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Elecsys Troponin T Gen 5 STAT

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REF	Σ	SYSTEM
07398000 160	100	**cobas e** 411 **cobas e** 601 **cobas e** 602

English

For use in the USA only

System information

For **cobas e** 411 analyzer: test number 1480
For **cobas e** 601 and **cobas e** 602 analyzers: Application Code Number 188

Intended 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 **e**lectro**c**hemi**l**uminescence **i**mmuno**a**ssay "ECLIA" is intended for use on **cobas e** immunoassay analyzers.

Summary

Troponin T (TnT) is a component of the contractile apparatus of the striated musculature. Although the function of TnT is the same in all striated muscles, the cardiac isoform of TnT originating exclusively from the myocardium (cardiac TnT, molecular weight 39.7 kDa) clearly differs from skeletal muscle TnT. As a result of its high tissue-specificity, cardiac troponin T (cTnT) is a cardio-specific, highly sensitive marker for myocardial damage. Cardiac troponin T increases rapidly (i.e. can be detected within 1 hour if using high-sensitivity assays)[1] after acute myocardial infarction (AMI) and may persist up to 2 weeks thereafter.[2,3] In contrast to ST-elevation myocardial infarction (STEMI), the diagnosis of non-ST elevation myocardial infarction (NSTEMI) relies heavily upon elevated cardiac troponin (cTn) concentrations in the appropriate clinical context. The Third Universal Definition of Myocardial Infarction (MI) has confirmed cTn as the biomarker of choice.[4] Diagnosis of MI is made with acute changes in cTn concentrations with at least one serial sample above the 99th percentile upper reference limit (URL), taken together with evidence of myocardial ischemia (symptoms, electrocardiogram (ECG) changes or imaging results). Various guidelines and publications recommend the optimal imprecision (coefficient of variation) of cTn assays at the 99th percentile upper reference limit be less than or equal to 10 %.[1,4,5,6,7]

The 99th percentile upper reference limit is derived from a reference control group of normal, non-diseased individuals.[4,7,8]

Several guidelines and research activities recognize that improved analytical sensitivity of cTn assays over the last several years has allowed for detection of other etiologies. Chronic cTn elevations can be detected in clinically stable patients such as patients with ischemic or non-ischemic heart failure,[9,10] patients with different forms of cardiomyopathy,[11] renal failure,[12,13,14,15,16,17,18] sepsis[19] and diabetes.[20]

Elevated concentrations of cTn can also occur in other clinical conditions such as myocarditis,[21] heart contusion,[22] pulmonary embolism[23] and drug-induced cardiotoxicity.[24]

To distinguish between acute and chronic cTn elevations, the Universal Definition of MI stresses the need for serial sampling to observe a rise and/or fall of cTn above the 99th percentile upper reference limit consistent with the clinical assessment, including ischemic symptoms and electrocardiographic changes.[4]

Troponin elevations may persist for up to 14 days or occasionally longer.[4] Other diagnostic tests such as NT-proBNP and CRP can complement the diagnostic and prognostic information of cTnT in different indications.

The Elecsys Troponin T Gen 5 STAT assay employs two monoclonal antibodies specifically directed against human cardiac troponin T.[25,26] The antibodies recognize two epitopes (amino acid position 125-131 and 136-147) located in the central part of the cardiac troponin T protein, which consists of 288 amino acids.

The Elecsys Troponin T Gen 5 STAT calibrators (CalSet Troponin T Gen 5 STAT) contain recombinant human cardiac troponin T (rec. hcTnT). The rec. hcTnT is isolated from cell culture of E. coli BL21 containing a pET vector with human cardiac troponin T isoform 3 gene. After fermentation, the cells are disrupted by sonication and rec. hcTnT is purified by ion exchange chromatography. Purified rec. hcTnT is further characterized by SDS PAGE, Western blotting, immunological activity, and protein content.[27]

