Troponin: Testing Today

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Disclosures

Joshua Soldo is an employee of Roche Diagnostics within the division of Medical Scientific Affairs.

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Medical and Scientific Liaisons (MSL)

Cardiac subject matter experts within the division of Medical and Scientific Affairs

- We deliver medical value through fair and balanced scientific exchange regarding the latest evidenced-based medicine on cardiovascular disease states, cardiac biomarkers and associated practice guidelines
- We identify and interact with regional, national and international Key Opinion Leaders (KOL), gaining the knowledge and innovation that influences clinical practice

Clinical Interactions: Cardiology, Emergency Medicine, Pathology, Nurse Practitioners, Nursing and other Healthcare Professionals
Analytical Considerations
Troponin Structure
Cardiac Muscle Contraction Regulated by 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

Cardiac Troponin Subunits in Circulation

Circulatory Release Patterns of cTn After Myocyte Necrosis

Cardiac Troponin T:
- Free TnT
- Tertiary TnT:C:I
- Fragments

Cardiac Troponin I:
- Free TnI
- Binary TnI:C (Predominant)
- Tertiary TnI:C:T
- Reduced/Oxidized
- Phosphorylated
- Fragments

Free TnT (37 kDa)
Free TnI (22 kDa)
Free TnC (18 kDa)

Epitope Site Selection/Antibody Detection\textsuperscript{1-4}


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Cardiac Troponin T

- M7: 125 - 131
- M11-7: 136 - 147

N-terminal Stable Area

Affinity for Proteolysis

Single Manufacturer

Cardiac Troponin I

- 211

Epitope Binding Sites

Central Stable Area

30 - 110

Affinity for Proteolysis

Multiple Manufacturers
# Analytic Characteristics of Contemporary Cardiac Troponin Assays

## Cardiac Troponin Concentration at:

<table>
<thead>
<tr>
<th>Company/platform/assay</th>
<th>LoD, μg/L</th>
<th>99th Percentile, μg/L (CV)</th>
<th>10% CV concentration μg/L</th>
<th>Amino acid residues of epitopes recognized by capture (C) and detection (D) MAbs</th>
</tr>
</thead>
<tbody>
<tr>
<td>Abbott ARCHITECT</td>
<td>0.009</td>
<td>0.028 (14%)</td>
<td>0.032</td>
<td>C: 87–91, 24–40; D: 41–49</td>
</tr>
<tr>
<td>Abbott i-STAT</td>
<td>0.02</td>
<td>0.08 (16.5%)</td>
<td>0.10</td>
<td>C: 41–49, 88–91; D: 28–39, 62–78</td>
</tr>
<tr>
<td>Beckman Access 2 AccuTnl+3</td>
<td>0.01</td>
<td>0.02 (20%)</td>
<td>0.04</td>
<td>C: 41–49; D: 24–40</td>
</tr>
<tr>
<td>Beckman Dxl AccuTnl+3</td>
<td>0.01</td>
<td>0.03 (20%)</td>
<td>0.04</td>
<td>C: 41–49; D: 24–40</td>
</tr>
<tr>
<td>Ortho Vitros Eci ES</td>
<td>0.012</td>
<td>0.034 (10%)</td>
<td>0.16</td>
<td>C: 24–40, 41–49; D: 87–91</td>
</tr>
<tr>
<td>Siemens Centaur Ultra</td>
<td>0.006</td>
<td>0.04 (8.8%)</td>
<td>0.03</td>
<td>C: 41–49, 87–91; D: 27–40</td>
</tr>
<tr>
<td>Siemens Dimension RxL</td>
<td>0.04</td>
<td>0.07 (20%)</td>
<td>0.14</td>
<td>C: 27–32; D: 41–56</td>
</tr>
<tr>
<td>Siemens Immulite 2500</td>
<td>0.1</td>
<td>0.2 (NA)</td>
<td>0.42</td>
<td>C: 87–91; D: 27–40</td>
</tr>
<tr>
<td>Siemens Stratus CS</td>
<td>0.03</td>
<td>0.07 (10%)</td>
<td>0.06</td>
<td>C: 27–32; D: 41–56</td>
</tr>
<tr>
<td>Siemens Vista</td>
<td>0.015</td>
<td>0.045 (10%)</td>
<td>0.04</td>
<td>C: 27–32; D: 41–56</td>
</tr>
</tbody>
</table>

*LoD, limit of detection; NA, not available; Gen 4, fourth-generation assay.

