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Analytical performance evaluation of the Elecsys® Troponin T Gen 5 STAT assay

Robert L. Fitzgerald[a,*], Judd E. Hollander[b], W. Frank Peacock[c], Alexander T. Limkakeng[d], Nancy Breitenbeck[e], Kareen Blechschmidt[f], Michael Laimighofer[f], Christopher deFilippi[g]

[a] Department of Pathology, UC San Diego Health, San Diego, CA, United States of America
[b] Department of Emergency Medicine, Thomas Jefferson University, Philadelphia, PA, United States of America
[c] Department of Emergency Medicine, Baylor College of Medicine, Houston, TX, United States of America
[d] Division of Emergency Medicine, Duke University, Durham, NC, United States of America
[e] NB Research Inc., Indianapolis, IN, United States of America
[f] Roche Diagnostics GmbH, Penzberg, Germany
[g] Inova Heart and Vascular Institute, Falls Church, VA, United States of America

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ABSTRACT

Background: We report the analytical performance of the Elecsys® Troponin T Gen 5 STAT (TnT Gen 5 STAT; Roche Diagnostics) assay.

Methods: Measuring limits/ranges were determined in lithium-heparin plasma samples per Clinical and Laboratory Standards Institute (CLSI) EP17-A2. Precision was evaluated per CLSI EP05-A2 using lithium-heparin plasma/quality control samples on cobas e 411/cobas e 601 analyzers; two duplicated runs per day for 21 days (n = 84). Cross-reactivity with other troponin forms and interference from endogenous substances/drugs was tested; recovery criterion for no cross-reactivity was within ± 10%.

Results: Coefficients of variation (CV) for repeatability/intermediate precision were 0.7–5.6%/1.4–10.3% (cobas e 411; mean cardiac troponin T [cTnT]: 7.3–9341 ng/L) and 0.7–3.0%/1.5–6.4% (cobas e 601; mean cTnT: 7.4–9455 ng/L). There was no cross-reactivity with skeletal muscle troponin T (≤ 10,000 ng/L), skeletal muscle troponin I (≤ 100,000 ng/L), cardiac troponin I (≤ 10,000 ng/L), or human troponin C (≤ 80,000 ng/L). No interference was observed with biotin (≤ 20 ng/mL) or 34 drugs.

Conclusion: The TnT Gen 5 STAT assay demonstrated a CV of < 10% at the 99th percentile upper reference limit, meeting precision requirements (Fourth Universal Definition of Myocardial Infarction) for high-sensitivity troponin assays.

1. Introduction

Increased blood concentrations of cardiac troponin (cTn) I and T are indicative of myocardial injury and are the preferred biomarkers for diagnosing acute myocardial infarction (AMI) [1]. The diagnosis of AMI requires a rise and/or fall in serial cTn measurements, in addition to other clinical features and/or electrocardiogram or myocardial imaging changes [1,2]. The assay-specific 99th percentile upper reference limit (URL) determined in a healthy population is the recommended diagnostic threshold for defining an elevated cTn [1,3].

The introduction of high-sensitivity assays has enabled the rapid detection of subtle changes in cTn and facilitated earlier decision-making in the management of patients with suspected AMI, compared with less sensitive contemporary assays [4–9]. The Fourth Universal Definition of Myocardial Infarction stipulates the minimum analytical performance criteria that cTn assays should meet, such as sufficient precision to report the 99th percentile URL [1]. High-sensitivity assays should measure the 99th percentile URL with a coefficient of variation (CV) ≤ 10% [3].

The Elecsys® Troponin T Gen 5 Short Turn Around Time (TnT Gen 5 STAT; Roche Diagnostics GmbH, Mannheim, Germany) assay received US Food and Drug Administration (FDA) clearance in January 2017.

Abbreviations: AMI, acute myocardial infarction; CLSI, Clinical and Laboratory Standards Institute; cTnI, T, cardiac troponin I and T; FDA, Food and Drug Administration; HAMA, human anti-mouse antibodies; LoB, LoD, LoQ, limits of blank, detection and quantitation; TnT Gen 5 STAT, Troponin T Gen 5 Short Turn Around Time; URL, upper reference limit

* Corresponding author at: Department of Pathology, UC San Diego Health, 200 W Arbor Drive, San Diego, CA 92103, United States of America.
E-mail address: rfitzgerald@ucsd.edu (R.L. Fitzgerald).