By current universal definition of the disease (AMI), the 99th percentile URL should be used as a diagnostic cutoff of AMI,[4] and is endorsed by major local guidelines.[1,7,28] Yet, the International Federation of Clinical Chemistry (IFCC) recently observed that only 11 out of 31 of contemporary troponin tests can measure the 99th percentile URL with adequate precision (≤ 10 % CV at the cutoff) and therefore many tests are used with higher cutoffs (such as their analytical 10 % CV).[8] Higher cutoffs produce higher estimates of clinical specificity and positive predictive value (PPV), but tend to underestimate clinical sensitivity and negative predictive value (NPV).[29] When switching to the Elecsys Troponin T Gen 5 STAT assay, users should be aware that the guideline compliant test using the 99th percentile URL as a diagnostic cutoff, can lead to a relative increase in the diagnosis of acute MIs compared to contemporary assays using other, often higher cutoffs.[1,30,31,32]

Test principle

Sandwich principle. Total duration of assay: 9 minutes.

cobas e 411 analyzer:

- 1st incubation: 50 µL of sample, a biotinylated monoclonal anti-cardiac troponin T-specific antibody, and a monoclonal anti-cardiac troponin T-specific antibody labeled with a ruthenium complex[a)] react to form a sandwich complex.
- 2nd incubation: After addition of streptavidin-coated microparticles, the complex becomes bound to the solid phase via interaction of biotin and streptavidin.

cobas e 601 and **cobas e** 602 analyzers:

- During a 9 minute incubation, antigen in the sample (50 µL), a biotinylated monoclonal anti-cardiac troponin T-specific antibody, a monoclonal anti-cardiac troponin T-specific antibody labeled with a ruthenium complex[a)], and streptavidin-coated microparticles react to form a sandwich complex, which is bound to the solid phase.

All analyzers:

- The reaction mixture is aspirated into the measuring cell where the microparticles are magnetically captured onto the surface of the electrode. Unbound substances are then removed with ProCell/ProCell M. Application of a voltage to the electrode then induces chemiluminescent emission which is measured by a photomultiplier.
- Results are determined via a calibration curve which is instrument-specifically generated by 2-point calibration and a master curve provided via the reagent barcode or e-barcode.

a) Tris(2,2-bipyridyl)ruthenium(II)-complex ($Ru(bpy)_3^{2+}$)

Reagents - working solutions

The reagent rackpack is labeled as TNT-G5ST.

M Streptavidin-coated microparticles (transparent cap), 1 bottle, 6.5 mL: Streptavidin-coated microparticles 0.72 mg/mL; preservative.

R1 Anti-troponin T-Ab~biotin (gray cap), 1 bottle, 8 mL: Biotinylated monoclonal anti-cardiac troponin T-antibody (mouse) 2.5 mg/L; phosphate buffer 100 mmol/L, pH 6.0; preservative; inhibitors.

R2 Anti-troponin T-Ab~$Ru(bpy)_3^{2+}$ (black cap), 1 bottle, 8 mL: Monoclonal chimeric anti-cardiac troponin T-antibody (mouse/human) labeled with ruthenium complex 2.5 mg/L; phosphate buffer 100 mmol/L, pH 6.0; preservative.

Precautions and warnings

For in vitro diagnostic use.
Exercise the normal precautions required for handling all laboratory reagents.
Disposal of all waste material should be in accordance with local guidelines.
Safety data sheet available for professional user on request.

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For USA: Caution: Federal law restricts this device to sale by or on the order of a physician.

Avoid foam formation in all reagents and sample types (specimens, calibrators and controls).

Reagent handling

The reagents in the kit have been assembled into a ready-for-use unit that cannot be separated.

All information required for correct operation is read in from the respective reagent barcodes.

Storage and stability

Store at 2-8 °C.

Do not freeze.

Store the Elecsys reagent kit **upright** in order to ensure complete availability of the microparticles during automatic mixing prior to use.

Stability:	
unopened at 2-8 °C	up to the stated expiration date
after opening at 2-8 °C	12 weeks
on the analyzers	4 weeks

Specimen collection and preparation

Only the specimen listed below was tested and found acceptable:

Li-heparin plasma.