*CV at 99th percentile.
Clinical Benefits of Troponin Testing in ACS Patients
Third Universal Definition of Myocardial Infarction

Detection of a rise and/or fall of cardiac biomarker values with at least one value above the 99th percentile upper reference limit (URL) and with at least one of the following:

- Symptoms of ischemia
- New or presumed new significant ECG changes
  - ST-segment–T wave changes
  - New left bundle branch block (LBBB)
  - Development of pathological Q waves
- Imaging
  - Evidence of new loss of viable myocardium or new regional wall motion abnormality
  - Identification of an intracoronary thrombus by angiography or autopsy
- Arbitrary values for:
  - Percutaneous Coronary Intervention (PCI) related MI
  - Stent thrombosis associated with MI
  - Coronary artery bypass grafting (CABG) related MI.¹

Third Universal Definition of Myocardial Infarction

- The guidelines for the Universal Definition of Myocardial Infarction call out both Troponin T and Troponin I\(^1\)
- The preferred biomarker is cardiac troponin (T or I), which has high myocardial tissue specificity as well as high clinical sensitivity\(^2\)
  - Detection of a rise and/or fall of cardiac biomarkers is essential to the diagnosis of acute MI
- Evidence-based data supports the use of assays with a CV of ≤20% at the 99th percentile\(^3\)

**Universal Classification of Myocardial Infarction**

**Type 1:** Ischemic myocardial necrosis secondary to plaque rupture (ACS)

**Type 2:** Ischemic myocardial necrosis not due to ACS (e.g., supply/demand mismatch, coronary spasm, embolism, ↑ or ↓BP, anemia, arrhythmia)

**Type 3:** Sudden cardiac death

**Type 4:** Procedure related
   - Secondary to PCI
   - From stent thrombosis

**Type 5:** CABG related

Differentiation of MI Types 1 and 2 Based on Coronary Artery Condition

- **MI Type 1:** Plaque Rupture With Thrombus
- **MI Type 2:** Vasospasm or Endothelial Dysfunction
- **MI Type 2:** Fixed Atherosclerosis and Supply-Demand Imbalance
- **MI Type 2:** Supply-Demand Imbalance Alone

Elevations of Cardiac Troponin Values Due to Myocardial Injury

Myocardial Injury with cell death marked by cardiac troponin elevation

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

Practical Considerations – Interpretation of Troponin Elevations
*Kinetic Pattern of Cardiac Markers: Rise and Fall Patterns*

Practical Considerations – Interpretation of Troponin Elevations

Kinetic Pattern of Cardiac Markers: Rise and Fall Patterns

- Myocardial Infarction
- CHF

2014 ACCF/AHA NSTE-ACS Guidelines
Serial Measurements

Class I:

- Measure cardiac-specific troponin (troponin I or T) at presentation and 3–6 hours after symptom onset in all patients with suspected ACS to identify pattern of values (Level of Evidence: A)

- Obtain additional troponin levels beyond 6 hours in patients with initial normal serial troponins with electrocardiographic changes and/or intermediate/high risk clinical features (Level of Evidence: A)

- Consider time of presentation the time of onset with ambiguous symptom onset for assessing troponin values (Level of Evidence: A)

Serial sampling allows for biological variation, improved identification of an acute event and differentiation of acute versus chronic troponin elevation. There is evidence to support defining criteria for a significant change or delta.
Cardiac Biomarker Kinetics
Comparing Troponin with CK-MB

According to the 2014 AHA/ACC Guideline for the Management of Patients With Non-ST-Elevation Acute Coronary Syndromes:

• “When using a contemporary troponin assay, creatine kinase myocardial isoenzyme (CK-MB) and myoglobin are not useful for diagnosis of ACS.”

