September 21, 2021

Roche Diagnostics
Jane Phillips, Ph.D.
Senior Regulatory Program Manager
9115 Hague Road
Indianapolis, IN 46250

Re: K201441
Trade/Device Name: Elecsys Troponin T Gen 5
Regulation Number: 21 CFR 862.1215
Regulation Name: Creatine Phosphokinase/Creatine Kinase Or Isoenzymes Test System
Regulatory Class: Class II
Product Code: MMI
Dated: December 2, 2020
Received: December 3, 2020

Dear Jane Phillips:

We have reviewed your Section 510(k) premarket notification of intent to market the device referenced above and have determined the device is substantially equivalent (for the indications for use stated in the enclosure) to legally marketed predicate devices marketed in interstate commerce prior to May 28, 1976, the enactment date of the Medical Device Amendments, or to devices that have been reclassified in accordance with the provisions of the Federal Food, Drug, and Cosmetic Act (Act) that do not require approval of a premarket approval application (PMA). You may, therefore, market the device, subject to the general controls provisions of the Act. Although this letter refers to your product as a device, please be aware that some cleared products may instead be combination products. The 510(k) Premarket Notification Database located at https://www.accessdata.fda.gov/scripts/cdrh/cfdocs/cfpmn/pmn.cfm identifies combination product submissions. The general controls provisions of the Act include requirements for annual registration, listing of devices, good manufacturing practice, labeling, and prohibitions against misbranding and adulteration. Please note: CDRH does not evaluate information related to contract liability warranties. We remind you, however, that device labeling must be truthful and not misleading.

If your device is classified (see above) into either class II (Special Controls) or class III (PMA), it may be subject to additional controls. Existing major regulations affecting your device can be found in the Code of Federal Regulations, Title 21, Parts 800 to 898. In addition, FDA may publish further announcements concerning your device in the Federal Register.

Please be advised that FDA's issuance of a substantial equivalence determination does not mean that FDA has made a determination that your device complies with other requirements of the Act or any Federal

statutes and regulations administered by other Federal agencies. You must comply with all the Act's requirements, including, but not limited to: registration and listing (21 CFR Part 807); labeling (21 CFR Part 801 and Part 809); medical device reporting (reporting of medical device-related adverse events) (21 CFR 803) for devices or postmarketing safety reporting (21 CFR 4, Subpart B) for combination products (see https://www.fda.gov/combination-products/guidance-regulatory-information/postmarketing-safety-reporting-combination-products); good manufacturing practice requirements as set forth in the quality systems (QS) regulation (21 CFR Part 820) for devices or current good manufacturing practices (21 CFR 4, Subpart A) for combination products; and, if applicable, the electronic product radiation control provisions (Sections 531-542 of the Act); 21 CFR 1000-1050.

Also, please note the regulation entitled, "Misbranding by reference to premarket notification" (21 CFR Part 807.97). For questions regarding the reporting of adverse events under the MDR regulation (21 CFR Part 803), please go to https://www.fda.gov/medical-devices/medical-device-safety/medical-device-reporting-mdr-how-report-medical-device-problems.

For comprehensive regulatory information about medical devices and radiation-emitting products, including information about labeling regulations, please see Device Advice (https://www.fda.gov/medical-devices/device-advice-comprehensive-regulatory-assistance) and CDRH Learn (https://www.fda.gov/training-and-continuing-education/cdrh-learn). Additionally, you may contact the Division of Industry and Consumer Education (DICE) to ask a question about a specific regulatory topic. See the DICE website (https://www.fda.gov/medical-devices/device-advice-comprehensive-regulatory-assistance/contact-us-division-industry-and-consumer-education-dice) for more information or contact DICE by email (DICE@fda.hhs.gov) or phone (1-800-638-2041 or 301-796-7100).

Sincerely,

Marianela Perez-Torres, Ph.D.
Deputy Director
Division of Chemistry
and Toxicology Devices
OHT7: Office of In Vitro Diagnostics
and Radiological Health
Office of Product Evaluation and Quality
Center for Devices and Radiological Health

Enclosure

DEPARTMENT OF HEALTH AND HUMAN SERVICES
Food and Drug Administration

Indications for Use

Form Approved: OMB No. 0910-0120
Expiration Date: 06/30/2023
See PRA Statement below.

510(k) Number *(if known)*
k201441

Device Name
Elecsys Troponin T Gen 5

Indications for Use *(Describe)*
Immunoassay for the in vitro quantitative determination of cardiac troponin T (cTnT) in lithium heparin plasma. The immunoassay is intended to aid in the diagnosis of myocardial infarction.
The electrochemiluminescence immunoassay "ECLIA" is intended for use on cobas e immunoassay analyzers.

Type of Use *(Select one or both, as applicable)*

☒ Prescription Use (Part 21 CFR 801 Subpart D) ☐ Over-The-Counter Use (21 CFR 801 Subpart C)

CONTINUE ON A SEPARATE PAGE IF NEEDED.

This section applies only to requirements of the Paperwork Reduction Act of 1995.

DO NOT SEND YOUR COMPLETED FORM TO THE PRA STAFF EMAIL ADDRESS BELOW.

The burden time for this collection of information is estimated to average 79 hours per response, including the time to review instructions, search existing data sources, gather and maintain the data needed and complete and review the collection of information. Send comments regarding this burden estimate or any other aspect of this information collection, including suggestions for reducing this burden, to:

Department of Health and Human Services
Food and Drug Administration
Office of Chief Information Officer
Paperwork Reduction Act (PRA) Staff
PRAStaff@fda.hhs.gov

"An agency may not conduct or sponsor, and a person is not required to respond to, a collection of information unless it displays a currently valid OMB number."