Stable for 24 hours at 2-8 °C,[33] 12 months at -20 °C (± 5 °C). Freeze only once.

The sample types listed were tested with a selection of sample collection tubes that were commercially available at the time of testing, i.e. not all available tubes of all manufacturers were tested. Sample collection systems from various manufacturers may contain differing materials which could affect the test results in some cases. When processing samples in primary tubes (sample collection systems), follow the instructions of the tube manufacturer.

Centrifuge samples containing precipitates before performing the assay.

Do not use samples and controls stabilized with azide.

Ensure the samples, calibrators and controls are at 20-25 °C prior to measurement.

Due to possible evaporation effects, samples, calibrators and controls on the analyzers should be analyzed/measured within 2 hours.

The claims, including those pertaining to sample stability made in the labeling of the cleared/approved reagents of Roche Diagnostics are part of the clearance of the overall IVD test system (assay). Sample stability was tested only for the temperatures/time frame as claimed by the manufacturer under the conditions claimed in the method sheet. It is the responsibility of the individual laboratory to use all available references and/or its own studies to determine specific stability criteria for its laboratory.

Materials provided

See "Reagents – working solutions" section for reagents.

Materials required (but not provided)

- REF 07398271160, CalSet Troponin T Gen 5 STAT, for 4 x 1.0 mL
- REF 05095107160, PreciControl Troponin, for 4 x 2.0 mL
- REF 03609987190, Diluent MultiAssay, 2 x 16 mL sample diluent
- General laboratory equipment
- **cobas e** 411, **cobas e** 601 or **cobas e** 602 analyzer

Accessories for **cobas e** 411 analyzer:

- REF 11662988122, ProCell, 6 x 380 mL system buffer
- REF 11662970122, CleanCell, 6 x 380 mL measuring cell cleaning solution
- REF 11930346122, Elecsys SysWash, 1 x 500 mL washwater additive
- REF 11933159001, Adapter for SysClean
- REF 11706802001, AssayCup, 60 x 60 reaction cups
- REF 11706799001, AssayTip, 30 x 120 pipette tips
- REF 11800507001, Clean-Liner

Accessories for **cobas e** 601 and **cobas e** 602 analyzers:

- REF 04880340190, ProCell M, 2 x 2 L system buffer
- REF 04880293190, CleanCell M, 2 x 2 L measuring cell cleaning solution
- REF 03023141001, PC/CC-Cups, 12 cups to prewarm ProCell M and CleanCell M before use
- REF 03005712190, ProbeWash M, 12 x 70 mL cleaning solution for run finalization and rinsing during reagent change
- REF 03004899190, PreClean M, 5 x 600 mL detection cleaning solution
- REF 12102137001, AssayTip/AssayCup, 48 magazines x 84 reaction cups or pipette tips, waste bags
- REF 03023150001, WasteLiner, waste bags
- REF 03027651001, SysClean Adapter M

Accessories for all analyzers:

- REF 11298500160, ISE Cleaning Solution/Elecsys SysClean, 5 x 100 mL system cleaning solution

Assay

For optimum performance of the assay follow the directions given in this document for the analyzer concerned. Refer to the appropriate operator's manual for analyzer-specific assay instructions.

Resuspension of the microparticles takes place automatically prior to use. Read in the test-specific parameters via the reagent barcode. If in exceptional cases the barcode cannot be read, enter the 15-digit sequence of numbers (except for the **cobas e** 602 analyzer).

cobas e 601 and **cobas e** 602 analyzers: PreClean M solution is necessary.

Bring the cooled reagents to approximately 20 °C and place on the reagent disk (20 °C) of the analyzer. Avoid foam formation. The system automatically regulates the temperature of the reagents and the opening/closing of the bottles.

Calibration

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

Every Elecsys reagent set has a barcoded label containing specific information for calibration of the particular reagent lot. The predefined master curve is adapted to the analyzer using the relevant CalSet.

