



## Overview of Next Generation Cardiac Troponin T High Sensitivity

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Roche Diagnostics


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## Disclosures

*Arleen Francis* is an employee of Roche Diagnostics and a member of the Medical & Scientific Affairs Department.

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## Course Objectives:

*high sensitive Troponin T assay*

- Distinguish between contemporary and high sensitive Troponin assay
- Identify the disciplines involved in implementing assay successfully into clinical practice
- Summarize the value of utilizing a more sensitive assay in acute care settings


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### Biomarkers: Acute Coronary Syndrome

AMI redefinition: Impact on Elecsys TnT test evolution

The timeline shows the evolution of Cardiac Troponin T and I tests. Key milestones include:

- 1988: Myocardial Infarction Redefined—A Consensus Document of The Joint European Society of Cardiology/American College of Cardiology Committee for the Redefinition of Myocardial Infarction
- 1999: Myocardial Infarction Redefined: Role of Cardiac Troponin Testing
- 2000: Universal Definition of Myocardial Infarction
- 2000: Expert Consensus Document
- 2000: Cardiac Troponins are preferred
- 2009: Cardiac Troponins are preferred..... Values presented as ng/L
- 2013: Third universal definition of myocardial infarction

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### Benefits of hs Troponin compared to contemporary

- Expedite disposition ED patient:
  - Reduce time between serial draws
  - Greater detection of MI from UA population
  - Facilitate treatment earlier
  - Reduce ED LoS 20%
  - Avoidance of unnecessary admissions
  - Safe discharge
  - Increased patient satisfaction

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### Shorter Time to MI Diagnosis by ~ 3 Hours

Hours after admission	conventional cTnT Gen 4 (%)	cTnT Gen 5 all (%)	cTnT Gen 5 early (<4h) (%)	cTnT Gen 5 late (>4h) (%)
1h	0	0	0	0
2h	0	0	0	0
3h	0	0	0	0
4h	0	0	0	0
5h	0	0	0	0
6h	0	0	0	0
24h	100	100	100	100

The cTnT Gen 5 identified >20% more patients with a final diagnosis of NSTEMI than cTnT Gen 4

Time to diagnosis of NSTEMI was made a mean of 175 min (~ 3 hours) earlier with cTnT Gen 5 than with cTnT Gen 4 (earlier presenters)

A 2<sup>nd</sup> measurement within 3-5 h after admission considerably improved cTnT Gen 5 clinical performance

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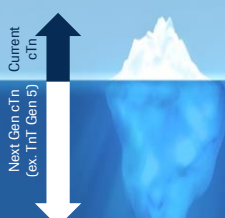
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**Higher Sensitivity of Next Gen Troponin** 

.....could reveal submerged evidence of myocardial injury



- AMI
- PE
- Myocarditis
- Myocardial injury
- Other cardiac issues

Biomarkers results always must be interpreted in relation to the clinical context and never in isolation.

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**Analytical Performance** 





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**Cardiac Troponin** 

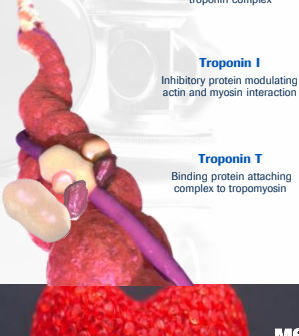
*Cardiac Muscle Contraction Regulated Troponin Complex*

- Troponin complex is made up of 3 protein subunits attached to tropomyosin on the actin filament.
- Essential for the regulation of striated muscle contraction.
  - Calcium-mediated
- Troponin T and I have different isoforms that are coded by separate genes in cardiac and in skeletal muscle.


**Troponin C**  
Calcium-binding subunit of the troponin complex

**Troponin I**  
Inhibitory protein modulating actin and myosin interaction

**Troponin T**  
Binding protein attaching complex to tropomyosin



Maron, DA. Cardiovascular Biomarkers, Pathophysiology and Disease Management. Elsevier, NJ, Harcourt, 2006:16.

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## Assay Format

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

- During a 9 minute incubation, antigen in the 50 µL sample, a biotinylated monoclonal anti-cardiac troponin T-specific antibody, a monoclonal anti-cardiac troponin T-specific antibody labeled with a ruthenium complex, and streptavidin-coated microparticles react to form a sandwich complex, which is bound to the solid phase.
- The reaction mixture is aspirated into a measuring cell where the microparticles are magnetically captured on 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.