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The assay provides a high negative predictive value (> 99%) for ruling out AMI using an overall cutoff of 19 ng/L for the 3-h TnT Gen 5 STAT result [10]. Due to the heterogeneity in cTn assays, and between-assay and between-laboratory variability in applied cutoffs, it is difficult to compare performance data across studies [11,12]. To facilitate inter-study comparisons, and to provide necessary information for local implementation, it is important that studies report relevant analytical parameters [13]. Therefore, we present the analytical performance of the TnT Gen 5 STAT assay, as well as potential interference from endogenous substances (including biotin) and therapeutic drugs commonly prescribed in the intended-use population. Analytical performance data were generated by Roche Diagnostics for submission to the FDA for clearance.

2. Materials and methods

2.1. Assay and analyzers

The TnT Gen 5 STAT assay is an electrochemiluminescence sandwich immunoassay, which uses two antibodies to form a sandwich complex with cTnT. A biotinylated monoclonal anti-cTnT-specific antibody and a monoclonal anti-cTnT-specific antibody labeled with ruthenium react to form a sandwich complex. The antibodies recognize two epitopes (amino acid positions 125–131 and 136–147) located in the central part of the cTnT protein, which consists of 288 amino acids [14]. After the addition of streptavidin-coated microparticles, the sandwich complex is bound to the solid phase via interaction of biotin and streptavidin. The reaction mixture is aspirated into the measuring cell where the microparticles are magnetically captured to the electrode surface. After removal of the unbound substances, a voltage is applied to induce chemiluminescent emission, which is measured by a photomultiplier.

2.2. Limits and ranges

The limit of blank (LoB), limit of detection (LoD), and limit of quantitation (LoQ) were determined in lithium-heparin plasma samples according to Clinical and Laboratory Standards Institute (CLSI) EP17-A2 guidelines [15]. The LoB is the highest observed measured value for analyte-free samples and was defined as the 95th percentile value of the measurement of blank samples. The LoB was determined using one preselected native human lithium-heparin plasma sample with 5-fold determination. The sample did not show any cTnT concentration using the TnT Gen 5 assay; however, it cannot be excluded that very low cTnT concentrations below the assay sensitivity were present. 6 runs were performed over a period of ≥3 days on two different instruments for cobas e 411 and cobas e 601 analyzers; 30 measurements were collected for each instrument for a total of 60 measured values.

The LoD was defined as the lowest plasma concentration of cTnT that could be detected above the LoB with 95% probability. The LoD was determined by measuring five samples (one determination each) with low cTnT concentrations. 6 runs were performed over a period of ≥3 days on two different instruments for cobas e 411 and cobas e 601 analyzers; 30 measurements were collected for each instrument for a total of 60 measured values. The LoD was established according to the following CLSI EP17-A2 calculation [15]: LoD = LoB + 1.653 x standard deviation total (low analyte samples).

The LoQ (functional sensitivity) was defined as the lowest analyte concentration that can be reproducibly measured with an intermediate precision CV of ≤20%. The LoQ was determined using 10 lithium-heparin plasma sample pools with cTnT concentrations at the lower end of the TnT Gen 5 assay's measuring range; two runs were performed per day over 21 days. For each sample pool, the mean cTnT concentration, repeatability (within-run precision) and intermediate precision (within-laboratory precision) were calculated. The mean cTnT concentration and intermediate precision were then plotted and fitted using the following CLSI EP17-A2 [15] function: % CV = A x concentration^B (if a visual inspection showed that this function did not fit the data well, another function was selected; all lots were fitted with the same function). From the fitted line, cTnT concentrations for which the 10% CV or 20% CV is achieved were calculated: $(10/A)^{1/B}$ = concentration or $(20/A)^{1/B}$ = concentration. The assay's measuring range was defined by the LoQ (lower limit) and the maximum of the master curve (upper limit).

2.3. Repeatability and intermediate precision

Repeatability (within-run precision) and intermediate precision (within-laboratory precision) were determined per CLSI EP05-A2 guidelines; two runs were performed per day in duplicate for 21 days (n = 84) [16]. Five different human lithium-heparin plasma sample pools, covering the TnT Gen 5 assay's measuring range (prepared internally by Roche Diagnostics), and two quality control samples with known cTnT concentrations (PreciControl Troponin; Roche Diagnostics GmbH, Mannheim, Germany) were run on cobas e 411 and cobas e 601 analyzers using 3 reagent lots. PreciControl Troponin is a lyophilized control serum, which is based on human serum with added recombinant human cTnT in two concentration ranges.