510(k) Summary of Safety and Effectiveness

This summary of 510(k) safety and effectiveness information is submitted in accordance with the requirements of SMDA 1990 and 21 CFR §807.92.

510(k) Number K201441

Manufacturer
Roche Diagnostics
9115 Hague Road
Indianapolis, IN 46250

Contact
Jane Ellen Phillips, PhD, Senior Regulatory Program Manager
9115 Hague Road, Building B
jane.phillips@roche.com
Phone: 317-521-3338
Fax; 317-521-2324

Date September 15th, 2021

Device Name
Proprietary name: Elecsys Troponin T Gen 5

Common name: Troponin T Gen 5

Device Classification Class II

Panel	Product Code	Classification Name	Regulation Citation
Clinical Chemistry	MMI	Creatine phosphokinase/creatine kinase or isoenzymes test system.	862.1215

Substantial Equivalence
The results presented in this 510(k) premarket notification demonstrate that the Elecsys Troponin T Gen 5 Immunoassay is substantially equivalent to the predicate Elecsys Troponin T Gen 5 STAT Immunoassay (k162895).

Substantial Equivalence Comparison		
Feature	**Candidate Device: Elecsys Troponin T Gen 5**	**Predicate Device: Elecsys Troponin T Gen 5 STAT Immunoassay (k162895)**
General Immunoassay Features		
Intended Use/ Indications for Use	Immunoassay for the in vitro quantitative determination of cardiac troponin T (cTnT) in lithium heparin plasma. The immunoassay is intended to aid in the diagnosis of myocardial infarction. The electrochemluminescence immunoassay "ECLIA" is intended for use on the cobas e 801 and 601 immunoassay analyzers.	Same
Immunoassay Protocol	Sandwich immunoassay	Same
Detection Protocol	Electrochemiluminescent immunoassay	Same
Assay Reaction Time	9 Minute	Same
Standardization	This method has been standardized against the Elecsys Troponin T STAT assay (4th generation). This in turn was originally standardized against the Enzymun-Test Troponin T (CARDIAC T) Test method.	Same
Epitopes	MAK-Biotinylated: aa 125-131 MAK-Ruthenium: aa 136-147	Same
Reagent Update	Addition of a monoclonal biotin scavenging antibody and increase in the length of the linker on the capture antibody.	Not present

Intended Use of the Device:

Immunoassay for the in vitro quantitative determination of cardiac troponin T (cTnT) in lithium heparin plasma. The immunoassay is intended to aid in the diagnosis of myocardial infarction.

The electrochemluminescence immunoassay "ECLIA" is intended for use on the **cobas e** immunoassay analyzers.

Device Description

The Elecsys Troponin T Gen 5 STAT Immunoassay is a one-step sandwich immunoassay on the **cobas e** 601 and **cobas e** 801 analyzer. The assay uses streptavidin-coated microparticles, a biotinylated monoclonal anti-cardiac Troponin T-specific antibody, a monoclonal anti-cardiac

Troponin T-specific antibody labeled with a ruthenium complex and electrochemiluminescence detection. Results are determined using a calibration curve that is generated specifically on each instrument by a 2-point calibration and a master curve (6-point calibration) provided with the reagent bar code.

Changes to Reagent Composition

The following changes were implemented to the reagent to block the potential interference of biotin with the Elecsys Troponin T Gen 5 assay. Briefly, we took a two-step approach by adding an antibody to bind free biotin in the sample, and changing the linker on the biotinylated capture antibody.

For the neutralization of free biotin in Li-Heparin plasma, Roche developed an antibody, which binds to free biotin. The antibodies are specific for free biotin and do not bind to or interact with the biotin-linker conjugates.

In the updated biotinylated antibody conjugate, the distance between biotin and the reactive MEA group is elongated by a PEG spacer, which provides the coupled biotin more flexibility for the interaction with the Streptavidin matrix. No change is made to the antibody (immunologically reactive component) which still recognizes the same epitope.

Summary of Analytical Performance

Precision/Reproducibility

<u>Repeatability and Intermediate Precision</u>

Precision measurements were conducted to evaluate repeatability (within-run precision) and the intermediate precision (within-laboratory precision) according to the CLSI guideline EP5-A3.

The precision of the Elecsys Troponin T Gen 5 assay was evaluated on one **cobas e** 801 analyzer at one internal site with three reagent lots over 21 days. Six human samples (sample pools) were run in randomized order on the analyzer. Native Li-Heparin plasma samples (≤ 100 ng/L cTnT) as well as spiked samples (> 100 ng/L cTnT) were used. Two replicates of each human Li-Heparin plasma sample and two levels of PreciControl Troponin were tested in two runs per day for 21 days. The analysis did not differentiate between runs conducted on the same day, therefore, the precision estimates reported below are for 21 days, 1 run/day, and 4 replicates. Calibration was performed once on day 1 according to the method sheet. No gel separator tubes were used in this experiment. Results from one representative lot of the three lots tested are described in the table below. Within laboratory precision includes within-run and between-day variability.

Table 1.1: Results for Precision with the Reagent Lot 344524 according to the 21d measuring model

Sample	Mean [ng/L]	Repeatability (within-part precision)		Intermediate precision (within-lab/total)	
		SD [ng/L]	CV [%]	SD [ng/L]	CV [%]
Sample 1	9.60	0.225	2.3	0.405	4.2
Sample 2	15.8	0.270	1.7	0.556	3.5
Sample 3	22.2	0.310	1.4	0.643	2.9
Sample 4	164	2.96	1.8	5.15	3.1
Sample 5	4877	97.9	2.0	153	3.1
Sample 6	9808	172	1.7	328	3.3
Control 1	26.6	0.370	1.4	0.866	3.3
Control 2	1962	27.2	1.4	51.7	2.6

Reproducibility

Measurements were conducted to evaluate reproducibility according to the CLSI guideline EP5-A3.