Calibration frequency: Calibration must be performed once per reagent lot using fresh reagent (i.e. not more than 24 hours since the reagent kit was registered on the analyzer).

Calibration interval may be extended based on acceptable verification of calibration by the laboratory.

Renewed calibration is recommended as follows:

- after 12 weeks when using the same reagent lot
- after 7 days (when using the same reagent kit on the analyzer)
- as required: e.g. quality control findings outside the defined limits

Quality control

For quality control, use PreciControl Troponin.

In addition, other suitable control material can be used.

Controls for the various concentration ranges should be run individually at least once every 24 hours when the test is in use, once per reagent kit, and following each calibration.

The control intervals and limits should be adapted to each laboratory's individual requirements. Values obtained should fall within the defined limits. Each laboratory should establish corrective measures to be taken if values fall outside the defined limits.

If necessary, repeat the measurement of the samples concerned.

Follow the applicable government regulations and local guidelines for quality control.

Calculation

The analyzer automatically calculates the analyte concentration of each sample:

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cobas e 411 analyzer	either in pg/mL, ng/L or in µg/L
cobas e 601 and **cobas e** 602 analyzers	either in pg/mL, ng/L, µg/L or in ng/mL

Limitations - interference

It is recommended to run serum indices on samples for troponin T measurement.

Samples showing visible signs of hemolysis may cause interference. Falsely depressed results are obtained when using samples with hemoglobin concentrations > 0.1 g/dL.

Endogenous substances

Compound	Concentration tested
Bilirubin	≤ 428 µmol/L or ≤ 25 mg/dL
Hemoglobin	≤ 0.062 mmol/L or ≤ 0.1 g/dL
Intralipid	≤ 1500 mg/dL
Biotin	≤ 82 nmol/L or ≤ 20 ng/mL
Rheumatoid factors	≤ 900 IU/mL
Serum albumin	≤ 7 g/dL
Cholesterol	≤ 310 mg/dL
HAMA (Human anti-mouse antibodies)	≤ 322 µg/mL

Criterion: Recovery within ± 1.4 ng/L with a concentration < 14 ng/L. Recovery within 100 ± 10 % with a concentration ≥ 14 ng/L.

In addition, the following commonly used pharmaceuticals and cardiac specific drugs were tested (using cTnT concentrations of approximately 15 ng/L and 9000 ng/L). No interference with the assay was found.

Drug interference (general drug panel)	
Drug	Concentration (mg/L)
Acetylcysteine	1660
Ampicillin-Na	1000
Ascorbic acid	300
Cyclosporine	5
Cefoxitin	2500
Heparin	5000 U
Levodopa	20
Methyldopa	20
Metronidazole	200
Phenylbutazone	400
Doxycycline	50
Acetylsalicylic acid	1000
Rifampicin	60
Acetaminophen	200
Ibuprofen	500
Theophylline	100

Drug interference (cardiac drug panel)	
Drug	Concentration (mg/L)
Carvedilol	37.5
Clopidogrel	75
Digoxin	0.25
Epinephrine	0.5

Drug interference (cardiac drug panel)	
Drug	Concentration (mg/L)
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
Torasemide	15
Verapamil	240

Recovery criteria:

Concentration of < 14 ng/L: recovery of ± 1.4 ng/L

Concentration of ≥ 14 ng/L: recovery 100 ± 10 %

Samples should not be taken from patients receiving therapy with high biotin doses (i.e. > 5 mg/day) until at least 8 hours following the last biotin administration.

There is no high-dose hook effect at cTnT concentrations up to 100000 ng/L.

For assays using antibodies, the possibility exists for interference by heterophile antibodies in the patient's sample. Patients who have been regularly exposed to animals or have received immunotherapy or diagnostic procedures using immunoglobulin or immunoglobulin fragments may produce antibodies, e.g. HAMA, that interfere with immunoassays. Carefully evaluate the results of patients suspected of having these antibodies.

In rare cases, interference due to extremely high titers of antibodies to analyte-specific antibodies, streptavidin or ruthenium can occur. The reagent has been formulated to minimize this effect.