2.4. Analytical specificity

Cross-reactivity with skeletal muscle troponin T and I, cTnI, and human troponin C was tested at cTnT concentrations of approximately 14, 4000 and 7000 ng/L (skeletal muscle troponin T materials were from Biodesign International, Inc., skeletal muscle troponin I, cTnI, and human troponin C materials were from HyTest Ltd.). Cross-reactivity was calculated as the recovery of the measured cTnT concentration of the sample spiked with cross-reacting substance to the measured cTnT concentration of the sample without cross-reacting substance. The recovery criterion for no cross-reactivity was within ± 10%.

2.5. Interference from endogenous substances and therapeutic drugs

Interference from endogenous substances (including biotin and human anti-mouse antibodies [HAMA]) was tested at three different cTnT concentrations (low, medium and high; different concentrations were used for different interferents). Endogenous substances were obtained internally from Roche Diagnostics or from Sigma-Aldrich). Interference from commonly used and cardiac-specific therapeutic drugs was tested at cTnT concentrations of 15 and 9000 ng/L per CLSI EP07-A2 guidelines [17]. Active components of the therapeutic drugs for testing were obtained from Sigma-Aldrich. All interference testing was performed in lithium-heparin plasma samples. Calculation and recovery criteria were the same as outlined above for analytical specificity.

2.6. Statistical analyses

To calculate intermediate precision and repeatability, a variance components analysis was performed per CLSI EP05-A2 guidelines [16]. A non-linear fitting model of regression was used to estimate the functional relationship between variation and analyte concentration [18]. All analyses were performed using R (ver 3.4.0; R Foundation for statistical computing).

3. Results

3.1. Limits and ranges

The specified LoB is 3 ng/L on the cobas e 411 analyzer and 2.5 ng/L on the cobas e 601 analyzer. The specified LoD is 5 ng/L on the cobas e 411 analyzer and 3 ng/L on the cobas e 601 analyzer. The measured

Table 1
Determination of the limit of blank and limit of detection of the Elecsys® Troponin T Gen 5 Short Turn Around Time assay on the cobas e 411 analyzer[a].

Sample/determination	Cardiac troponin T concentration, ng/L					
	Run 1	Run 2	Run 3	Run 4	Run 5	Run 6
Limit of blank: 3.0 ng/L[b]						
Instrument 1						
1	0	3.3	1.6	1.5	2.7	1.5
2	0	3.4	0.6	0.9	2.2	0.7
3	0	2.9	0.4	1.7	2.5	2.2
4	0	2.8	1.0	1.1	2.6	1.4
5	0	3.1	1.3	1.3	2.1	2.4
Instrument 2						
1	1.6	2.0	2.4	2.6	1.4	0.4
2	1.5	1.5	2.3	2.1	1.6	0.2
3	1.3	1.4	1.5	1.6	0.6	0.8
4	0.2	1.4	1.3	1.3	0.8	0
5	0	1.0	1.3	1.7	1.2	0.9
Limit of detection: 4.5 ng/L[c]						
Instrument 1						
1	5.6	5.0	4.6	3.0	4.6	3.5
2	6.6	6.5	7.0	4.7	6.2	6.6
3	9.3	8.6	8.8	7.9	9.0	8.8
4	12.0	11.2	10.4	11.1	10.8	11.4
5	14.3	13.9	13.5	13.0	13.6	14.2
Instrument 2						
1	2.7	4.5	4.4	3.6	3.1	4.0
2	3.8	5.3	5.3	6.9	4.2	5.2
3	6.6	8.6	7.6	8.0	6.6	8.0
4	9.5	11.1	10.0	10.9	10.0	11.3
5	11.4	13.0	11.8	12.2	12.5	13.2
Variation						
Sample	1	2	3	4	5	Total
SD	0.9	1.1	0.9	0.7	0.9	0.9
SD x calculation factor (1.653)						1.5

[a] Representative data from one reagent lot are shown.
[b] One preselected native human lithium-heparin plasma sample with five-fold determination for 6different runs.
[c] Five low-analyte lithium-heparin plasma samples with one-fold determination for 6 different runs.