A reproducibility panel consisting of five human Li-Heparin plasma samples and two levels of PreciControl Troponin was measured in five-fold determinations for 5 days on three **cobas e** 801 analyzers at three different sites (one internal and two external sites). The five native or spiked Li-Heparin plasma samples used were generated from sample pools. Samples ≤ 100 ng/L were native. For the higher concentrations, samples were spiked.

One **cobas e** 801 with a unique serial number per site was used with one reagent lot spanning one calibration cycle as recommended in the Method Sheet. Calibration was performed once on day one according to the method sheet. Repeatability, intermediate precision and reproducibility were calculated according to CLSI EP05-A3. Reproducibility includes repeatability, between day, and between site variance components.

Table 1.2: Results of Repeatability and Intermediate Precision per Site

Sample Material	Mean [ng/L]	Repeatability (Within-run Precision)		Intermediate Precision	
		SD [ng/L]	CV [%]	SD [ng/L]	CV [%]
Site 1					
Sample 1	9.03	0.297	3.3	0.297	3.3
Sample 2	22.0	0.323	1.5	0.350	1.6
Sample 3	174	5.47	3.2	5.47	3.2

Sample Material	Mean [ng/L]	Repeatability (Within-run Precision)		Intermediate Precision	
		SD [ng/L]	CV [%]	SD [ng/L]	CV [%]
Sample 4	4890	44.2	0.9	62.2	1.3
Sample 5	9540	165	1.7	165	1.7
PreciControl 1	25.8	0.455	1.8	0.469	1.8
PreciControl 2	1960	28.7	1.5	34.0	1.7
Site 2					
Sample 1	7.95	0.263	3.3	0.323	4.1
Sample 2	20.9	0.418	2.0	0.418	2.0
Sample 3	169	4.46	2.6	6.64	3.9
Sample 4	4670	94.5	2.0	143	3.1
Sample 5	9310	192	2.1	192	2.1
PreciControl 1	24.3	0.594	2.5	0.868	3.6
PreciControl 2	1860	37.3	2.0	60.7	3.3
Site 3					
Sample 1	9.24	0.281	3.0	0.444	4.8
Sample 2	21.8	0.376	1.7	0.832	3.8
Sample 3	170	3.28	1.9	5.61	3.3
Sample 4	4860	67.6	1.4	174	3.6
Sample 5	9620	103	1.1	355	3.7
PreciControl 1	26.1	0.378	1.5	0.877	3.4
PreciControl 2	1950	24.0	1.2	58.0	3.0

Table 1.3: Results for Reproducibility

Sample Material	Mean [ng/L]	Reproducibility			
		SD estimate [ng/L]	SD [ng/L] 95% UCL	CV estimate (%)	CV (%) 95% UCL
Sample 1	8.74	0.770	2.80	8.8	32.0
Sample 2	21.6	0.820	1.85	3.8	8.6
Sample 3	171	5.99	7.74	3.5	4.5

Sample Material	Mean [ng/L]	Reproducibility			
		SD estimate [ng/L]	SD [ng/L] 95% UCL	CV estimate (%)	CV (%) 95% UCL
Sample 4	4806	174	342	3.6	7.1
Sample 5	9490	282	441	3.0	4.7
PreciControl 1	25.4	1.19	3.02	4.7	11.9
PreciControl 2	1923	71.8	154	3.7	8.0

Method Comparisons

Excellent comparison of the assay to the predicate device Elecsys Troponin T Gen 5 STAT is needed in order to ensure a transfer of the Roche clinical data from the existing and cleared assay to the biotin update.

To this end, method comparison studies were performed between one lot of the predicate device on the **cobas e** 601 and three lots of the Troponin T Gen 5 assay on the **cobas e** 801.

A method comparison (MC) study was performed to compare the Elecsys Troponin T Gen 5 immunoassay on the **cobas e** 801 (Y-axis) with the predicate device (X-axis) on the **cobas e** 601.

One set of samples was tested with one lot (predicate device, one internal site) and three different lots with the Elecsys Troponin T Gen 5 on the **cobas e** 801 (one internal site) as follows:

One set of 299 samples was tested with each reagent; at least 264 samples were detected within the measuring range. One lot of predicate device was taken as the reference value used for comparison to the values generated with each of the three lots of Elecsys Troponin T Gen 5 on the **cobas e** 801.

Table 1.4: Regression Analyses (n=264) Representative Method Comparison

	Passing-Bablok	Weighted Deming
Slope	**0.993**	**0.995**
95% LCL	0.979	0.987
95% UCL	1.01	1.00
Y-intercept [ng/L]	**1.34**	**1.26**
95% LCL	1.07	1.10
95%UCL	1.53	1.41
Absolute Bias at cutoff 14 [ng/L]	**1.24**	**1.19**
95% LCL	1.09	1.09

	Passing-Bablok	Weighted Deming
95% UCL	1.32	1.30
Absolute Bias at cutoff 19 [ng/L]	**1.21**	**1.17**
95% LCL	1.02	1.06
95% UCL	1.30	1.28
Absolute Bias at cutoff 22 [ng/L]	**1.19**	**1.16**
95% LCL	0.978	1.03
95% UCL	1.31	1.27
Correlation Coefficient	0.976 (Kendall's tau)	1.00 (Pearson's r)

Limit of Blank (LoB)
LoB of the Elecsy Troponin T Gen 5 assay on the **cobas e** 801 was determined according to CLSI EP17-A2.

