well as state-of-the-art interference elimination against anti-Streptavidin antibody interference. These results indicate that any subsequent hemolysis of the blood sample would have very limited impact on assay measurements, even where the samples are markedly hemolyzed. Furthermore, the Troponin T hs Gen 6 assay maintained resistance to all other interferents, including biotin, consistent with the predecessor assay. Low lot-to-lot variability further supports the robustness of the Troponin T hs Gen 6 assay.

The Troponin T hs Gen 6 assay has higher analytical sensitivity (LoB/LoD/LoQ) than the fifth generation cTnT hs assay, enabling quantification of cTnT with high precision at lower concentrations that would otherwise be undetectable with the previous assay. Improved analytical sensitivity will enable better differentiation of low cTnT values at or below the 99$^{th}$ percentile URL, which may improve the definition and performance of optimized risk stratification thresholds and delta values, thereby facilitating early and safe rule-out

**Table 1. Repeatability and precision of the Troponin T hs Gen 6 assay on the Cobas e 801 and e 402 analyzers.**

| | | Repeatability | | Intermediate precision | |
|---|---|---|---|---|---|
| Sample | Mean, ng/L | SD estimate, ng/L | CV % | SD estimate, ng/L | CV % |
| **Cobas e 801 analyzer** | | | | | |
| Reagent Lot 1 | | | | | |
| Human LiHep plasma 1 | 2.8 | 0.1 | 5.1 | 0.3 | 9.0 |
| Human LiHep plasma 2 | 4.6 | 0.2 | 5.3 | 0.4 | 7.9 |
| Human LiHep plasma 3 | 8.2 | 0.2 | 2.8 | 0.4 | 4.6 |
| Human LiHep plasma 4 | 20.4 | 0.5 | 2.5 | 0.7 | 3.5 |
| Human LiHep plasma 5 | 22.0 | 0.6 | 2.9 | 0.9 | 4.1 |
| Human LiHep plasma 6 | 30.9 | 0.7 | 2.2 | 1.0 | 3.4 |
| Human LiHep plasma 7 | 87.8 | 1.9 | 2.1 | 2.9 | 3.3 |
| Human LiHep plasma 8 | 143.0 | 2.4 | 1.7 | 4.3 | 3.0 |
| Human LiHep plasma 9 | 360.0 | 6.0 | 1.7 | 10.1 | 2.8 |
| Human LiHep plasma 10 | 4105.0 | 66.3 | 1.6 | 138.0 | 3.4 |
| Human LiHep plasma 11 | 9223.0 | 190.0 | 2.1 | 298.0 | 3.2 |
| Human LiHep plasma 12 | 9230.0 | 172.0 | 1.9 | 411.0 | 4.5 |
| PreciControl 1 | 4.9 | 0.2 | 4.2 | 0.3 | 6.3 |
| PreciControl 2 | 35.4 | 0.5 | 1.5 | 1.1 | 3.0 |
| PreciControl 3 | 221.0 | 2.6 | 1.2 | 6.4 | 2.9 |
| Reagent Lot 2 | | | | | |
| Human LiHep plasma 1 | 2.6 | 0.2 | 5.7 | 0.2 | 8.0 |
| Human LiHep plasma 2 | 4.4 | 0.2 | 3.6 | 0.2 | 5.4 |
| Human LiHep plasma 3 | 8.0 | 0.2 | 2.0 | 0.3 | 3.5 |
| Human LiHep plasma 4 | 20.2 | 0.5 | 2.4 | 0.5 | 2.7 |
| Human LiHep plasma 5 | 21.9 | 0.5 | 2.5 | 0.7 | 3.1 |
| Human LiHep plasma 6 | 30.9 | 0.6 | 1.9 | 0.8 | 2.7 |
| Human LiHep plasma 7 | 87.8 | 1.7 | 1.9 | 2.5 | 2.9 |
| Human LiHep plasma 8 | 143.0 | 2.3 | 1.6 | 3.7 | 2.6 |
| Human LiHep plasma 9 | 362.0 | 6.3 | 1.7 | 8.9 | 2.5 |
| Human LiHep plasma 10 | 4157.0 | 63.1 | 1.5 | 108.0 | 2.6 |
| Human LiHep plasma 11 | 9091.0 | 177.0 | 1.9 | 226.0 | 2.5 |
| Human LiHep plasma 12 | 9136.0 | 167.0 | 1.8 | 318.0 | 3.5 |
| PreciControl 1 | 4.7 | 0.1 | 3.1 | 0.2 | 5.1 |
| PreciControl 2 | 35.4 | 0.5 | 1.5 | 0.8 | 2.3 |
| PreciControl 3 | 222.0 | 2.3 | 1.1 | 4.5 | 2.0 |
| Reagent Lot 3 | | | | | |
| Human LiHep plasma 1 | 2.6 | 0.2 | 5.8 | 0.2 | 7.6 |
| Human LiHep plasma 2 | 4.4 | 0.2 | 3.6 | 0.2 | 5.2 |
| Human LiHep plasma 3 | 8.0 | 0.2 | 2.4 | 0.3 | 3.7 |