For diagnostic purposes, the results should always be assessed in conjunction with the patient's medical history, clinical examination and other findings.

The positive predictive value for females using the lower sex-specific cutoff (14 ng/L) is lower when compared to the higher cutoff of 19 ng/L. When looking at the lower bound of the 95 % CI, up to 69 %, 82 % and 78 % of positive test results for females are non-MI. Troponin results should always be used in conjunction with clinical signs and symptoms.

Limits and ranges

Measuring range

6-10000 ng/L (defined by the Limit of Quantitation and the maximum of the master curve). Values below 6 ng/L are reported as < 6 ng/L. Values above the measuring range are reported as > 10000 ng/L (or up to 100000 ng/L for 10-fold diluted samples).

Lower limits of measurement

Limit of Blank, Limit of Detection and Limit of Quantitation

	cobas e 411	**cobas e** 601 and **cobas e** 602
Limit of Blank	3 ng/L	2.5 ng/L
Limit of Detection	5 ng/L	3 ng/L
Limit of Quantitation	6 ng/L (20 % CV)	

The Limit of Blank, Limit of Detection and Limit of Quantitation were determined in accordance with the CLSI (Clinical and Laboratory Standards Institute) EP17-A2 requirements.

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The Limit of Blank is the 95th percentile value from n ≥ 60 measurements of analyte-free samples over several independent series. The Limit of Blank corresponds to the concentration below which analyte-free samples are found with a probability of 95 %.

The Limit of Detection is determined based on the Limit of Blank and the standard deviation of low concentration samples. The Limit of Detection corresponds to the lowest analyte concentration which can be detected (value above the Limit of Blank with a probability of 95 %).

The Limit of Quantitation (functional sensitivity) is the lowest analyte concentration that can be reproducibly measured with an intermediate precision CV of ≤ 20 %.

Dilution

Samples with cTnT concentrations above the measuring range can be diluted with Diluent MultiAssay. The recommended dilution is 1:10 (either automatically by the analyzers, or manually). The concentration of the diluted sample must be > 1000 ng/L.

Dilution experiments were performed using three Li-heparin plasma samples run in triplicate on two **cobas e** 411 and two **cobas e** 601 analyzers. Automatic dilution was compared to manual dilution.

After manual dilution, multiply the result by the dilution factor.

After dilution by the analyzers, the software automatically takes the dilution into account when calculating the sample concentration.

Expected values

A total of 1301 healthy subjects (656 females, 645 males), were enrolled and included in the testing for determining the 99th percentile upper reference limit of a normal US population (age range 21 to 89 years) using the Elecsys Troponin T Gen 5 STAT assay and heparin plasma samples on both the **cobas e** 411 and the **cobas e** 601 analyzers. The 99th percentile upper reference limits were determined to be:

- 19 ng/L for both genders (n = 1301)
- 14 ng/L for females (n = 656)
- 22 ng/L for males (n = 645)

The joint ESC/ACCF/AHA/WHF task force for the Universal Definition of myocardial infarction and the IFCC recommend using a troponin test that can measure the 99th percentile upper reference limit with an analytical imprecision of ≤ 10 % (% CV; coefficient of variation).[4,8] The 10 % CV (total imprecision) for Elecsys Troponin T Gen 5 STAT assay was measured to be 11 ng/L.

There is an international consensus that the 99th percentile upper reference limits should be reported as whole numbers in ng/L units. In addition, the IFCC defines a high-sensitivity troponin test as one that can measure cTn above the Limit of Detection in ≥ 50 % of healthy subjects.[8]

In the reported reference cohort, a fraction of 55.1 % of healthy subjects was measured with cTnT levels above 3 ng/L, which is the Limit of Detection of the **cobas e** 601 and **cobas e** 602 analyzers. According to the IFCC definition, this corresponds to a "high sensitivity" troponin test on the **cobas e** 601 and **cobas e** 602.[8] A fraction of 35.0 % of healthy subjects was measured with cTnT levels above 5 ng/L, which is the Limit of Detection of the **cobas e** 411 analyzer.