Limit of Blank determines the highest observed measurement values for samples free of analyte. The Limit of Blank was determined as the 95th percentile of measurements of blank samples.

The distribution of values for the analyte-free Li-Heparin plasma sample was determined with three reagent lots on one **cobas e** 801 analyzer with six run over at least three days. The sample was measured in ten-fold determinations in each run.

In summary, 60 measurements were collected for the determination of LoB. The data were evaluated according to EP 17-A2, chapter 5.3.3.1 as the linear interpolation of the 57th and 58th ranked observation. The sample used was an analyte free native lithium-heparin sample.

Conclusion:

All lots met the predetermined acceptance criterion of ≤ 2.5 ng/L. The LoB claim in the labeling will be set to 2.5 ng/L.

Limit of Detection (LoD) (CLSI EP17-A2)

LoD of the Elecsys Troponin T Gen 5 assay on the **cobas e** 801 was determined according to CLSI EP17-A2. The LoD determines the lower limit for samples with analyte. The LoD was determined as the lowest amount of analyte in a sample that can be detected with a 95% probability.

Methods

The distribution of values for five low-analyte Li-Heparin plasma samples were determined using three reagent lots on one cobas e 801 instrument.

Five low-analyte Li-Heparin plasma samples were measured in two-fold determination, six runs over at least three days. In total, 60 determinations per reagent lot were performed.

In addition, we did testing for LoD on 3 different instruments by running samples on two additional e 801 instruments. Pooled estimate of the precision (SD total) of the five samples was calculated. The LoD was established according to the following EP17-A2 calculation:

LoD = LoB + 1.653 x SD total (of low analyte samples)

Conclusion: All lots met the predetermined acceptance criterion of ≤ 3 ng/L. The LoD claim in the labeling will be set to 3 ng/L.

Limit of Quantitation (CLSI EP17-A2)

The LoQ of the Elecsys Troponin T Gen 5 assay was determined on the **cobas e** 801 analyzer according to CLSI Guideline EP17-A2.

LoQ determines the lowest amount of analyte that can be quantitatively determined with stated accuracy and stated experimental conditions. The LoQ was determined as the lowest concentration of analyte which can be quantified with a CV (intermediate precision) of no more than 20%.

Methods

Functional sensitivity has been used as a detection capability performance attribute for the TnT Gen 5 Assay. It represents the measurand concentration associated with a desired within-lab (intermediate) precision, based upon a precision profile experiment in the low-end region of the measuring interval. This performance attribute, however, simply represents a limiting for the limit of quantitation (LoQ) in which the acceptable accuracy goal is based solely upon a precision requirement. This is suggested by CLSI EP17-A2 for troponin.

The performance goal was defined as intermediate precision equal to 20% CV.

Ten native Li-Heparin plasma pools were prepared across the low-end of the measuring range of the assay. In addition, two controls were included. Data were collected using three lots over 21 days with two runs per day. Estimates of the mean and within-lab precision were calculated for each sample for each reagent lot.

Total CV is based on the total variance, calculated as the sum of the variance components from day, run and within run (21 * 2 * 2 = 84 measurements). The square root from the total variance was divided by the grand mean times 100 is the result for the total CV (%). The calculation of

the variance components is based on a strict hierarchical model with random factors according to CLSI EP5-A3.

The functional relationship between CV and concentration is modeled according the suggestion in EP17 with:

%CV = A*conc B

To simplify the fit of the data, the CV and the mean are log-transformed. "Log" here refers to the natural logarithm. After log-transformation, a linear regression using least squares can be performed and the model looks like this:

$$\boldsymbol{log}(\%\boldsymbol{CV}) = \widetilde{\boldsymbol{A}} + \boldsymbol{B} * \boldsymbol{log}(\boldsymbol{concentration})$$

With $\widetilde{\boldsymbol{A}} = \boldsymbol{log}(\boldsymbol{A})$

Results are visually assessed and the goodness of fit of the data is evaluated.

The concentration where a 20% CV is achieved is calculated by:

Concentration = exp $(\frac{\boldsymbol{\log(20) - \widetilde{A}}}{\boldsymbol{B}})$

Conclusion:

All lots met the predetermined acceptance criterion of 6 ng/L. The LoQ claim in the labeling will be set to 6 ng/L.

Linearity/Assay Reportable Range

The linearity study was conducted to demonstrate the measurements across the claimed measuring range for each parameter to prove linearity. The study was performed according to CLSI guideline EP6-A.

Three high analyte Li-Heparin plasma samples from pools spiked with recombinant Troponin T were diluted with three low analyte Li-Heparin plasma samples from native pools. At least sixteen concentrations (15 dilutions) throughout the measuring range were prepared.

Samples were assayed in 3-fold determinations within a single run using one lot. Data from one representative sample is shown below

In the first step, a linearity check was performed with a first order (linear) regression analysis and then with higher order models (quadratic and cubic).

Data were analyzed using linear, quadratic and cubic order least square regression analysis according to CLSI protocol EP6-A.

$\mathbf{y = a + b1 * x}$ (first order polynomial or linear fit)

$\mathbf{y = a + b1 * x + b2 * x^2}$ (second order polynomial fit [quadratic])

$\mathbf{y = a + b1 * x + b2 * x^2 + b3 * x^3}$ (third order polynomial fit [cubic])

Parameters of regression line

Intercept 2.94 ng/L, slope 0.966

The linear range is from 6 to 13766 ng/L

The coefficient b1 in the first order model is significant at the 5% level and no improvement of the goodness-of-fit for higher order models are observed. Therefore, it is used for the calculation of deviation from linearity.