(continued)

**Table 1.** Continued

| Sample | Mean, ng/L | Repeatability | | Intermediate precision | |
|---|---|---|---|---|---|
| | | SD estimate, ng/L | CV % | SD estimate, ng/L | CV % |
| Human LiHep plasma 4 | 20.2 | 0.4 | 1.9 | 0.6 | 3.0 |
| Human LiHep plasma 5 | 21.8 | 0.6 | 2.6 | 0.7 | 3.1 |
| Human LiHep plasma 6 | 30.3 | 0.6 | 1.9 | 0.8 | 2.8 |
| Human LiHep plasma 7 | 86.4 | 1.4 | 1.6 | 2.7 | 3.1 |
| Human LiHep plasma 8 | 141.0 | 2.1 | 1.5 | 4.3 | 3.0 |
| Human LiHep plasma 9 | 355.0 | 5.6 | 1.6 | 9.3 | 2.6 |
| Human LiHep plasma 10 | 4135.0 | 59.0 | 1.4 | 113.0 | 2.7 |
| Human LiHep plasma 11 | 9127.0 | 202.0 | 2.2 | 252.0 | 2.8 |
| Human LiHep plasma 12 | 9143.0 | 184.0 | 2.0 | 343.0 | 3.8 |
| PreciControl 1 | 4.7 | 0.1 | 2.7 | 0.2 | 5.0 |
| PreciControl 2 | 35.3 | 0.5 | 1.4 | 0.8 | 2.3 |
| PreciControl 3 | 220.0 | 2.3 | 1.0 | 4.7 | 2.1 |
| **Cobas e 402 analyzer** | | | | | |
| Reagent Lot 1 | | | | | |
| Human LiHep plasma 1 | 3.0 | 0.1 | 3.5 | 0.1 | 4.5 |
| Human LiHep plasma 2 | 4.8 | 0.1 | 2.6 | 0.2 | 3.7 |
| Human LiHep plasma 3 | 8.6 | 0.1 | 1.5 | 0.2 | 2.3 |
| Human LiHep plasma 4 | 21.5 | 0.2 | 1.1 | 0.4 | 1.9 |
| Human LiHep plasma 5 | 23.3 | 0.3 | 1.3 | 0.4 | 1.8 |
| Human LiHep plasma 6 | 32.4 | 0.3 | 1.0 | 0.7 | 2.2 |
| Human LiHep plasma 7 | 93.1 | 1.0 | 1.1 | 2.6 | 2.7 |
| Human LiHep plasma 8 | 150.0 | 1.9 | 1.3 | 3.8 | 2.5 |
| Human LiHep plasma 9 | 374.0 | 3.6 | 1.0 | 7.4 | 2.0 |
| Human LiHep plasma 10 | 4161.0 | 51.9 | 1.2 | 80.3 | 1.9 |
| Human LiHep plasma 11 | 9479.0 | 106.0 | 1.1 | 279.0 | 2.9 |
| Human LiHep plasma 12 | 9470.0 | 81.4 | 0.9 | 328.0 | 3.5 |
| PreciControl 1 | 5.1 | 0.2 | 3.1 | 0.2 | 3.8 |
| PreciControl 2 | 36.8 | 0.4 | 1.0 | 0.7 | 1.9 |
| PreciControl 3 | 229.0 | 2.9 | 1.3 | 5.3 | 2.3 |
| Reagent Lot 2 | | | | | |
| Human LiHep plasma 1 | 3.2 | 0.1 | 3.1 | 0.1 | 4.2 |
| Human LiHep plasma 2 | 4.9 | 0.1 | 2.2 | 0.1 | 2.8 |
| Human LiHep plasma 3 | 8.7 | 0.2 | 2.0 | 0.2 | 2.3 |
| Human LiHep plasma 4 | 21.4 | 0.3 | 1.4 | 0.4 | 1.7 |
| Human LiHep plasma 5 | 23.2 | 0.2 | 1.0 | 0.4 | 1.6 |
| Human LiHep plasma 6 | 32.1 | 0.4 | 1.1 | 0.6 | 2.0 |
| Human LiHep plasma 7 | 91.7 | 1.1 | 1.2 | 2.7 | 2.9 |