FOA 510a Summary for (http://www.accessdata.fda.gov/drugs/foia/docs/2012/01/20120001.pdf)

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## Summary of Troponin T Gen 4 & Gen 5

Feature	cTnT Gen 4	cTnT Gen 5
<b>Claim(s)</b>	Aid in Diagnosis; Risk Stratification of: ACS; Chronic Renal Failure Selection of More Intensive Therapy or Intervention	Aid in Diagnosis
<b>Sample type</b>	Serum; K2/K3-EDTA; Citrate; Li-Hep	Li-Hep only
<b>Measuring Range</b>	0.01 – 25 ng/mL (10-25,000 ng/L)	6-10,000 ng/L
<b>Optimal Precision (10% CV)</b>	0.03 ng/mL	11 ng/L (cobas e 411) 5 ng/L (cobas e 601)
<b>99<sup>th</sup> Percentile URL</b>	0.01 ng/mL	19 ng/L (both sexes) 14 ng/L (females) 22 ng/L (males)

Troponin T (cTnT) (Serum) (HedP) 2012-01-31; Interim; All Roche Diagnostics Corporation 2012  
FOA 510a Summary for (http://www.accessdata.fda.gov/drugs/foia/docs/2012/01/20120001.pdf)

\* Values below 6 ng/L and above 10,000 ng/L are reported as < 6 ng/L and > 10,000 ng/L.

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## Development and Enhancements in cTnT Gen 5

### Improving Signal Detection

- Modified Sample Sizes**
  - 15 µL → 50 µL on the **cobas e 411** and **cobas e 601/602** analyzers
- Enhanced Antibody Design**
  - Mouse → Chimeric mouse/human antibody (interference)
  - Conjugate was changed to protect Ruthenium center
  - Non-specific antibody added to reduce background noise
- Improved Blocking Reagents**
  - HAMA blockers



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## Limitations - Interference



**Specification:**


Concentrations of <14 ng/L:  
Recovery  $\pm$  1.4 ng/L

Endogenous Substance	No Interference Up To
Bilirubin	25 mg/dL
Biotin	20 ng/mL
Lipemia (Intralipid®)	1,500 mg/dL
Rheumatoid Factor	900 IU/mL
Hemoglobin	100 mg/dL
Cholesterol	310 mg/dL

Concentrations of  $\geq$ 14 ng/L:  
Recovery within 100  $\pm$  10%

**\* Samples showing visible signs of hemolysis may cause interference. Falsely depressed results are obtained when using samples with hemoglobin concentrations  $>$  .1 g/dL.**

FOA 51003 Summary for X12205 (http://www.accessdata.fda.gov/drugs\_ftp/x12205.pdf)

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## Roche Immunoassays




**SANDWICH ASSAY**

This will lead to falsely decreased results in sandwich assays


Excessive free Biotin in the sample will compete with the biotinylated, pre-formed immunocomplex for binding sites on the streptavidin coated microparticles

The Streptavidin coated microparticles have a very high binding capacity for Biotin and therefore provide some buffer capacity for free Biotin in the sample. Interference will only become visible with high Biotin concentrations in the sample which are above the published threshold for each specific assay.



Streptavidin      Antigen      Ruthenium

← Detection

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## IFCC Definition of High Sensitivity



- International consensus states 99th percentile upper reference limits should be reported as whole numbers in ng/L units.
- IFCC recommends a troponin test that can measure the 99th percentile URL with an analytical imprecision  $\leq$  10 %.
- Gen 5 assay 99th percentile URLs are:
  - 19 ng/L for both sexes (n = 1301), Total CV 6%, 3% (e411, e601/2)
  - 14 ng/L for females (n = 656), Total CV 8%, 4% (e411, e601/2)
  - 22 ng/L for males (n = 645), Total CV 5%, 3% (e411, e601/2)
- The IFCC defines a hs troponin test as one that measures cTn above the Limit of Detection in  $\geq$  50% of healthy subjects.
- In the cTnT Gen 5 assay's reported reference cohort:
  - 35.0% of healthy subjects measured with cTnT levels above 5 ng/L (e411 LoD)
  - 55.1% of healthy subjects measured with cTnT levels above 3 ng/L (e601/602 LoD)
- TnT Gen 5 meets the IFCC definition on the cobas e 601/602

Clinical Biochemistry (2015) 48 (4-5), 201-203  
http://www.ncbi.nlm.nih.gov/pubmed/25615015