Conclusions

The deviation from linearity is within specifications.

High Dose Hook Effect

Methods

The high dose hook effect of the Elecsys Troponin T Gen 5 assay was assessed on the **cobas e** 801 immunoassay analyzer.

Two low analyte Li-Heparin plasma samples from single donors were spiked with recombinant Troponin T to achieve high Troponin T concentrations. For each sample, a dilution series was performed using human Li-Heparin plasma, which contained no Troponin T.

The hook concentration reported corresponds to the analyte concentration with a signal corresponding to at least 10% above the highest master calibrator.

Conclusion:

No High dose Hook effect was seen up to 111326 ng/L

Human Anti-Mouse Antibodies (HAMA)

Methods

The effect of the presence of human anti-mouse-antibodies on the Troponin T Gen 5 assay was assessed on the **cobas e** 801 analyzer. Two different samples with low levels of Troponin T were spiked with potential interfering HAMA and tested in duplicate. The HAMA interferents used

for the interference testing have been quantified externally with a commercial assay and yielded a HAMA concentration of 644 µg/L.

Conclusion:

Specifications were met for the two native Li-Heparin plasma samples. A maximum of 10% deviation from the expected concentration was seen for a HAMA concentration of 644 µg/L (80%). In the labeling the testing of the specified HAMA concentration of 322 µg/L is reported.

Endogenous Interference Studies

The purpose of this study was to evaluate endogenous substances for potential interference with the parameters measured on the cobas e 801.

Methods

The effect on quantitation of analyte in the presence of endogenous interfering substances using the Elecsys Troponin T Gen 5 assay was determined on the **cobas e** 801 immunoassay analyzer for the following seven interfering substances: Intralipid, biotin, bilirubin, hemoglobin, rheumatoid factors, cholesterol and human serum albumin.

Three native Li-Heparin plasma samples (one low, one medium and one high concentration of Troponin T) were used to prepare dilution series. The series were tested with one lot.

Since biotin is of particular interest, we tested this kind of potential interference with three lots.

The following samples were used in this study:

> **Low sample:** Native Li-Heparin plasma pool with analyte concentration of ~20 ng/L near the cut-off
>
> **Medium sample:** Li-Heparin plasma pool of native samples with analyte concentration of ~100 ng/L in the slightly elevated part of the measuring range
>
> **High sample:** Li-Heparin plasma pool of native samples with analyte concentration of ~900 ng/L in the elevated part of the measuring range

One part of each sample pool (low, medium, high) is spiked with the interfering endogenous substance and is used as “interference pool”. Another part of the same sample pool is spiked with the same volume of the solvent of the interfering endogenous substance (without interfering substance) and is used as the related “dilution pool”.

A series of nine dilution steps was prepared by mixing the interference pools and the related dilution pools.

Each sample was measured in 3-fold determination. For each interferent concentration level, the recovery compared to the “dilution pool” (without interfering substance) was calculated. For 3-fold determinations, recovery calculation is based on the mean value.

Biotin concentrations of the samples were:50, 100, 500, 1000, 1250, 1500, 1750, 2000, 2500, 3600 ng/mL

Biotin Results

% Bias for samples containing various concentrations of biotin					
Samples TnT concentration (ng/L)	**Biotin concentration (ng/mL)**				
	50	**100**	**500**	**1000**	**1250**
18.9	-0.5	-1.3	2.0	-1.4	-2.5
108	0.3	1.8	1.7	-0.5	-2.2
870	-0.4	1.1	2.0	-0.1	-2.6

% Bias for samples containing various concentrations of biotin					
Samples TnT concentration (ng/L)	**Biotin concentration (ng/mL)**				
	1500	**1750**	**2000**	**2500**	**3600**
18.9	-6.4	-12.2	-18.6	-39.7	-96.2
108	-6.9	-13.4	-20.9	-40.6	-93.2
870	-7.5	-12.7	-20.4	-40.6	-92.9

Note that patients with compromised renal function may have higher concentrations of biotin in their circulation.

Some studies have shown that serum concentrations of biotin can reach up to 355 ng/mL within the first hour after biotin ingestion for subjects consuming supplements of 20 mg of biotin per dayand up to 1160 ng/mL for subjects after a single dose of 300 mg biotin.

In addition, the following commonly used pharmaceuticals and cardiac specific drugs were tested (using cTnT concentrations of approximately 20 ng/L and 9000 ng/L). No interference with the assay was found.

Conclusions

No interference was seen from the potential interferents at the levels tabulated below.

Table 1.5: Summary of Results for all Tested Interferents

Interferent Tested	No interference seen up to	
Biotin	1200	ng/mL
Intralipid® (Lipemia)	2000	mg/dL
Bilirubin	66.0	mg/dL
Hemoglobin	200	mg/dL
Rheumatic Factor	1200	IU/mL
Human Serum Albumin	7.00	g/dL
Cholesterol	310	mg/dL

Cross-Reactivity
This study was conducted to evaluate the Elecsys Troponin T Gen 5 assay on the **cobas e** 801 for potential cross-reactivity.

Methods

The analytical specificity of the Troponin T Gen 5 assay was assessed on the **cobas e** 801 analyzer using three concentrations of troponin in Li-Heparin plasma samples spiked with the potential cross-reacting compounds listed below:

Table 1.6: Cross-Reactants Tested

Interfering Substance	Spiked Concentration
Skeletal muscle TnT	30 ng/mL
Skeletal muscle TnI	100 ng/mL
Cardiac TnI	25 ng/mL
Human TnC	100 ng/mL

Description of samples used in cross-reactivity study: The spiked and non-spiked samples were tested in three-fold determination on the **cobas e** 801 analyzer.