(continued)

**Table 1. Continued**

| Sample | Mean, ng/L | Repeatability | | Intermediate precision | |
|---|---|---|---|---|---|
| | | SD estimate, ng/L | CV % | SD estimate, ng/L | CV % |
| Human LiHep plasma 8 | 148.0 | 1.7 | 1.2 | 3.7 | 2.5 |
| Human LiHep plasma 9 | 367.0 | 3.5 | 0.9 | 7.4 | 2.0 |
| Human LiHep plasma 10 | 4177.0 | 52.0 | 1.2 | 82.6 | 2.0 |
| Human LiHep plasma 11 | 9341.0 | 99.5 | 1.1 | 286.0 | 3.1 |
| Human LiHep plasma 12 | 9352.0 | 83.6 | 0.9 | 311.0 | 3.3 |
| PreciControl 1 | 5.3 | 0.1 | 2.1 | 0.2 | 3.0 |
| PreciControl 2 | 36.8 | 0.3 | 0.8 | 0.7 | 1.9 |
| PreciControl 3 | 227.0 | 2.7 | 1.2 | 5.6 | 2.5 |
| Reagent Lot 3 | | | | | |
| Human LiHep plasma 1 | 3.0 | 0.1 | 3.4 | 0.2 | 7.2 |
| Human LiHep plasma 2 | 4.8 | 0.1 | 2.3 | 0.3 | 5.8 |
| Human LiHep plasma 3 | 8.6 | 0.1 | 1.5 | 0.3 | 3.2 |
| Human LiHep plasma 4 | 21.5 | 0.3 | 1.3 | 0.4 | 2.0 |
| Human LiHep plasma 5 | 23.3 | 0.3 | 1.4 | 0.5 | 2.0 |
| Human LiHep plasma 6 | 32.9 | 0.3 | 1.0 | 0.8 | 2.3 |
| Human LiHep plasma 7 | 93.9 | 1.1 | 1.1 | 2.4 | 2.6 |
| Human LiHep plasma 8 | 151.0 | 1.7 | 1.1 | 3.3 | 2.2 |
| Human LiHep plasma 9 | 378.0 | 4.1 | 1.1 | 6.0 | 1.6 |
| Human LiHep plasma 10 | 4233.0 | 53.9 | 1.3 | 78.7 | 1.9 |
| Human LiHep plasma 11 | 9395.0 | 96.1 | 1.0 | 253.0 | 2.7 |
| Human LiHep plasma 12 | 9426.0 | 88.6 | 0.9 | 300.0 | 3.2 |
| PreciControl 1 | 5.2 | 0.1 | 2.4 | 0.3 | 5.3 |
| PreciControl 2 | 37.2 | 0.4 | 1.0 | 0.7 | 1.8 |
| PreciControl 3 | 230.0 | 1.8 | 0.8 | 3.7 | 1.6 |

Abbreviation: LiHep, lithium-heparin.

of AMI (32). Higher sensitivity may also allow more precise determination of the 99$^{th}$ percentile in both males and females and increase the number of measurements above the LoD (33). Additionally, higher analytical sensitivity and accuracy, especially at lower concentrations and improved interference tolerance, is likely to improve clinical performance for current and future applications for hs-cTnT testing in practice (34).