• This is now a “Class III: No Benefit” (Level of Evidence: A)
Clinical Considerations
Serial Troponin Values Grouped by Final Diagnosis

Verification of FRISC II Findings in Gusto IV

Sensitivity of Troponin T for Detections of Death and MI at 30 Days in FRISC II and GUSTO IV

Risk Stratification in ACS Patients
30-Day Risk of Death or MI in the GUSTO IV Trial

Data presented was generated in subset of GUSTO IV patient population (7,033 patients). These findings confirmed the results from the FRISC II patient population (1,074 patients).

Proposed Algorithm for Troponin in Therapeutic Decision Making

**Elevated troponin**

- **Pretest probability of ACS** (risk factors, symptoms, ECG)
  - **Low**
    - ACS unlikely
  - **High**
    - Global risk
      - **Low**
        - Early conservative or invasive strategy
      - **High**
        - Early invasive strategy

- **Pretest probability of ACS** (risk factors, symptoms, ECG)
  - **Low**
    - ACS unlikely
  - **High**
    - Global risk
      - **Low**
        - Early conservative or invasive strategy
      - **High**
        - Early invasive strategy

Proposed Algorithm for Troponin in Therapeutic Decision Making

“Global risk assessment rather than any single risk marker should be the main driver of therapeutic decision making”

- Elevated troponin
  - ACS unlikely
    - Low: Early conservative or invasive strategy
    - High: Early invasive strategy
  - Global risk
    - Low: Early conservative or invasive strategy
    - High: Early invasive strategy

Yes

ACS unlikely

Global risk

ACS unlikely

Search for other causes

Global risk

Low

High

Low

High

Early conservative or invasive strategy

Early invasive strategy

Early conservative or invasive strategy

Early invasive strategy

Clinical Implications of Elevated TnT in Patients With ACS
*Tactics TIMI18 Clinical Trial*

At 30 Days

**TnT > 0.01 ng/mL**
- Primary Endpoint: 7.9 vs 16.2 (Odds Ratio: 0.44 [95% CI: 0.30-0.66])
- Death/Non-fatal MI: 5.3 vs 10.6 (Odds Ratio: 0.47 [95% CI: 0.29-0.77])

**TnT ≤ 0.01 ng/mL**
- Primary Endpoint: 6.0 vs 5.6 (Odds Ratio: 1.08 [95% CI: 0.60-1.92])
- Death/Non-fatal MI: 2.9 vs 3.1 (Odds Ratio: 0.95 [95% CI: 0.43-2.10])

Primary endpoint includes death, non-fatal MI, and rehospitalization

Clinical Implications of Elevated TnT in Patients With ACS

*Tactics TIMI18 Clinical Trial*

**At 30 Days**

“Patients with TnT levels >0.01ng/mL benefit from invasive therapy, as shown with a 50% reduction in death and MI at 30 days”

Primary endpoint includes death, non-fatal MI, and rehospitalization

Chronic Kidney Disease in ACS

- High prevalence of CKD with ACS
- Almost 40% of NSTEMI patients, and 30% of STEMI patients have CKD as defined by eGFR
- CKD is a potent and independent risk factor for adverse outcomes in ACS patients
- Among ACS patients, CKD doubles mortality rates

What About cTnT in Symptomatic Patients?

Adjusted Odds Ratio for Death or Myocardial Infarction among Patients With Abnormal Troponin T (≥0.1 ug/L) Levels in Relation to Creatinine Clearance Rates

Non-ischemic Troponin Elevations

Kidney Disease

- TnI-Ultra did not differ significantly between CKD stages \( (P = 0.6) \)
- TnI (Siemens assay study) levels increase in approximately 1/3 of predialysis patients

**CKD 3** (GFR <60-30)
**CKD 4** (GFR 29-15)
**CKD 5** (GFR <15 or “ON DIALYSIS”)
Non-ischemic Troponin Elevations

Kidney Disease

• TnI-Ultra did not differ significantly between CKD stages \((P = 0.6)\)
• TnI (Siemens assay study)

“Cardiac Troponin I Concentration is Commonly Increased in Nondialysis Patients with CKD: Experience with a Sensitive Assay”

CKD 3 (GFR <60-30)
CKD 4 (GFR 29-15)
CKD 5 (GFR <15 or “ON DIALYSIS”)
In regards to MI, the preferred biomarker is cardiac troponin (T or I).