Table 2
Determination of the limit of blank and limit of detection of the Elecsys® Troponin T Gen 5 Short Turn Around Time assay on the **cobas e** 601 analyzer[a].

Sample/determination	Cardiac troponin T concentration, ng/L					
	Run 1	Run 2	Run 3	Run 4	Run 5	Run 6
Limit of blank: 1.3 ng/L[b]						
Instrument 1						
1	0	1.2	1.1	0	1.3	0.7
2	0	1.0	0.9	0	0.8	0.2
3	0.2	1.3	1.0	0	1.3	0.3
4	0	1.1	1.1	0	1.4	0.2
5	0.2	1.3	0.6	0	0.6	0.9
Instrument 2						
1	0	0	0	0	0.6	0.8
2	0	0	0	0	0.8	0.5
3	0	0	0	0	0.4	0.4
4	0	0	0	0.2	0.6	0
5	0	0	0	0	0.7	0.8
Limit of detection: 2.0 ng/L[c]						
Instrument 1						
1	4.6	4.3	5.3	4.3	5.4	4.7
2	5.5	5.4	5.9	5.6	6.0	5.5
3	8.1	7.9	8.8	8.0	8.7	8.4
4	9.5	9.4	9.9	9.4	9.6	9.4
5	10.8	11.2	11.5	10.9	11.7	11.3
Instrument 2						
1	4.5	4.3	4.2	4.4	4.7	5.2
2	5.3	5.7	5.1	5.3	5.9	6.2
3	8.0	8.2	8.3	7.9	9.1	9.1
4	8.9	9.2	8.8	8.9	9.5	10.0
5	11.1	11.2	10.5	11.1	11.6	11.8
Variation						
Sample	1	2	3	4	5	Total
SD	0.4	0.3	0.4	0.4	0.4	0.4
SD x calculation factor (1.653)						0.6

[a] Representative data from one reagent lot are shown.
[b] One preselected native human lithium-heparin plasma sample with 5-fold determination for 6 different runs.
[c] Five low-analyte lithium-heparin plasma samples with one-fold determination for 6 different runs.

LoB and LoD data on both analyzers were well within these specifications (Tables 1 and 2). The LoQ was 6 ng/L (Table 3) and the measuring range was 6–10,000 ng/L on both analyzers. Limits and ranges for the cobas e 601 analyzer are also applicable to the cobas e 602 analyzer.

3.2. Repeatability and intermediate precision

Representative precision data from one reagent lot are shown in Table 4. On the cobas e 411 analyzer, repeatability CVs were 0.7–5.6% and intermediate precision CVs were 1.4–10.3% for mean cTnT concentrations of 7.3–9341 ng/L in lithium-heparin plasma samples. On the cobas e 601 analyzer, repeatability CVs were 0.7–3.0% and intermediate precision CVs were 1.5–6.4% for mean cTnT concentrations of 7.4–9455 ng/L in lithium-heparin plasma samples. The 10% CV (total imprecision) was 11 ng/L; CVs ranged ~1–3% for cTnT concentrations of 50–10,000 ng/L (Fig. 1). Precision data measured on the cobas e 601 analyzer are also applicable to the cobas e 602 analyzer.

3.3. Analytical specificity

At cTnT concentrations of approximately 14, 4000 and 7000 ng/L, no cross-reactivity was observed with skeletal muscle troponin T (up to 10,000 ng/L) or I (up to 100,000 ng/L), cTnI (up to 10,000 ng/L), or human troponin C (up to 80,000 ng/L). The recovery criterion for no cross-reactivity was within ± 10%.

3.4. Interference from endogenous substances and therapeutic drugs

No interference was observed with any of the endogenous substances tested, including biotin up to 82 nmol/L (20 ng/mL), hemoglobin up to 0.1 g/dL (visual to moderate hemolysis) and HAMA up to 322 µg/mL (Table 5). At cTnT concentrations of 15 ng/L and 9000 ng/L, no interference was observed with each of the 16 commonly used and 18 cardiac-specific drugs tested (Table 6). The recovery criterion for no interference was within ± 10%.