It should be noted that the Troponin T hs Gen 6 assay uses the same antibodies and recognizes the same epitopes as the fifth generation cTnT hs assay (11, 35); therefore, both assays are able to detect intact and fragmented cTnT. However, the assays were standardized against different reference materials. The fifth generation cTnT hs assay was standardized against previous assay generations, with a traceability chain tracing back to bovine cTnT (36). Bovine cTnT was used as no international cTnT reference materials were established at the time of standardizing the first generation cTnT assay (36). As human samples display a higher level of uncertainty over generations (37), the Troponin T hs Gen 6 assay was standardized using a sample panel

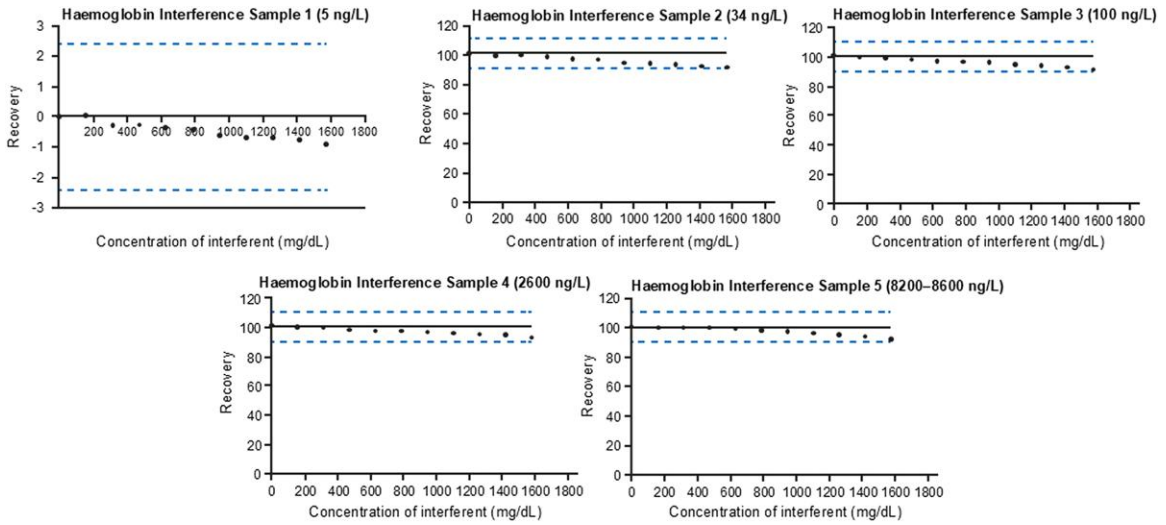


**Fig. 4. Interference of hemoglobin investigated according to the Glick model using freshly prepared hemolysates in concentration ranges from 0 to 1571 mg/dL (reagent lot 2) in LiHep plasma samples. Samples 1 to 5 are representative of low (5, 34, 100 ng/L), medium (2600 ng/L), and high cTnT concentrations (8200–8600 ng/L). Acceptance criteria (shown as lines below and above the *x*-axis) for the lowest concentration sample were recovery within absolute values of ±2.4 ng/L, and ±10% recovery for the other concentrations, as changes in the lower ranges are equal to higher percentage changes. All experiments performed with 3 reagent lots on one analyzer. Abbreviations: cTnT, cardiac troponin T; LiHep, lithium-heparin.**

(secondary calibrator II) spiked with recombinant human cTnT that is traceable to NIST materials to establish metrological traceability (38). NIST materials SRM350b and 84 were used rather than SRM2921 (human cTn complex) as SRM2921 demonstrates noncertified reference values (39). In addition, if SRM2921 was used for standardization, protein-specific biases could be introduced; as cTnT fragments over time, recombinant human cTnT best represents the available circulating form of cTnT 12 h after the onset of AMI (40).

Given the restandardization and traceability (per regulatory requirements) of the new Troponin T hs Gen 6 assay to recombinant human cTnT, the reported values for the same sample will be different for these assays. However, the Troponin T hs Gen 6 assay and the fifth generation cTnT hs assay showed a positive monotonic nonlinear relationship. The restandardization of the Troponin T hs Gen 6 assay may impact decision thresholds for established diagnostic pathways and increase detection of patients who display cTnT measurements below the 99$^{th}$ percentile URL; future studies are required to define new decision thresholds and assess the clinical performance of the assay.