Serial troponin sampling allows for differentiation of acute versus chronic troponin elevation – “look for a rise and/or a fall”

The multiplicity of TnI assays makes standardization of TnI results problematic – “many different analytical sensitivities”

Troponin I tests results are not always interchangeable because of the differences in antibody reactivity and heterogeneity of TnI.

The 4th generation Troponin T assay is the ONLY troponin test cleared by the FDA for stratification of cardiac risk in patients with chronic renal failure.
Next Generation Troponin Assays

• Higher sensitive cTn assays that meet the definition of AMI can improve earlier detection and potentially accelerate the evaluation of patients with chest pain

• Differences in the sensitivity and specificity of high sensitive assays for the diagnosis of AMI may in large part be due to the choice of the population to determine the 99th percentile

• Improved sensitivity of cTn assays may result in detection of a variety of non ACS cardiac etiologies and will require more rigorous interpretation in possible ACS settings

• Patients with renal disease will frequently have elevated values, but with adjustment of the AMI cut-off will have an accuracy that approaches or is similar to that in patients without renal disease
Elecsys Troponin T Gen 5 STAT Assay Overview

• Test Principle

• Assay Enhancements

• Analytical Performance
  - 3rd Universal Definition of MI
  - IFCC Definition of high sensitivity

• Clinical Performance
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 electrochemiluminescence immunoassay “ECLIA” is intended for use on the cobas e 411 and cobas e 601 analyzers.

- For cobas e 411 analyzer, cobas e 601 analyzer and cobas e 602 analyzer.
Assay Format

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, and streptavidin-coated microparticles react to form a sandwich complex, which is bound to the solid phase.

• 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.

FDA 510(k) Summary for K162895 (http://www.accessdata.fda.gov/cdrh_docs/pdf16/K162895.pdf)
The cTnT Gen 5 assay recognizes the **exact same troponin T epitopes** as the Gen 4 assay.

**Cardiac Troponin T**

- **M7**: 125 - 131
- **M11-7**: 136 - 147

**Affinity for Proteolysis**

cTnT Gen 5 Assay Enhancements

• Sample Size
  - 15 µl ➔ 50 µl

• Antibody Design
  - Mouse ➔ Chimeric mouse/human antibody
  - Conjugate was changed to protect Ruthenium center
  - Non-specific antibody added to reduce background noise

• Blocking Reagents
  - HAMA blockers
Sample size increased from 15 μL (Gen 4) to **50 μL** (Gen 5) to improve assay sensitivity.
The chimeric F(ab’)2 labeled with a ruthenium complex is now a **polymeric antibody** to amplify signal and improve assay sensitivity.
The capture antibody is now a strictly mono-biotinylated $F(ab)$ to increase the signal to noise ratio by lowering of background signal.
The detection antibody is a **chimeric F(ab’)2** genetically re-engineered to further minimize endogenous interferences such as HAMA.

** troponin T **

** mouse antibody (F(ab’)2) **

** chimeric mouse/human antibody **

** FDA 510(k) Summary for K162895 (http://www.accessdata.fda.gov/cdrh_docs/pdf16/K162895.pdf) **
Blocking Reagents

The cTnT Gen 5 assay uses the same blocking reagents and antibody design as Gen 4 to block heterophilic antibodies and unspecific interference.

- Use of Fab and F(ab’)2 for minimizing HAMA and RF interference

cTnT Gen 5 adds **new blocking reagents** and uses **new antibody design** to minimize additional interference mechanisms.

- Use of chimeric signal antibody for minimizing HAMA interference
- Mono-biotinylation of capture antibody to reduce matrix effects
False elevations caused by:

- Heterophile Antibodies*
- Human Anti-Mouse Antibodies*
- Rheumatoid Factor (RF)*
- Fibrin Interference

Roche solution:

- Effects minimized by suitable test design

*No significant interference with TnT²

Autoantibodies Are Detectable With Greater Prevalence During an Acute Cardiac Event

- Levels of autoantibodies against troponin I increase in acute cardiac events\(^1\)
- Results between TnI assays are not comparable because of the antibody reactivity differences and the heterogeneity of TnI\(^2\)
- One study showed more TnI autoantibodies than TnT autoantibodies in two groups of cardiac patients\(^3\)

Interpretation of Results:

- 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. Li-heparin plasma was used and measurements were performed 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 sexes (n = 1301)
  - 14 ng/L for females (n = 656)
  - 22 ng/L for males (n = 645)
**Precision**

- Intermediate precision was measured using the CLSI EP5-A2 protocol (2 runs per day in duplication each for 21 days (N=84)).