The Universal Definition of AMI takes into consideration the ESC/ACC/AHA/WHF definition recommending the detection of a rise and/or fall of cardiac troponin in the clinical setting with at least one value above the 99th percentile upper reference limit.[34,35]

Due to the release kinetics of cardiac troponin T, an initial test result may not be definitive in diagnosing MI. Serial cardiac troponin measurements are suggested.

Factors associated with elevated values[36,37,38,39,40,41,42]

Published clinical studies have shown elevations of cardiac troponin T in patients with myocardial injury, as seen in stable or unstable angina, heart failure, myocarditis, pulmonary embolism, pericarditis, arrhythmias, cardiac contusions, and cardiac transplants. Elevations are also notable in patients with rhabdomyolysis and polymyositis.

Specific performance data

Representative performance data on the analyzers are given below. Results obtained in individual laboratories may differ.

Precision

Precision was determined using Elecsys reagents, samples (Li-heparin plasma) and controls in a protocol (EP5-A2) of the CLSI (Clinical and Laboratory Standards Institute): 2 runs per day in duplicate each for 21 days (n = 84). Human plasma samples and PreciControl Troponin were run on both the **cobas e** 411 analyzer and the **cobas e** 601 analyzer using three lots of reagents. The following representative results were obtained:

cobas e 411 analyzer					
		Repeatability		**Intermediate precision**	
Sample (Li-heparin plasma)	Mean ng/L	SD ng/L	CV %	SD ng/L	CV %
Human plasma 1	7.27	0.408	5.6	0.746	10.3
Human plasma 2	12.2	0.373	3.1	0.717	5.9
Human plasma 3	152	1.43	0.9	2.20	1.4
Human plasma 4	4673	38.2	0.8	117	2.5
Human plasma 5	9341	64.5	0.7	262	2.8
PreciControl TN[b)]1	20.0	0.452	2.3	0.740	3.7
PreciControl TN2	1739	15.5	0.9	36.3	2.1

b) TN = Troponin

cobas e 601 and **cobas e** 602 analyzer					
		Repeatability		**Intermediate precision**	
Sample (Li-heparin plasma)	Mean ng/L	SD ng/L	CV %	SD ng/L	CV %
Human plasma 1	7.42	0.224	3.0	0.473	6.4
Human plasma 2	13.5	0.252	1.9	0.558	4.1
Human plasma 3	154	1.23	0.8	2.24	1.5
Human plasma 4	4831	38.0	0.8	124	2.6
Human plasma 5	9455	62.7	0.7	256	2.7
PreciControl TN1	24.2	0.270	1.1	0.774	3.2
PreciControl TN2	1971	13.3	0.7	45.0	2.3

Analytical specificity

The Elecsys Troponin T Gen 5 STAT assay does not show any significant cross-reaction with the following substances (tested with cTnT concentrations of approximately 14 ng/L, 4000 ng/L and 7000 ng/L).

Criterion: Recovery within ± 1.4 ng/L with a concentration < 14 ng/L. Recovery within 100 ± 10 % with a concentration ≥ 14 ng/L.

Interfering substance	No interference seen up to
Skeletal muscle troponin T	10000 ng/L
Skeletal muscle troponin I	100000 ng/L
Cardiac troponin I	10000 ng/L
Human troponin C	80000 ng/L

Diagnostic sensitivity and specificity

APACE Trial: The Advantageous Predictors of Acute Coronary Syndromes Evaluation (APACE) study is an international, multicentric 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 was kidney failure that required dialysis. 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[43] including

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ECG changes, symptoms characteristic for ischemia and elevations of cardiac troponin. All 718 subjects had a baseline test result. Five hundred fifteen (515) of the 718 subjects had a second test result at the 3 hour time point and 310 (of the 718) subjects had a test result at the 6 hour time point.