This study has several strengths, including adherence to CLSI protocols for analytical performance assessments and standardization to new state-of-the-art metrological traceability to NIST reference materials, which can improve the correlation between different assays. In addition, the 3-level QC system enables better monitoring of sensitivity across the clinically relevant measuring range, better detection of trends or issues throughout the measuring range, and provides a more comprehensive picture of overall test system performance compared with a 2-level

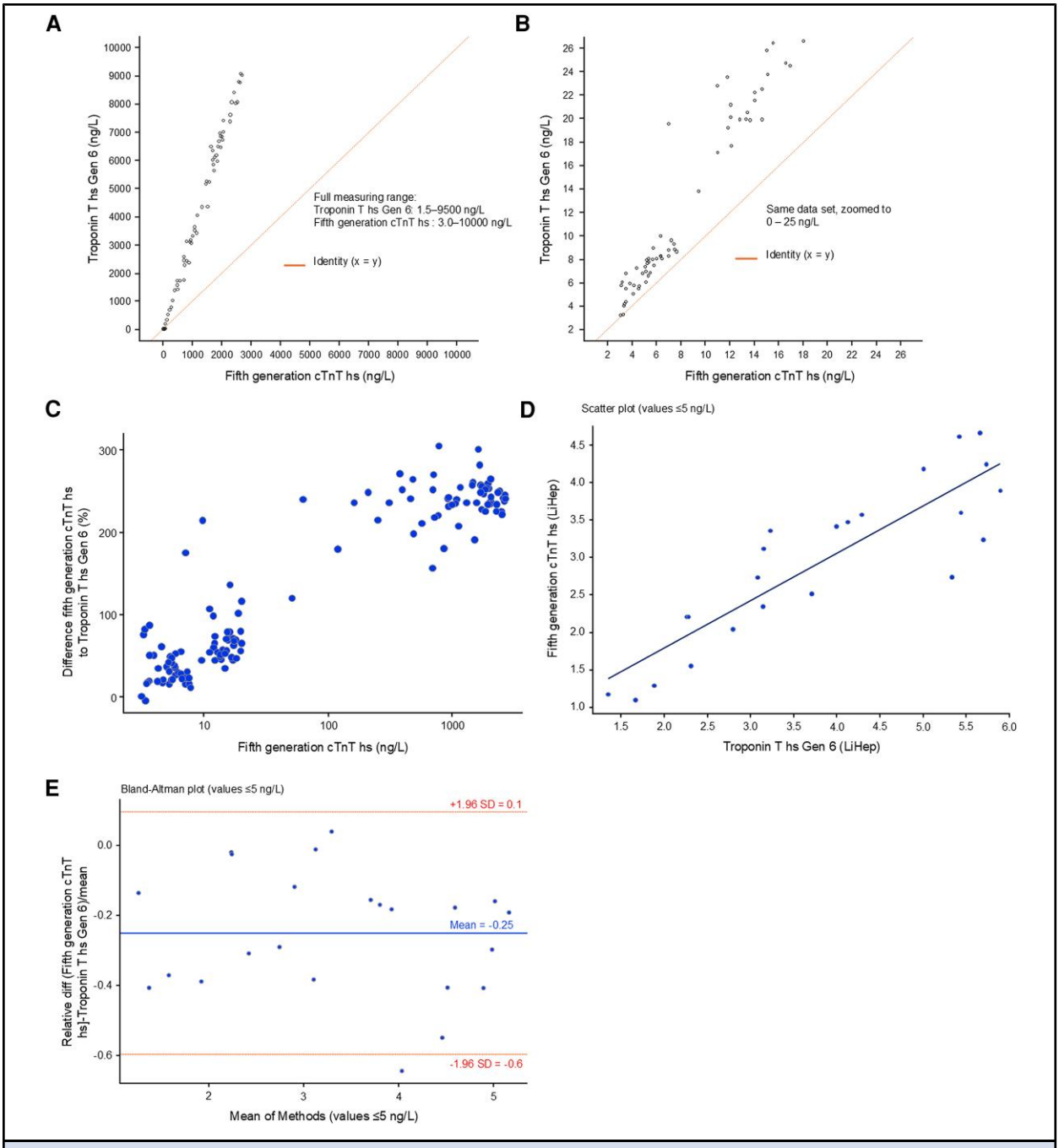


**Fig. 5. Correlation of the fifth generation cTnT hs and Troponin T hs Gen 6 assays across the measuring range. Data shown for 147 samples across the measuring range (A), 0 to 25 ng/L (B), the difference of values (C), comparison at the low end of the measuring range (1.5 to 5 ng/L) (D), a Bland-Altman plot for values ≤5 ng/L (E). Plot of Troponin T hs Gen 6 value range correlation with fifth generation cTnT hs: the values of the 2 assays are not comparable (i.e., not standardized) but highly correlated to each other. Abbreviations: cTnT, cardiac troponin T; hs, high sensitivity; LiHep, lithium-heparin.**

system (41). Furthermore, assessment of Troponin T hs Gen 6 assay performance was done using more than one analyzer and several reagent lots to reflect the variability expected in clinical practice.