Conclusion:

The specifications were met for all substances tested. See the following results summarized for the highest interference level tested and the resulting claim in the method sheet.

Table 1.7: Summary of Cross-Reactivity Study

Interfering Substance	No interference seen up to	Method Sheet claim
Skeletal muscle TnT	30 ng/mL	30 ng/mL
Skeletal muscle TnI	100 ng/mL	100 ng/mL
Cardiac TnI	12.5 ng/mL	12.5 ng/mL
Human TnC	100 ng/mL	100 ng/mL

Exogenous Interference – Drugs

The purpose of this study was to evaluate drugs for potential interference with the Elecsys Troponin T Gen 5 assay on the **cobas e** 801.

Methods

Seventeen common pharmaceutical compounds were spiked into Li-Heparin plasma samples.

Native Li-Heparin plasma samples were used as the low sample. The high sample was spiked with recombinant Troponin T.

The analyte concentrations were approximately 20 ng/L and 9000 ng/L.

In addition, 22 cardiac drugs were assessed by spiking into human Li-Heparin plasma samples. The analyte concentrations were approximately 19 ng/L and 8200 ng/L.

Li-Heparin plasma samples were divided into aliquots and spiked with the potential interferents. The reference sample without interferent was spiked with the respective amount of solvent only.

Testing was performed in 3-fold determination with one reagent lot in one run on two **cobas e** 801 analyzer.

Recovery was calculated based on the mean values of the 3-fold determinations.

Conclusion:

The specifications were met for all exogenous interfering substances tested.

Calibration and Traceability

This method has been standardized against the Elecsys Troponin T STAT assay (Mat# 04660307, 4th generation). This in turn was originally standardized against the Enzymun Test Troponin T (CARDIAC T) method.

Clinical Data--APACE Study

Diagnostic sensitivity and specificity

APACE Trial: The Advantageous Predictors of Acute Coronary Syndromes Evaluation (APACE) study is an international, multicenter prospective trial of acute chest pain patients that is currently continuing enrollment. (ClinicalTrials.gov number NCT00470587). The sites enrolled all patients who presented to the emergency department with symptoms of chest pain and angina pectoris. Peak symptoms had to have occurred within the last 12 hours (onset of symptoms reported ranged from 0 to 72 hours). The only exclusion criterion were kidney failure that required dialysis and age less than 18 years. Diagnosis of MI was done through an independent adjudication committee which included cardiologists. This included 60 day follow-up information on each subject. In the case of a disagreement, a third independent cardiologist was used as the tie breaker. The subjects were diagnosed with acute MI by using the diagnostic criteria described in the ACC/ESC/AHA guidelines[5] including ECG changes, symptoms characteristic for ischemia and elevations of cardiac troponin. 1074 subjects were enrolled with 3023 available samples. There were 188 adjudicated diagnoses of MI.

The clinical performance (clinical sensitivity, clinical specificity, positive predictive value [PPV] and negative predictive value [NPV]) of the Elecsys Troponin T Gen 5 STAT assay in the diagnosis of MI in this trial is shown below using a single 99th percentile cutoff 19 ng/L for all patients:

Performance data at all three cutoffs are presented below.

	Diagnosis	
	MI	Non-MI
cTnT positive	A	B
cTnT negative	C	D

Clinical performance of single 99th percentile cutoff (19 ng/L) for aid in diagnosis of AMI in both sexes							
Instrument	**BCT[a)]**	**MDT[b)]**	**N[c)]**	**Sens[d)] % 95 % CI[e)]**	**Spec[f)] % 95 % CI**	**PPV[g)] % 95 % CI**	**NPV[h)] % 95 % CI**
cobas e 801 analyzer	<=1.5	0.74	968	80.0 (73.2-85.7)	86.0 (83.4-88.3)	54.8 (48.4-61.1)	95.3 (93.5-96.7)
	>1.5-<=2.5	1.99	796	91.3 (84.6-95.8)	86.6 (83.8-89.1)	53.6 (46.3-60.7)	98.3 (97.0-99.2)
	>2.5-<=3.5	2.95	596	96.5 (90.1-99.3)	85.9 (82.6-88.8)	53.5 (45.4-61.6)	99.3 (98.0-99.9)
	>3.5	3.96	351	91.8 (81.9-97.3)	82.4 (77.5-86.6)	52.3 (42.5-62.1)	98.0 (95.3-99.3)

a) BCT = Blood collection time in hours since presentation

b) MDT = Mean draw time in hours

c) N = number of draws

d) Sensitivity = 100xA/(A+C)

e) CI = confidence interval

f) Specificity = 100xD/(B+D)

g) Positive predictive value = 100xA/(A+B)

h) Negative predictive value = 100xD/(D+C)

Clinical performance of single 99th percentile cutoff (19 ng/L) for aid in diagnosis of AMI in women							
Instrument	**BCT[a)]**	**MDT[b)]**	**N[c)]**	**Sens[d)] % 95 % CI[e)]**	**Spec[f)] % 95 % CI**	**PPV[g)] % 95 % CI**	**NPV[h)] % 95 % CI**
cobas e 801 analyzer	<=1.5	0.75	308	74.3 (56.7-87.5)	86.4 (81.8-90.3)	41.3 (29.0-54.4)	96.3 (93.1-98.3)
	>1.5-<=2.5	2.00	255	82.6 (61.2-95.0)	87.5 (82.5-91.5)	39.6 (25.8-54.7)	98.1 (95.1-99.5)