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:

All patients using 19 ng/L cutoff

	Sensitivity		Specificity		PPV		NPV	
Time-point	%	95 % CI	%	95 % CI	%	95 % CI	%	95 % CI
Base line	93.5 (115/123)	87.6-97.2	86.4 (514/595)	83.4-89.0	58.7 (115/196)	51.4-65.6	98.5 (514/522)	97.0-99.3
3 hours	98.3 (59/60)	91.1-100	85.1 (387/455)	81.4-88.2	46.5 (59/127)	37.6-55.5	99.7 (387/388)	98.6-100
6 hours	100 (37/37)	90.5-100	82.4 (225/273)	77.4-86.7	43.5 (37/85)	32.8-54.7	100 (225/225)	98.4-100

The clinical performance using gender specific cutoffs 14 ng/L for females and 22 ng/L for males is provided below:

Females using 14 ng/L cutoff

	Sensitivity		Specificity		PPV		NPV	
Time-point	%	95 % CI	%	95 % CI	%	95 % CI	%	95 % CI
Base line	97.1 (34/35)	85.1-99.9	77.4 (164/212)	71.1-82.8	41.5 (34/82)	30.7-52.9	99.4 (164/165)	96.7-100
3 hours	100 (17/17)	80.5-100	75.2 (124/165)	67.8-81.5	29.3 (17/58)	18.1-42.7	100 (124/124)	97.1-100
6 hours	100 (15/15)	78.2-100	72.3 (68/94)	62.2-81.1	36.6 (15/41)	22.1-53.1	100 (68/68)	94.7-100

The positive predictive value for females using the lower sex-specific cutoff (14 ng/L) is lower when compared to the higher cutoff of 19 ng/L. When looking at the lower bound of the 95 % CI, up to 69 %, 82 % and 78 % of positive test results for females are non-MI. Troponin results should always be used in conjunction with clinical signs and symptoms. These observations underline the Universal AMI guideline requirements to use troponin results always in conjunction with at least one of the following criteria: 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.

Males using 22 ng/L cutoff

	Sensitivity		Specificity		PPV		NPV	
Time-point	%	95 % CI	%	95 % CI	%	95 % CI	%	95 % CI
Base line	90.9 (80/88)	82.9-96.0	89.3 (342/383)	85.8-92.2	66.1 (80/121)	57.0-74.5	97.7 (342/350)	95.5-99.0
3 hours	97.7 (42/43)	87.7-99.9	86.9 (252/290)	82.5-90.6	52.5 (42/80)	41.0-63.8	99.6 (252/253)	97.8-100
6 hours	100 (22/22)	84.6-100	86.0 (154/179)	80.1-90.8	46.8 (22/47)	32.1-61.9	100 (154/154)	97.6-100

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. 1679 of these subjects were evaluated on the **cobas e** 411 analyzer and 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 (i.e. 19 ng/L) for all patients:

Number of myocardial infarctions based on adjudicated diagnosis							
cobas e 411 analyzer					**cobas e** 601 analyzer		
	MI	non-MI	Total			MI	non-MI	Total
N	173	1506	1679		N	173	1502	1675
%	10.3	89.7	100		%	10.3	89.7	100

Clinical performance of single 99th percentile cutoff (19 ng/L) for aid in diagnosis of AMI in both genders						
Instrument	**Time point**	**n**	**Sens[c] % 95 % CI[d]**	**Spec[e] % 95 % CI**	**PPV[f] % 95 % CI**	**NPV[g] % 95 % CI**
cobas e 411 analyzer	Base-line	1628	86.7 (80.5-91.5)	87.8 (86.0-89.5)	44.5 (39.0-50.2)	98.3 (97.5-98.9)
	3 hours	1429	94.4 (89.2-97.5)	87.4 (85.5-89.2)	45.3 (39.5-51.1)	99.3 (98.6-99.7)
	6-9 hours	1178	94.2 (88.9-97.5)	85.3 (83.0-87.4)	45.9 (40.0-51.9)	99.1 (98.2-99.6)
	12-24 hours	887	92.9 (86.4-96.9)	81.7 (78.8-84.3)	42.3 (36.0-48.7)	98.8 (97.6-99.5)
cobas e 601 analyzer	Base-line	1600	86.0 (79.7-90.9)	88.0 (86.2-89.6)	44.9 (39.3-50.6)	98.2 (97.3-98.9)
	3 hours	1415	94.3 (89.1-97.5)	86.6 (84.6-88.4)	43.6 (37.9-49.4)	99.3 (98.6-99.7)
	6-9 hours	1158	94.9 (89.8-97.9)	85.3 (83.0-87.4)	46.6 (40.7-52.6)	99.2 (98.4-99.7)
	12-24 hours	872	91.9 (85.2-96.2)	80.6 (77.6-83.3)	40.8 (34.6-47.2)	98.6 (97.3-99.3)