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## Clinical Performance



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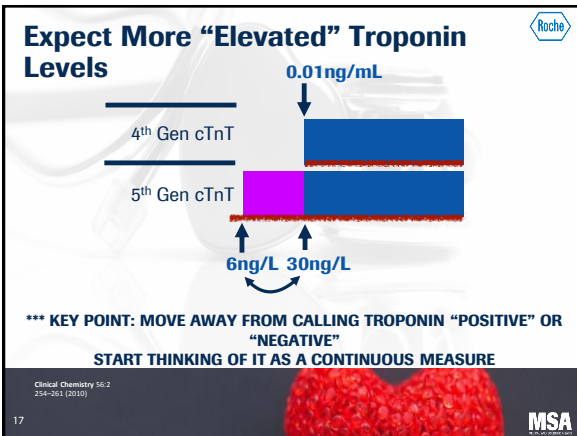
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## Expect More "Elevated" Troponin Levels



0.01ng/mL

4<sup>th</sup> Gen cTnT

5<sup>th</sup> Gen cTnT

6ng/L 30ng/L

\*\*\* KEY POINT: MOVE AWAY FROM CALLING TROPONIN "POSITIVE" OR "NEGATIVE"  
START THINKING OF IT AS A CONTINUOUS MEASURE

Clinical Chemistry 56:2  
254-261 (2010)

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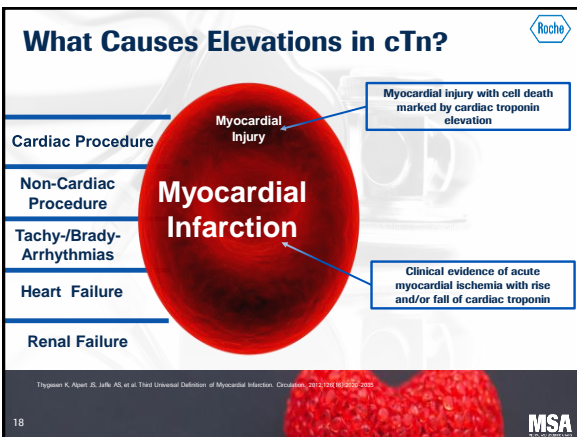
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## What Causes Elevations in cTn?



Myocardial Injury

Myocardial injury with cell death marked by cardiac troponin elevation

Myocardial Infarction

Clinical evidence of acute myocardial ischemia with rise and/or fall of cardiac troponin

- Cardiac Procedure
- Non-Cardiac Procedure
- Tachy-/Brady-Arrhythmias
- Heart Failure
- Renal Failure

Thygesen K, Alpert JS, Jaffe AS, et al. Third Universal Definition of Myocardial Infarction. Circulation. 2012;126:2020-2028

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## Third Universal Definition of Myocardial Infarction

*An Expert Consensus Document*



- **Evidence of myocardial necrosis in a clinical setting consistent with acute myocardial ischemia**
  - Rise and/or fall of cardiac troponin with at least one value > 99<sup>th</sup> percentile URL
  - WITH
  - Symptoms of ischemia
  - New or presumed new ECG changes
  - New Q waves, imaging evidence myocardial loss, or intracoronary thrombus on angiography or autopsy

ACD/UA 2012 Third Universal Definition of Myocardial Infarction. JACC. 2012; Vol.6, No.16  
Third universal definition of myocardial infarction. Circulation.2012;226:25-35.



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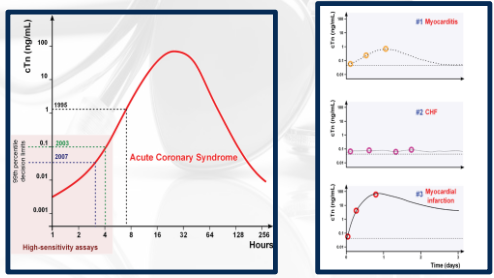
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## Defining Discriminating Results

*Between Acute and Nonacute Causes for Troponin Elevations*





Muller et al. Circ. 2011



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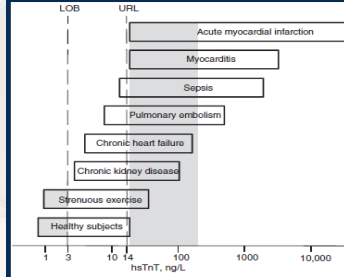
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## TnT Gen 5 Concentrations for Cardiac & Non-Cardiac Conditions





Jardin. Clin Chem Lab Med 2015; 53(3): 625-632



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### APACE Study Performance

Sex-specific 99<sup>th</sup> percentile cutoffs for aiding in the diagnosis of AMI