4. Discussion

High-sensitivity cTn assays can facilitate early diagnosis of AMI by detecting smaller changes in cTn versus contemporary assays [4–9]. However, when comparing the clinical performance of cTn assays, some tests may lack sufficient precision to measure the 99th percentile URL accurately, and the clinical performance data may differ considerably due to imprecise cutoffs [19]. A comparison of different cTn assays' precision at the 99th percentile URL showed that many do not meet the CV ≤ 10% criterion [20]. In addition to promoting confidence in the cutoff, assay precision is important when deciding whether serial cTn elevations represent an acute change or a stable elevation [21].

We evaluated the analytical performance of the Elecsys® TnT Gen 5 STAT assay according to CLSI guidelines (overall and sex-specific reference ranges have been previously reported [10,22]). The assay demonstrated a CV of < 10% at the 99th percentile URL on both cobas e 411 and cobas e 601 analyzers, supporting the FDA clearance of the assay as an aid in the diagnosis of AMI. Our findings are consistent with

Table 3
Repeatability (within-run precision) and intermediate precision (within-laboratory precision) for calculation of the limit of quantitation of the Elecsys® Troponin T Gen 5 Short Turn Around Time assay on cobas e 411 and cobas e 601 analyzers[a].

Lithium-heparin plasma sample pool	Mean cTnT concentration from 84 determinations, ng/L	Repeatability		Intermediate precision	
		SD, ng/L	CV, % (95% upper confidence limit)	SD, ng/L	CV, % (95% upper confidence limit)
cobas e 411 analyzer					
1	1.8	0.6	34.1 (43.4)	0.8	43.8 (53.7)
2	2.5	0.6	24.2 (30.8)	0.7	26.5 (31.4)
3	4.1	0.6	15.1 (19.2)	0.7	16.0 (19.0)
4	4.8	0.5	10.4 (13.2)	0.7	14.0 (16.7)
5	13.0	0.4	3.2 (4.1)	0.6	4.5 (5.4)
6	4.3	0.7	15.6 (19.8)	0.8	17.9 (21.3)
7	5.2	0.5	10.0 (12.7)	0.7	13.8 (16.5)
8	6.2	0.6	9.3 (11.8)	0.6	10.1 (12.0)
9	5.8	0.6	10.3 (13.1)	0.8	14.4 (17.6)
10	7.0	0.5	6.6 (8.4)	0.7	9.4 (11.4)
cobas e 601 analyzer					
1	1.3	0.2	17.5 (22.2)	0.4	33.3 (42.6)
2	2.2	0.2	9.0 (11.4)	0.4	19.2 (24.8)
3	4.3	0.2	4.9 (6.2)	0.4	8.5 (10.7)
4	5.4	0.2	4.0 (5.1)	0.4	6.8 (8.8)
5	14.5	0.3	2.4 (3.0)	0.4	2.7 (3.2)
6	5.1	0.2	4.2 (5.3)	0.4	7.2 (9.2)
7	6.0	0.2	4.0 (5.0)	0.3	5.8 (7.1)
8	6.8	0.2	3.5 (4.4)	0.3	5.1 (6.5)
9	7.9	0.2	2.9 (3.7)	0.3	4.3 (5.4)
10	8.0	0.2	3.1 (3.9)	0.4	4.5 (5.6)

cTnT, cardiac troponin T; CV, coefficient of variation; SD, standard deviation.
[a] Representative data from one reagent lot are shown.

Table 4
Repeatability (within-run precision) and intermediate precision (within-laboratory precision) of the Elecsys® Troponin T Gen 5 Short Turn Around Time assay on cobas e 411 and cobas e 601 analyzers (per Clinical and Laboratory Standards Institute EP05-A2 guidelines [16])[a].

Sample	Mean cTnT concentration, ng/L	Repeatability		Intermediate precision	
		SD, ng/L	CV, %	SD, ng/L	CV, %
cobas e 411 analyzer					
Quality control samples[b]					
1	20.0	0.5	2.3	0.7	3.7
2	1739	15.5	0.9	36.3	2.1
Lithium-heparin plasma sample pool[c]					
1	7.3	0.4	5.6	0.7	10.3
2	12.2	0.4	3.1	0.7	5.9
3	152	1.4	0.9	2.2	1.4
4	4673	38.2	0.8	117	2.5
5	9341	64.5	0.7	262	2.8
cobas e 601 analyzer[d]					
Quality control samples[b]					
1	24.2	0.3	1.1	0.8	3.2
2	1971	13.3	0.7	45.0	2.3
Lithium-heparin plasma sample pool[c]					
1	7.4	0.2	3.0	0.5	6.4
2	13.5	0.3	1.9	0.6	4.1
3	154	1.2	0.8	2.2	1.5
4	4831	38.0	0.8	124	2.6
5	9455	62.7	0.7	256	2.7