Clinical performance of single 99th percentile cutoff (19 ng/L) for aid in diagnosis of AMI in women							
Instrument	**BCT[a)]**	**MDT[b)]**	**N[c)]**	**Sens[d)] % 95 % CI[e)]**	**Spec[f)] % 95 % CI**	**PPV[g)] % 95 % CI**	**NPV[h)] % 95 % CI**
	>2.5-<=3.5	2.96	181	88.9 (65.3-98.6)	89.6 (83.8-93.8)	48.5 (30.8-66.5)	98.6 (95.2-99.8)
	>3.5	4.01	113	86.7 (59.5-98.3)	86.7 (78.4-92.7)	50.0 (29.9-70.1)	97.7 (91.9-99.7)

Clinical performance of single 99th percentile cutoff (19 ng/L) for aid in diagnosis of AMI in men							
Instrument	**BCT[a)]**	**MDT[b)]**	**N[c)]**	**Sens[d)] % 95 % CI[e)]**	**Spec[f)] % 95 % CI**	**PPV[g)] % 95 % CI**	**NPV[h)] % 95 % CI**
cobas e 801 analyzer	<=1.5	0.73	660	81.5 (73.9-87.6)	85.7 (82.4-88.6)	59.5 (52.0-66.6)	94.7 (92.3-96.6)
	>1.5-<=2.5	1.98	541	93.5 (86.3-97.6)	86.2 (82.7-89.2)	58.1 (49.7-66.2)	98.5 (96.7-99.4)
	>2.5-<=3.5	2.95	415	98.5 (92.1-100.0)	84.1 (79.9-87.8)	54.9 (45.7-63.9)	99.7 (98.1-100.0)
	>3.5	3.93	238	93.5 (82.1-98.6)	80.2 (73.9-85.6)	53.1 (41.7-64.3)	98.1 (94.5-99.6)

Clinical performance of sex-specific 99th percentile cutoff (14 ng/L) for aid in diagnosis of AMI in females							
Instrument	**BCT[a)]**	**MDT[b)]**	**N[c)]**	**Sens[d)] % 95 % CI[e)]**	**Spec[f)] % 95 % CI**	**PPV[g)] % 95 % CI**	**NPV[h)] % 95 % CI**
cobas e 801 analyzer	<=1.5	0.75	308	80.0 (63.1-91.6)	76.9 (71.5-81.8)	30.8 (21.5-41.3)	96.8 (93.5-98.7)

Clinical performance of sex-specific 99th percentile cutoff (14 ng/L) for aid in diagnosis of AMI in females							
Instrument	**BCT[a)]**	**MDT[b)]**	**N[c)]**	**Sens[d)] % 95 % CI[e)]**	**Spec[f)] % 95 % CI**	**PPV[g)] % 95 % CI**	**NPV[h)] % 95 % CI**
	>1.5-<=2.5	2.00	255	95.7 (78.1-99.9)	76.3 (70.3-81.6)	28.6 (18.8-40.0)	99.4 (96.9-100.0)
	>2.5-<=3.5	2.96	181	94.4 (72.7-99.9)	81.6 (74.8-87.2)	36.2 (22.7-51.5)	99.3 (95.9-100.0)
	>3.5	4.01	113	93.3 (68.1-99.8)	74.5 (64.7-82.8)	35.9 (21.2-52.8)	98.6 (92.7-100.0)

Clinical performance of sex-specific 99th percentile cutoff (22 ng/L) for aid in diagnosis of AMI in males							
Instrument	**BCT[a)]**	**MDT[b)]**	**N[c)]**	**Sens[d)] % 95 % CI[e)]**	**Spec[f)] % 95 % CI**	**PPV[g)] % 95 % CI**	**NPV[h)] % 95 % CI**
cobas e 801 analyzer	<=1.5	0.73	660	80.7 (73.1-87.0)	89.0 (86.0-91.5)	65.3 (57.5-72.5)	94.7 (92.4-96.5)
	>1.5-<=2.5	1.98	541	88.0 (79.6-93.9)	88.9 (85.6-91.6)	61.8 (52.9-70.2)	97.3 (95.3-98.7)
	>2.5-<=3.5	2.95	415	95.6 (87.6-99.1)	88.5 (84.6-91.6)	61.9 (51.9-71.2)	99.0 (97.2-99.8)
	>3.5	3.93	238	93.5 (82.1-98.6)	83.3 (77.3-88.3)	57.3 (45.4-68.7)	98.2 (94.7-99.6)

At least one of the following criteria should be met: symptoms of ischemia, ECG changes (ST and/or Q wave), left bundle branch block, imaging evidence of viable myocardium loss, wall motion abnormality or intracoronary thrombus to clarify the origin of myocardial injury.

In a second multicenter study, a total of 1679 subjects presenting emergently with chest pain were enrolled. The trial excluded chest pain subjects with an MI within the last 3 months,

subjects with surgery or hospitalization within the last 3 months, subjects with revascularization or percutaneous coronary intervention (PCI) within the last 3 months, subjects with an established acute non-cardiac primary illness and subjects transferred from another hospital or facility. **These excluded subjects could be expected to have elevated troponin concentrations that would likely reflect cardiac comorbidities besides MI, and yield positive results; therefore specificity estimates and the positive predictive values of this trial may be overestimated.** Within this population, there were 173 adjudicated MIs. 1675 subjects were evaluated on the **cobas e** 601 analyzer. Final diagnoses were determined by an independent adjudication committee which included cardiologists and emergency medicine physicians using the universal guidelines.