**Females using 14 ng/L cutoff**

Time	Sensitivity % (95% CI)	Specificity % (95% CI)	PPV % (95% CI)	NPV % (95% CI)
Base-line	97.1% (85.1-99.9)	77.4% (71.1-82.8)	41.5% (30.7-52.9)	99.4% (96.7-100)
3 hours	100% (80.5-100)	75.2% (67.8-81.5)	29.3% (18.1-42.7)	100% (97.1-100)
6 hours	100% (78.2-100)	72.3% (62.2-81.1)	36.6% (22.1-53.1)	100% (94.7-100)

**Males using 22 ng/L cutoff**

Time	Sensitivity % (95% CI)	Specificity % (95% CI)	PPV % (95% CI)	NPV % (95% CI)
Base-line	90.9% (80/88)	89.3% (342/383)	66.1% (80/121)	97.7% (342/350)
3 hours	97.7% (87.7-99.9)	86.9% (82.5-90.6)	52.5% (42/80)	99.6% (252/253)
6 hours	100% (84.6-100)	86.0% (80.1-90.6)	46.8% (32.1-61.9)	100% (154/154)

FOA 510(k) Summary for K12202 [http://www.accessdata.fda.gov/cdrh\_cdrh/pdfs/K12202.pdf]

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### Roche Clinical Study Performance

Sex-specific 99<sup>th</sup> percentile cutoff for aiding in the diagnosis of AMI


**Females using 14 ng/L cutoff on cobas e 601 analyzer**

Time	n	Sensitivity % (95% CI)	Specificity % (95% CI)	PPV % (95% CI)	NPV % (95% CI)
Base-line	771	85.7 (74.6-93.3)	88.1 (85.5-90.4)	39.1 (30.9-47.6)	98.6 (97.3-99.3)
3 hours	682	91.8 (80.4-97.7)	86.9 (84.0-89.4)	35.2 (26.3-44.1)	99.3 (98.2-99.8)
6-9 hours	536	91.3 (79.2-97.6)	85.5 (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 (26.5-45.0)	99.0 (97.1-99.8)

**Males using 22 ng/L cutoff on cobas e 601 analyzer**

Time	n	Sensitivity % (95% CI)	Specificity % (95% CI)	PPV % (95% CI)	NPV % (95% CI)
Base-line	829	85.1 (76.7-91.4)	87.2 (84.6-89.6)	48.0 (40.5-56.0)	97.7 (96.2-99.3)
3 hours	733	95.6 (89.1-98.8)	86.3 (83.4-88.9)	42.1 (42.1-57.4)	98.2 (98.2-98.6)
6-9 hours	622	92.5 (86.3-97.6)	82.3 (76.7-85.4)	47.8 (40.3-55.3)	98.6 (97.1-99.3)
12-24 hours	473	94.4 (86.4-98.5)	80.0 (75.8-83.9)	45.9 (37.7-54.3)	98.8 (98.8-99.7)

FOA 510(k) Summary for K12202 [http://www.accessdata.fda.gov/cdrh\_cdrh/pdfs/K12202.pdf]

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### Implement hsTnT into Clinical Practice

**Pre-Implementation**

- Multidisciplinary team
- Clinical guidance
- Modify work flow
- Educate clinical staff


**Implementation**

- Oversight by clinical & laboratory champions
- Ongoing education
- Provide decision support

**Post-Implementation**

- Ongoing verification of assay performance
- Monitoring key clinical outcomes
- TAT, 30 MACE, LOS

Kohley et al. JACC 2016

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**Medical and Scientific Liaisons (MSL)** 

 <b>SCIENTIFIC EXCHANGE</b>	 <b>CLINICAL EDUCATION</b>	 <b>KOL MANAGEMENT &amp; NETWORKING</b>	 <b>CARDIAC ASSAY CONVERSION</b>	 <b>RESEARCH</b>
Evidence-Based Medicine Trials & Studies Off-Label Inquiries	Practice Guidelines Multidisciplinary Grand Rounds Presentations Ongoing PACE	Facilitate Relationships Global, National & Regional Conferences Collaboration	FDA Claim Discussions Clinical Correlations Case Reviews Staff Training	Investigator Initiated Studies (IIS) Roche Sponsored Studies (RSS)

Clinical Interactions: Cardiology, Emergency Medicine, Pathology, Nurse Practitioners, Nursing and other Healthcare Professionals

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*Doing now what patients need next*

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