However, a limitation of the assay is that the Troponin T hs Gen 6 values are not comparable to fifth generation cTnT hs values as the assays were standardized against different reference materials, leading to a new measuring range and necessitating determination of new sex-specific 99$^{th}$ percentile URLs in a separate reference range study (42, 43). Therefore, accelerated diagnostic pathways for the use of hs-cTnT for AMI will need to be updated accordingly. An additional limitation is that the sample collection date for some samples was not provided by the vendor; therefore, we could not verify the age of such samples. The use of Intralipid to assess lipid-based interference, which does not replicate triglyceride interference in clinical practice, is another limitation. However, Intralipid is the recommended surrogate for establishing lipemia interference as it provides a standardized, reproducible, and stable matrix for creating turbidity, representing the "worst-case" scenario for turbidity-based interference, ensuring that the Troponin T hs Gen 6 assay is robust against light-scattering effects. Furthermore, interferences such as macrotroponin, autoantibodies, and heterophile antibodies need to be assessed in detail in future studies, as has previously been done for the fifth generation cTnT hs assay. Although we demonstrate the analytical performance of the Troponin T hs Gen 6 assay, further studies are needed to determine the clinical performance of the assay.

## CONCLUSION

The Troponin T hs Gen 6 assay on Cobas e 801, e 402, and Pro analyzers displays high sensitivity; high precision at the low end and across the measuring range; and robust resistance to hemoglobin, bilirubin, and biotin interference, fulfilling the analytical criteria set by the IFCC for hs-cTn assays (42, 43).

## SUPPLEMENTAL MATERIAL

Supplemental material is available at *The Journal of Applied Laboratory Medicine* online.

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**Data Availability:** Requests concerning the data supporting the findings of this study can be directed to rotkreuz.datasharingrequests@roche.com for consideration.

**Nonstandard Abbreviations:** cTn, cardiac troponin; AMI, acute myocardial infarction; hs, high sensitivity; URL, upper reference limit; LoD, limit of detection; LiHep, lithium-heparin; LoB, limit of blank; LoQ, limit of quantitation; ISO, International Organization for Standardization; cTnT, cardiac troponin T.

**Author Contributions:** *The corresponding author takes full responsibility that all authors on this publication have met the following required criteria of eligibility for authorship: (a) significant contributions to the conception and design, acquisition of data, or analysis and interpretation of data; (b) drafting or revising the article for intellectual content; (c) final approval of the published article; and (d) agreement to be accountable for all aspects of the article thus ensuring that questions related to the accuracy or integrity of any part of the article are appropriately investigated and resolved. Nobody who qualifies for authorship has been omitted from the list.*

Marko Knoll (Conceptualization-Equal, Data curation-Equal, Funding acquisition-Equal, Investigation-Equal, Methodology-Equal, Project administration-Equal, Resources-Equal, Visualization-Equal, Writing—original draft-Equal, Writing—review & editing-Equal), Lori Daniels (Writing—original draft-Equal, Writing—review & editing-Equal), Christian Mueller (Writing—original draft-Equal, Writing—review & editing-Equal), Nicholas Mills (Writing—original draft-Equal, Writing—review & editing-Equal),