- 10 human plasma pools with troponin concentrations at the low end of the measuring range were measured using 2 lots of reagent.

<table>
<thead>
<tr>
<th>Sample (Li-heparin plasma)</th>
<th>Repeatability</th>
<th>Intermediate Precision</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Mean ng/L</td>
<td>SD ng/L</td>
</tr>
<tr>
<td>Human plasma 1</td>
<td>7.42</td>
<td>0.224</td>
</tr>
<tr>
<td>Human plasma 2</td>
<td>13.5</td>
<td>0.252</td>
</tr>
<tr>
<td>Human plasma 3</td>
<td>154</td>
<td>1.23</td>
</tr>
<tr>
<td>Human plasma 4</td>
<td>4,831</td>
<td>38.0</td>
</tr>
<tr>
<td>Human plasma 5</td>
<td>9,455</td>
<td>62.7</td>
</tr>
<tr>
<td>PreciControl TN1</td>
<td>24.2</td>
<td>0.270</td>
</tr>
<tr>
<td>PreciControl TN2</td>
<td>1,971</td>
<td>13.3</td>
</tr>
</tbody>
</table>

FDA 510(k) Summary for K162895 (http://www.accessdata.fda.gov/cdrh_docs/pdf16/K162895.pdf)
**Optimal Precision (10% CV)**

- The value where a CV of ± 10% can be achieved was determined using the CLSI EP5-A2 20-day protocol to estimate intermediate precision.
- Ten Li-Heparin plasma pools were prepared across the low-end of the measuring range of the assay.
- Data were collected with two lots over 21 days, two runs per day with two replicates per run. Estimates of mean and intermediate precision were calculated for each sample.

<table>
<thead>
<tr>
<th>Analyzer</th>
<th>Lot</th>
<th>Æ</th>
<th>B</th>
<th>Troponin T [ng/L]</th>
<th>Intermediate precision CV [%]</th>
</tr>
</thead>
<tbody>
<tr>
<td>cobas e 411</td>
<td>170511</td>
<td>4.861</td>
<td>-1.091</td>
<td>10.4</td>
<td>10</td>
</tr>
<tr>
<td>cobas e 411</td>
<td>173678</td>
<td>4.407</td>
<td>-1.106</td>
<td>6.70</td>
<td>10</td>
</tr>
<tr>
<td>cobas e 601</td>
<td>170511</td>
<td>3.944</td>
<td>-1.052</td>
<td>4.76</td>
<td>10</td>
</tr>
<tr>
<td>cobas e 601</td>
<td>173678</td>
<td>3.780</td>
<td>-1.097</td>
<td>3.85</td>
<td>10</td>
</tr>
</tbody>
</table>

- The 10% CV for all analyzers is 11 ng/L, and 5 ng/L for the cobas e 601.
The Limit of Blank (LoB), Limit of Detection (LoD) and Limit of Quantitation (LoQ) were determined in accordance with CLSI EP17-A2 requirements.

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

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

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

<table>
<thead>
<tr>
<th></th>
<th>cobas e 411</th>
<th>cobas e 601</th>
</tr>
</thead>
<tbody>
<tr>
<td>Limit of Blank</td>
<td>3 ng/L</td>
<td>2.5 ng/L</td>
</tr>
<tr>
<td>Limit of Detection</td>
<td>5 ng/L</td>
<td>3 ng/L</td>
</tr>
<tr>
<td>Limit of Quantitation</td>
<td>6 ng/L (20 % CV)</td>
<td></td>
</tr>
</tbody>
</table>

- The LoQ was determined to be 5.5 ng/L on the cobas e 411 and 2.5 ng/L on the cobas e 601 analyzer. The LoQ for the assay will be labeled as 6 ng/L.