[a] Representative precision data from one reagent lot are shown.
[b] Quality control samples with known concentrations of cTnT (PreciControl Troponin; Roche Diagnostics GmbH, Mannheim, Germany). PreciControl Troponin is a lyophilized control serum, which is based on human serum with added recombinant human cTnT in two concentration ranges.
[c] Five different lithium-heparin plasma sample pools covering the assay's measuring range (prepared internally by Roche Diagnostics); [d]Measured precision data on the cobas e 601 analyzer are also applicable to the cobas e 602 analyzer.

a previous multicenter evaluation (3 US and 5 European sites) of the TnT Gen 5 assay by Saenger et al., which demonstrated assay linearity up to 10,000 ng/L, and observed greatest within-run and total CVs in the lowest concentration serum pools [23]. For cTnT concentrations at the lower end of the assay's analytical measuring range, we observed within-run and intermediate CVs of 5.6% and 10.3%, respectively, on the cobas e 411 analyzer (mean sample concentration: 7.3 ng/L), and 3.0% and 6.4%, respectively, on the cobas e 601 analyzer (mean sample concentration: 7.4 ng/L). In the Saenger et al. study, within-run and total CVs were 6.0–8.7% and 11.4–16.4%, respectively, on the cobas e 411 analyzer (mean sample concentration: 7.6–7.9 ng/L [the cobas e 411 analyzer was tested at two sites]), and 2.4% and 6.2%, respectively, on the cobas e 601 analyzer (mean sample concentration: 7.6 ng/L) [23]. It should be noted that our results were determined in lithium-heparin plasma samples, while the Saenger et al. study used serum samples. We demonstrated standard deviations (SDs) for repeatability and intermediate precision of ≤0.8 ng/L in samples with cTnT concentrations ≤10 ng/L (Table 3). These results support previous research proposing a total analytical error estimate of 3.4 ng/L (1.8 ng/L bias plus 2 SDs of 0.8 ng/L) for measuring cTnT concentrations ≤10 ng/L with high-sensitivity cTn assays, in order to help prevent erroneous result reporting [24].

In the present study, the specified LoB and LoD were 3 ng/L and 5 ng/L, respectively, on the cobas e 411 analyzer, and 2.5 ng/L and 3 ng/L, respectively, on the cobas e 601 analyzer. These limits are consistent with two previous studies of the assay's analytical performance on Elecsys® 2010/cobas e 411 and Modular® Analytics E170/cobas e 601 analyzers [23,25]. The measuring range determined in our study for use in the US (6–10,000 ng/L) has a slightly different lower limit than the measuring range used globally (3–10,000 ng/L) [25]. The reason for this is that the lower limit of the global measuring range (outside of the US) is based on the LoB (3 ng/L), while the lower limit of the US measuring range in our study was defined as the LoQ (6 ng/L), per FDA regulations. Although the American Association of Clinical Chemistry and the Committee (formerly Task Force) on Clinical Applications of Cardiac Bio-Markers of the International Federation of Clinical Chemistry and Laboratory Medicine recommend the LoD as the lowest analytical reportable limit for high-sensitivity cTn assays [3], the FDA does not allow reporting of results below the LoQ [13,26].

In our study, the TnT Gen 5 STAT assay showed a lack of cross-reactivity of cTnT with other troponin forms. This is consistent with a previous study of the assay, which also showed negligible cross-reactivity with skeletal muscle troponin T (0.003%) and I (0.003%), cTnI (0.2%) or human troponin C (< 0.001%) [25]. It has recently been suggested that the TnT Gen 5 assay may cross-react with skeletal troponins [27]; however, this was not observed in our study.