Evangelos Giannitsis (Writing—original draft-Equal, Writing—review & editing-Equal), Alisa Rösser (Conceptualization-Equal, Data curation-Equal, Methodology-Equal, Writing—original draft-Equal, Writing—review & editing-Equal), Dunja Kurtoic (Formal analysis-Equal, Investigation-Equal, Methodology-Equal, Software-Equal, Supervision-Equal, Validation-Equal, Visualization-Equal, Writing—original draft-Equal, Writing—review & editing-Equal), Annika Wahl (Formal analysis-Equal, Methodology-Equal, Supervision-Equal, Validation-Equal, Writing—original draft-Equal, Writing—review & editing-Equal), Richard Body (Writing—review & editing-Equal), Robert Christenson (Writing—review & editing-Equal), Christa Cobbaert (Writing—review & editing-Equal), Christopher R Defilippi (Writing—review & editing-Equal), Kai Eggers (Writing—review & editing-Equal), Kenji Inoue (Writing—review & editing-Equal), Allan Jaffe (Writing—review & editing-Equal), Cian McCarthy (Writing—review & editing-Equal), James McCord (Writing—review & editing-Equal), Johannes Neumann (Writing—review & editing-Equal), Torbjorn Omland (Writing—review & editing-Equal), Cynthia Papendick (Writing—review & editing-Equal), Yader Sandoval (Writing—review & editing-Equal), Jack Wei Chieh Tan (Writing—review & editing-Equal), Martin Than (Writing—review & editing-Equal), Raphael Twerenbold (Writing—review & editing-Equal), William Peacock (Writing—original draft-Equal, Writing—review & editing-Equal), and Steven Meex (Writing—original draft-Equal, Writing—review & editing-Equal)

**Authors' Disclosures or Potential Conflicts of Interest:** *Upon manuscript submission, all authors completed the author disclosure form.*

**Research Funding:** This study was funded by Roche Diagnostics GmbH(Penzberg, Germany).

**Disclosures:** M. Knoll, A.F.A. Rösser, A. Wahl, and D. Kurtoic are employees of Roche Diagnostics GmbH. L.B. Daniels has served as a consultant for QuidelOrtho, Roche, and Siemens; has served on clinical endpoint adjudication committees for Abbott, Pfizer, QuidelOrtho, and Tosoh; and has received research funding and/or served as an investigator for clinical studies for Cardiosense, DalCor Pharmaceuticals, and Roche. C. Mueller reports research support from the Swiss National Science Foundation, the Swiss Heart Foundation, the University of Basel, the University Hospital Basel, the KTI, Abbott, Beckman Coulter, BRAHMS, Idorsia, Ortho Diagnostics, Novartis, Roche, Siemens, and Singulex and speaker/consulting honoraria from Amgen, AstraZeneca, Bayer, Bristol-Myers Squibb, Boehringer Ingelheim, Daiichi Sankyo, Idorsia, Novartis, Osler, Roche, and Sanofi, all outside the submitted work. N.L. Mills is supported by the British Heart Foundation through a Chair Award (CH/F/21/90010), a Programme Grant (RG/F/25/110169), and a Research Excellence Award (RE/24/130012) and reports receiving honoraria or speaker fees from Abbott Diagnostics, Siemens Healthineers, and Roche Diagnostics within the last 3 years. E. Giannitsis received honoraria for lectures from Roche Diagnostics, AstraZeneca, Bayer, Daiichi-Sankyo, and Lilly Eli Deutschland; served as a consultant for Roche Diagnostics, BRAHMS Thermo Fisher Scientific, and Boehringer Ingelheim; and has received research funding from BRAHMS Thermo Fisher Scientific, Roche Diagnostics, Bayer Vital, and Daiichi Sankyo. R. Body has consulted for Siemens, Roche, Beckman, Singulex, LumiraDx, Radiometer, and Abbott and has received research support from Roche, Siemens, Abbott, Singulex, and LumiraDx. R.H. Christenson is a consultant for and receives funding/support from Roche Diagnostics, Siemens Healthineers, Beckman Coulter Diagnostics, Becton Dickinson and Company, QuidelOrtho Corp., and Sphingotec GmbH and was editor-in-chief for *The Journal of Applied Laboratory Medicine* (ADLM) through 2025. C. Cobbaert states no conflict of interest related to this study and declares a research collaboration with Roche Diagnostics and received lecture fees, all fully used for further research on cardiac biomarkers. C.R. deFilippi reports receiving grants from Roche Diagnostics, QuidelOrtho, Siemens Healthineers, FujiRebio, and Abbott Diagnostics; receiving personal fees from Roche Diagnostics, Abbott Diagnostics, Siemens Healthineers, QuidelOrtho, Tosoh, and FujiRebio; and holding a patent that assesses differential risk for developing heart failure. K.M. Eggers reports receiving personal fees from Roche Diagnostics. K. Inoue received a research grant from Grant-in-Aid for Scientific Research C (No. 18K09954), Roche Diagnostics, SB Bioscience Co., Ltd., Fujirebio Inc., Bayel Sysmex, and Kanto Chemical Co. Inc. A.S. Jaffe has consulted or presently consults for most of the major diagnostics companies, including Abbott, Siemens, ET Healthcare, Roche, Radiometer, Mindray, 4Teen4, and Moderna, and has stock options in RCE Technologies. C.P. McCarthy reported receiving a grant from the National Heart, Lung, And Blood Institute (K23HL167659) and personal fees or honorarium from Roche Diagnostics, Abbott Laboratories, Alnylam Pharmaceuticals, Cleerly Health, Corcept Therapeutics, New Amsterdam Pharma, and HeartFlow. J. McCord receives research funding/support from Beckman Coulter, Roche Diagnostics, Abbott Laboratories, and Siemens and is a consultant for Beckman Coulter, Roche Diagnostics, and Siemens. J.T. Neumann reports receiving personal fees from Roche, Siemens, Abbott, and PHC; having a patent pending on the use of a computing device to estimate the probability of myocardial infarction; and being a cofounder and shareholder of ART-EMIS Hamburg GmbH. T. Omland reports receiving personal fees from Abbott Laboratories, Roche Diagnostics, CardiNor, SpinChip, and Novo Nordisk; receiving grants from Abbott Laboratories; and receiving nonfinancial support from Roche Diagnostics, Novartis, ChromaDex Inc., a Niagen Bioscience company, and CardiNor. C. Papendick reports receiving speaker and advisory board fees from Roche Diagnostics and Abbott Diagnostics. Y. Sandoval reports receiving consulting fees from Abbott Vascular, Boston Scientific, CathWorks, GE Healthcare, HeartFlow, Philips, Roche Diagnostics, and Siemens; speaker fees from Abbott Vascular, CathWorks, Cleerly, GE Healthcare, HeartFlow, Medtronic, Philips, and Roche Diagnostics; advisory board fees from Abbott Vascular, GE Healthcare, HeartFlow, Philips, Roche Diagnostics, and Siemens; a research grant from Cleerly; and an educational grant from Shockwave Medical; he and others hold US patent 20210401347. J.W.C. Tan has received honoraria from Amgen,