FDA 510(k) Summary for K162895 (http://www.accessdata.fda.gov/cdrh_docs/pdf16/K162895.pdf)
# Troponin T Generation 4 vs Generation 5

<table>
<thead>
<tr>
<th>Feature</th>
<th>Gen 4</th>
<th>Gen 5</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Claim(s)</strong></td>
<td>Aid in Diagnosis Risk Stratification of: ACS; Chronic renal failure</td>
<td>Aid in Diagnosis</td>
</tr>
<tr>
<td><strong>Sample type</strong></td>
<td>Serum; K2/K3-EDTA; Citrate; Li-Hep</td>
<td>Li-Hep only</td>
</tr>
<tr>
<td><strong>Measuring Range</strong></td>
<td>0.01 – 25 ng/mL (10-25,000 ng/L)</td>
<td>6-10,000 ng/L</td>
</tr>
<tr>
<td><strong>Optimal Precision (10% CV)</strong></td>
<td>0.03 ng/mL</td>
<td>11 ng/L</td>
</tr>
<tr>
<td><strong>99th Percentile URL</strong></td>
<td>0.01 ng/mL</td>
<td>19 ng/L (both sexes)</td>
</tr>
<tr>
<td></td>
<td></td>
<td>14 ng/L (females)</td>
</tr>
<tr>
<td></td>
<td></td>
<td>22 ng/L (males)</td>
</tr>
</tbody>
</table>

FDA 510(k) Summary for K162895 (http://www.accessdata.fda.gov/cdrh_docs/pdf16/K162895.pdf)
### Endogenous Interferences

<table>
<thead>
<tr>
<th>Interfering Substance</th>
<th>No interference up to</th>
</tr>
</thead>
<tbody>
<tr>
<td>Bilirubin</td>
<td>25 mg/dL</td>
</tr>
<tr>
<td>Biotin</td>
<td>20 ng/mL</td>
</tr>
<tr>
<td>Lipemia (Intralipid®)</td>
<td>1,500 mg/dL</td>
</tr>
<tr>
<td>Rheumatoid Factor</td>
<td>900 IU/mL</td>
</tr>
<tr>
<td>Hemoglobin</td>
<td>100 mg/dL</td>
</tr>
</tbody>
</table>

**Specification:**

Concentrations of < 14 ng/L: Recovery ±1.4 ng/L

Concentrations of ≥14 ng/L: Recovery within 100 ±10%
Analytical Specificity

• Analytical Specificity/Cross-Reactivity

• The analytical specificity of the TnT Gen 5 STAT assay was assessed on the cobas e 411 and cobas e 601 analyzer using three concentrations (approximately 14.0 ng/L, 4000 ng/L and 7000 ng/L) of plasma samples spiked with potential cross-reacting compounds skeletal muscle TnT and TnI, cardiac TnI and human TnC.

• Specification:

  ▪ Concentrations of < 14 ng/L: absolute deviation ± 2.8 ng/L
  ▪ Concentrations of 14 - ≤ 100 ng/L: Recovery 100 ± 20%
  ▪ Concentrations of > 100 ng/L: Recovery 100 ± 10%

<table>
<thead>
<tr>
<th>Interfering Substance</th>
<th>No Interference Seen Up To</th>
</tr>
</thead>
<tbody>
<tr>
<td>Skeletal muscle TnT</td>
<td>50,000 ng/L</td>
</tr>
<tr>
<td>Skeletal muscle TnI</td>
<td>100,000 ng/L</td>
</tr>
<tr>
<td>Cardiac TnI</td>
<td>20,000 ng/L</td>
</tr>
<tr>
<td>Human TnC</td>
<td>100,000 ng/L</td>
</tr>
</tbody>
</table>

FDA 510(k) Summary for K162895 (http://www.accessdata.fda.gov/cdrh_docs/pdf16/K162895.pdf)
Cardiac Tn (T and I) are the preferred markers for the diagnosis of myocardial infarction (MI).

In a clinical setting consistent with myocardial ischemia, detection of a rise and/or fall of cTn with at least one value above the 99th percentile URL is the cornerstone of MI diagnosis.