c) Sensitivity = 100xA/A+C

d) CI = confidence interval

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e) Specificity = 100xD/B+D

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

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

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

Clinical performance of gender-specific 99th percentile cutoff (14 ng/L) for aid in diagnosis of AMI in females

Instrument	Time point	n	Sens % 95 % CI	Spec % 95 % CI	PPV % 95 % CI	NPV % 95 % CI
cobas e 411 analyzer	Baseline	787	87.3 (76.5-94.4)	87.6 (84.9-89.9)	37.9 (30.0-46.4)	98.8 (97.6-99.5)
	3 hours	687	92.0 (80.0-97.8)	86.2 (83.3-88.8)	34.3 (26.3-43.0)	99.3 (98.2-99.8)
	6-9 hours	553	91.7 (80.0-97.7)	85.1 (81.7-88.1)	37.0 (28.3-46.3)	99.1 (97.7-99.7)
	12-24 hours	410	92.3 (79.1-98.4)	79.8 (75.3-83.8)	32.4 (23.9-42.0)	99.0 (97.1-99.8)
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)

The positive predictive value for females using the lower sex-specific cutoff (14 ng/L) is lower when compared to the higher cutoff of 19 ng/L. When looking at the lower bound of the 95 % CI, up to 70 %, 74 %, 72 % and 76 % of positive test results for females (at baseline, 3 hours, 6-9 hours and 12-24 hours, respectively) are non-MI. Troponin results should always be used in conjunction with clinical signs and symptoms. These observations underline the Universal AMI guideline requirements to use troponin results always in conjunction with at least one of the following criteria: 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.

Clinical performance of gender-specific 99th percentile cutoff (22 ng/L) for aid in diagnosis of AMI in males

Instrument	Time point	n	Sens % 95 % CI	Spec % 95 % CI	PPV % 95 % CI	NPV % 95 % CI
cobas e 411 analyzer	Baseline	841	86.3 (78.0-92.3)	87.3 (84.7-89.6)	48.4 (40.9-55.9)	97.9 (96.5-98.8)
	3 hours	742	95.7 (89.2-98.8)	85.7 (82.8-88.3)	48.6 (41.1-56.1)	99.3 (98.2-99.8)
	6-9 hours	625	93.3 (86.1-97.5)	82.1 (78.5-85.2)	46.7 (39.2-54.2)	98.7 (97.1-99.5)
	12-24 hours	477	94.5 (86.6-98.5)	80.2 (76.0-84.0)	46.3 (38.1-54.7)	98.8 (96.9-99.7)
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)

Troponin values in other disease states

Troponins are released during the process of myocyte necrosis. While they are cardiac specific, they are not specific for MI and detectable levels may be seen in other disease states that involve the heart muscle (e.g. arrhythmia, acute aortic syndrome, acute heart failure, hypertensive crisis, myocarditis, pericarditis, pulmonary embolism and Takotsubo cardiomyopathy), so that ACC/ESC/AHA guidelines and the Universal Definition of MI recommend serial sampling with a rise or fall in troponin to distinguish between acute and chronic cTn elevations.

Results should be interpreted in conjunction with clinical presentation including medical history, signs and symptoms, ECG data and biomarker concentrations.

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