Medtronic, Abbott Vascular, Novartis, and Biosensors; research and educational grants from Medtronic, Edwards, Abbott, Menarini, and Roche; and consulting fees from J&J and Medtronic. M.P. Than has received research and education grants from Abbott, Alere, Beckman Coulter, QuidelOrtho, Radiometer, Roche, and Siemens Healthineers and has received consultant, speaker, and/or advisory board payments from Abbott, Alere, Beckman Coulter, QuidelOrtho, Radiometer, Roche, Siemens Healthineers, and Upstream Medical Technologies. R. Twerenbold reports receiving grants from the German Center for Cardiovascular Research, the Kühne Foundation, the Joachim Herz Foundation, the Swiss National Science Foundation, and the Swiss Heart Foundation; receiving personal fees from Abbott, Amgen, AstraZeneca, Psyros, Roche, Siemens, Singulex, and Thermo Scientific BRAHMS Biomarkers; having a patent pending on the use of a computing device to estimate the probability of myocardial infarction; and being a cofounder and shareholder of ART-EMIS Hamburg GmbH. W.F. Peacock is a consultant for Brainbox, Abbott, Werfen, Janssen, Osler, Roche, Siemens, CSL Behring, RCE Technologies, Trivarum, Upstream, and ScPharma and has ownership interest in Aseptoscope, Emergencies in Medicine, and Comprehensive Research Associates. S.J.R. Meex reports research support and consulting honoraria from Abbott Laboratories and Roche Diagnostics and lecture fees from Werfen, all paid to the institution.

**Role of Sponsor:** The funding organization played a direct role in the design of study, review and interpretation of data, and preparation of the manuscript. The funding organization played no role in the choice of enrolled patients or final approval of the manuscript.

**Acknowledgments:** Editorial support for this manuscript, under the direction of the authors, was provided by Estelle Challinor and Jade Drummond of Springer Health+ and was funded by Roche Diagnostics International AG (Rotkreuz, Switzerland). R. Body acknowledges the support (time and access to expertise) of the NIHR Manchester Biomedical Research Centre and the NIHR Healthtec Research Centre for Emergency and Acute Care.

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