We found no interference from endogenous substances or therapeutic drugs, including cardiac-specific drugs, and observed less interference from HAMA than has been reported with cTnI [28]. We also observed no interference from biotin up to 20 ng/mL. Biotin interference with immunoassays that utilize biotin-streptavidin/avidin bonding has recently become a cause of potential concern, due to the increasing popularity of over-the-counter biotin supplements and recent trials of high-dose biotin for the treatment of multiple sclerosis [29–34]. Although the adequate intake for biotin in adults is 30 µg/day [35], biotin doses in high-dose supplements (e.g. 30–500 µg in multivitamin preparations and 0.5–10 mg in single-ingredient preparations) and experimental compounds for the treatment of multiple sclerosis (up to 3 × 100 mg [29]) can far exceed this. A recent study, with the aim of clarifying the magnitude of assay susceptibility to biotin interference, evaluated a range of Roche assays and demonstrated high variability in biotin tolerance. At biotin concentrations of 15.6–31.1 ng/mL, simulating 5–10 mg intake, the TnT Gen 5 assay exhibited approximately

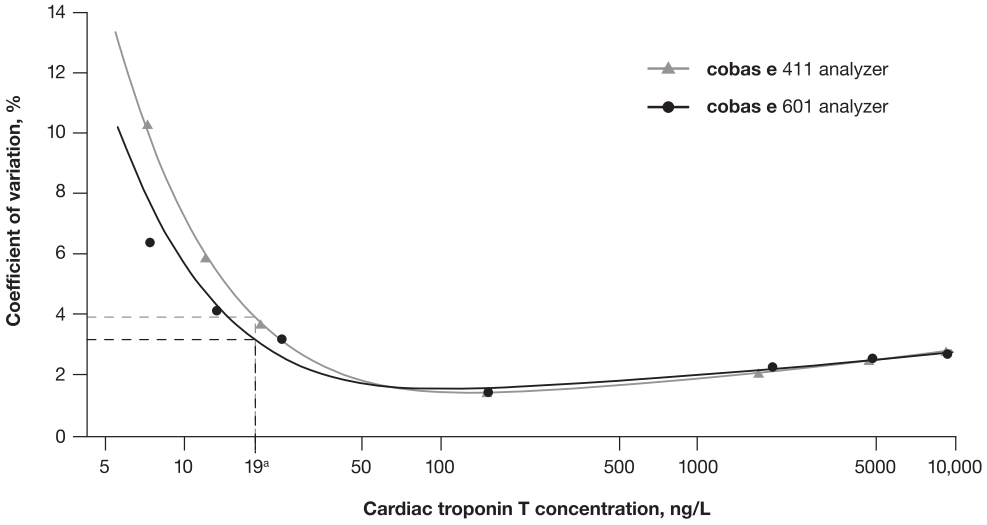

Fig. 1. Intermediate precision (within-laboratory precision) profiles of the Elecsys® Troponin T Gen 5 Short Turn Around Time assay on cobas e 411 and cobas e 601 analyzers (per Clinical and Laboratory Standards Institute EP05-A2 guidelines [16]).
[a]99th percentile upper reference limit for the Troponin T Gen 5 assay.
Measured precision data on the cobas e 601 analyzer are also applicable to the cobas e 602 analyzer.

Table 5
Evaluation of potential interference with the Elecsys® Troponin T Gen 5 Short Turn Around Time assay from endogenous substances.

Compound	Concentration tested at which no interference was observed[a]
Bilirubin[b]	428 µmol/L (25 mg/dL)
Biotin	82 nmol/L (20 ng/mL)
Total cholesterol[c]	310 mg/dL
Human anti-mouse antibodies[d]	322 µg/mL
Hemoglobin	0.062 mmol/L (0.1 g/dL)
Intralipid	1500 mg/dL
Rheumatoid factor	900 IU/mL
Serum albumin	7 g/dL

[a] Interference from endogenous substances was tested at three different cTnT concentrations (low, medium and high; different concentrations were used for different interferents); the recovery criterion for no interference was within ± 10%. All interference testing was performed in lithium-heparin plasma samples. Endogenous substances were obtained internally from Roche Diagnostics or from Sigma-Aldrich.
[b] 90% bilirubin and 10% ditaurobilirubin, corresponding to the naturally occurring ratio of unconjugated to conjugated bilirubin.
[c] Sigma Grade, ≥ 99% (C8667; Sigma-Aldrich).
[d] Serum concentration of human anti-mouse antibodies was determined using a commercial enzyme-linked immunosorbent assay (Medac GmbH).