The clinical performance of the Elecsys Troponin T Gen 5 STAT assay in the diagnosis of MI in this trial is shown below using a single 99th percentile cutoff (19 ng/L) for all patients and sex-specific cutoffs. The data are presented as timed draws (minutes) since presentation:

cobas e 601 analyzer			
	MI	non-MI	Total
N	173	1502	1675
%	10.3	89.7	100

The agreement between Elecsys Troponin T Gen 5 and clinical diagnosis is shown in the tables below:

	Diagnosis	
	MI	Non-MI
cTnT positive	A	B
cTnT negative	C	D

Clinical performance of single 99th percentile cutoff (19 ng/L) for aid in diagnosis of AMI in females						
Instrument	**BCT[a)]**	**N[b)]**	**Sens[c)] % 95 % CI[d)]**	**Spec[e)] % 95 % CI**	**PPV[f)] % 95 % CI**	**NPV[g)] % 95 % CI**
cobas e 601 analyzer	Baseline	771	82.5 (70.9-90.9)	91.9 (89.7-93.8)	47.7 (38.1-57.5)	98.3 (97.0-99.2)
	3 hours	682	91.8 (80.4-97.7)	90.2 (87.6-92.4)	42.1 (32.6-52.0)	99.3 (98.2-99.8)
	6-9 hours	536	91.3 (79.2-97.6)	90.8 (87.9-93.2)	48.3 (37.4-59.2)	99.1 (97.7-99.8)
	12-24 hours	399	87.2 (72.6-95.7)	85.3 (81.2-88.8)	39.1 (28.8-50.1)	98.4 (96.3-99.5)

a) BCT = Blood collection time in hours since presentation

b) N = number of draws

c) Sensitivity = 100xA/(A+C)

d) CI = confidence interval

e) Specificity = 100xD/(B+D)

f) Positive predictive value = 100xA/(A+B)

g) Negative predictive value = 100xD/(D+C)

Clinical performance of single 99th percentile cutoff (19 ng/L) for aid in diagnosis of AMI in males						
Instrument	**BCT[a)]**	**N[b)]**	**Sens[c)] % 95 % CI[d)]**	**Spec[e)] % 95 % CI**	**PPV[f)] % 95 % CI**	**NPV[g)] % 95 % CI**
cobas e 601 analyzer	Baseline	829	88.1 (80.2-93.7)	84.1 (81.2-86.7)	43.4 (36.5-50.5)	98.1 (96.7-99.0)
	3 hours	733	95.6 (89.1-98.8)	83.0 (79.9-85.8)	44.4 (37.3-51.6)	99.3 (98.1-99.8)

Clinical performance of single 99th percentile cutoff (19 ng/L) for aid in diagnosis of AMI in males						
Instrument	**BCT[a)]**	**N[b)]**	**Sens[c)] % 95 % CI[d)]**	**Spec[e)] % 95 % CI**	**PPV[f)] % 95 % CI**	**NPV[g)] % 95 % CI**
	6-9 hours	622	96.7 (90.8-99.3)	80.2 (76.5-83.5)	45.9 (38.7-53.2)	99.3 (98.0-99.9)
	12-24 hours	473	94.4 (86.4-98.5)	76.3 (71.8-80.4)	41.7 (34.1-49.7)	98.7 (96.7-99.6)

Clinical performance of sex-specific 99th percentile cutoff (14 ng/L) for aid in diagnosis of AMI in females						
Instrument	**BCT[a)]**	**N[b)]**	**Sens[c)] % 95 % CI[d)]**	**Spec[e)] % 95 % CI**	**PPV[f)] % 95 % CI**	**NPV[g)] % 95 % CI**
cobas e 601 analyzer	Baseline	771	85.7 (74.6-93.3)	88.1 (85.5-90.4)	39.1 (30.9-47.8)	98.6 (97.3-99.3)
	3 hours	682	91.8 (80.4-97.7)	86.9 (84.0-89.4)	35.2 (26.9-44.1)	99.3 (98.2-99.8)
	6-9 hours	536	91.3 (79.2-97.6)	86.5 (83.2-89.4)	38.9 (29.7-48.7)	99.1 (97.6-99.7)
	12-24 hours	399	92.3 (79.1-98.4)	81.4 (77.0-85.3)	35.0 (25.8-45.0)	99.0 (97.1-99.8)

Clinical performance of sex-specific 99th percentile cutoff (22 ng/L) for aid in diagnosis of AMI in males						
Instrument	**BCT[a)]**	**N[b)]**	**Sens[c)] % 95 % CI[d)]**	**Spec[e)] % 95 % CI**	**PPV[f)] % 95 % CI**	**NPV[g)] % 95 % CI**
cobas e 601 analyzer	Baseline	829	85.1 (76.7-91.4)	87.2 (84.6-89.6)	48.0 (40.5-55.6)	97.7 (96.2-98.7)
	3 hours	733	95.6 (89.1-98.8)	86.3 (83.4-88.9)	49.7 (42.1-57.4)	99.3 (98.2-99.8)
	6-9 hours	622	93.5 (86.3-97.6)	82.3 (78.7-85.4)	47.8 (40.3-55.3)	98.6 (97.1-99.5)
	12-24 hours	473	94.4 (86.4-98.5)	80.0 (75.8-83.9)	45.9 (37.7-54.3)	98.8 (96.9-99.7)

Conclusion

The Elecsys Troponin T Gen 5 on the cobas e 801 is substantially equivalent to the FDA cleared Elecsys Troponin T Gen 5 STAT assay (k162895).