10–20% negative bias [34]. The International Federation for Clinical Chemistry Committee on Cardiac Biomarkers recently collated biotin interference information from a wide range of assay manufacturers. This highlighted both the high variability in biotin interference thresholds and particular concerns regarding cTn and natriuretic peptide assays, which are widely used to guide critical clinical decisions [33]. The prevalence of high biotin concentrations in the intended-use population and potential clinical implications of biotin interference with the TnT Gen 5 assay are currently being investigated and will be discussed in a separate paper [36].

It should be noted that biotin interference occurs relatively infrequently compared with other analytical and pre-analytical factors that have been shown to affect cTn assay measurements, such as anti-cTn antibodies, hemolysis, icterus, and lipemia [37,38]. Hemolysis is one of the most commonly occurring pre-analytical interfering factors in clinical laboratories and should be routinely monitored [38,39]. In the present study, no interference was observed from hemoglobin up to 0.1 g/dL (visual to moderate hemolysis). In addition to hemolysis, fibrin compounds in the sample can adhere to the well of the plate and influence cTn measurements when using enzyme-linked immunosorbent assay technology [40]. However, the electrochemiluminescent-based

Table 6
Evaluation of potential interference with the Elecsys® Troponin T Gen 5 Short Turn Around Time assay from commonly used and cardiac-specific therapeutic drugs.

Compound	Concentration tested at which no interference was observed, mg/L (unless otherwise stated)[a]
Commonly used therapeutic drugs	
Acetaminophen	200
Acetylcysteine	1660
Acetylsalicylic acid	1000
Ampicillin-Na	1000
Ascorbic acid	300
Cyclosporine	5
Cefoxitin	2500
Doxycycline	50
Heparin	5000 IU
Ibuprofen	500
Levodopa	20
Methyldopa	20
Metronidazole	200
Phenylbutazone	400
Rifampicin	60
Theophylline	100
Cardiac-specific therapeutic drugs	
Carvedilol	37.5
Clopidogrel	75
Digoxin	0.25
Epinephrine	0.5
Insulin	1.6
Lidocaine	80
Lisinopril	10
Methylprednisolone	7.5
Metoprolol	150
Nifedipine	30
Phenprocoumon	3
Propafenone	300
Reteplase	33.3
Simvastatin	30
Spironolactone	75
Tolbutamide	1500
Torsemide	15
Verapamil	240

[a] Interference from commonly used and cardiac-specific therapeutic drugs was tested at cTnT concentrations of 15 ng/L and 9000 ng/L; the recovery criterion for no interference was within ± 10%. All interference testing was performed in lithium-heparin plasma samples. Active components of the therapeutic drugs for testing were from Sigma-Aldrich.

TnT Gen 5 STAT assay does not use plates and so is not affected by fibrin interference. Potential interfering antibodies, such as heterophile antibodies, are another challenging analytical factor, which bind non-specifically to cTn assay antibodies and may lead to false-positive results [41]. Assuming adequate monitoring for standard artifacts, the TnT Gen 5 STAT assay demonstrates low levels of cross-reactivity with other troponin forms and low levels of interference from endogenous substances/drugs.

A limitation of the present study is that we only tested interference from one type of HAMA. The reactivity of HAMA to immunoassay antibodies can vary depending on the specificity of the HAMA, as well as the configuration of the immunoassay, and the impact of these antibody interferences is difficult to predict [42,43]. Therefore, we cannot completely rule out interference from other HAMA or human anti-animal antibodies. Lastly, we only report precision data from testing in lithium-heparin plasma samples. Assay precision was also tested in serum samples, but the TnT Gen 5 STAT assay is not yet cleared by the FDA for use in serum; therefore, precision data from serum sample testing is not reported in the present paper.

5. Conclusion

The Elecsys® TnT Gen 5 STAT assay demonstrated good analytical performance on **cobas e 411 and cobas e 601 analyzers, with a CV of ≤ 10% at the 99th percentile URL.** These findings support the routine use of the assay in clinical laboratories in the US.

Declaration of interest

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- Nancy Breitenbeck: clinical trial enroller and institutional financial decision-making role.
- Kareen Blechschmidt: employee of Roche Diagnostics.
- Michael Laimighofer: employee of Roche Diagnostics.
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