The joint ESC/ACCF/AHA/WHF task force for the Universal Definition of myocardial infarction recommend using a troponin test that can measure the 99th percentile upper reference limit with an analytical imprecision ≤ 10 % (% CV; coefficient of variation).

- The 10% CV (total imprecision) for the Elecsys Troponin T Gen 5 STAT assay was measured to be 11 ng/L (5 ng/L on the cobas e 601 analyzer).
- The cTnT Gen 5 assay 99th percentile URLs are 14 ng/L (females), 19 ng/L (both sexes) and 22 ng/L (males).

cTnT Gen 5 is **Guideline Acceptable.**
IFCC Definition of High Sensitivity

- There is an international consensus that the 99th percentile upper reference limits should be reported as whole numbers in ng/L units.

- The IFCC recommends using a troponin test that can measure the 99th percentile upper reference limit with an analytical imprecision ≤ 10%.
  - The 10% CV (total imprecision) for the Elecsys Troponin T Gen 5 STAT assay was measured to be 11 ng/L (5 ng/L on the cobas e 601 analyzer).
  - The cTnT Gen 5 assay 99th percentile URLs are 14 ng/L (females), 19 ng/L (both sexes) and 22 ng/L (males).

- The IFCC defines a high-sensitivity troponin test as one that can measure cTn above the Limit of Detection in ≥ 50% of healthy subjects.
  - 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 analyzer.

- cTnT Gen 5 meets the IFCC definition on the cobas e 601 analyzer.
Troponin T Gen 5
Only FDA cleared assay that is compliant to IFCC guidelines

<table>
<thead>
<tr>
<th>Assay</th>
<th>99th Percentile</th>
<th>10% CV</th>
<th>Optimal Precision?</th>
<th>IFCC “hs”?</th>
</tr>
</thead>
<tbody>
<tr>
<td>Roche TnT Gen 5 STAT</td>
<td>19 ng/L</td>
<td>11 ng/L</td>
<td>YES</td>
<td>YES</td>
</tr>
<tr>
<td>Abbott Architect TnI</td>
<td>0.028 ng/mL</td>
<td>0.032 ng/mL</td>
<td>NO</td>
<td>NO</td>
</tr>
<tr>
<td>Abbott AxSYM ADV</td>
<td>0.040 ng/mL</td>
<td>0.16 ng/mL</td>
<td>NO</td>
<td>NO</td>
</tr>
<tr>
<td>Beckman Access Accu TnI</td>
<td>0.040 ng/mL</td>
<td>0.040 ng/mL</td>
<td>YES</td>
<td>NO</td>
</tr>
<tr>
<td>Beckman Dxl</td>
<td>0.030 ng/mL</td>
<td>0.040 ng/mL</td>
<td>NO</td>
<td>NO</td>
</tr>
<tr>
<td>Ortho VITROS TnI ES</td>
<td>0.034 ng/mL</td>
<td>0.034 ng/mL</td>
<td>YES</td>
<td>NO</td>
</tr>
<tr>
<td>Siemens ADVIA Centaur ULTRA-TnI</td>
<td>0.040 ng/mL</td>
<td>0.030 ng/mL</td>
<td>YES</td>
<td>NO</td>
</tr>
<tr>
<td>Siemens Dimension VISTA TnI</td>
<td>0.045 ng/mL</td>
<td>0.040 ng/mL</td>
<td>YES</td>
<td>NO</td>
</tr>
</tbody>
</table>

http://www.ifcc.org/media/276661/IFCC%20Troponin%20Tables%20ng_L%20DRAFT%20Update%20NOVEMBER%202014.pdf
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 NCT004705876.6).

The subjects were diagnosed with acute MI by using the diagnostic criteria described in the ACC/ESC/AHA guidelines including ECG changes, symptoms characteristic for ischemia and elevations of cardiac troponin.

### All patients using 19 ng/L cutoff

<table>
<thead>
<tr>
<th>Time-point</th>
<th>n</th>
<th>Sensitivity</th>
<th>Specificity</th>
<th>PPV</th>
<th>NPV</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>%</td>
<td>%</td>
<td>%</td>
<td>%</td>
</tr>
<tr>
<td></td>
<td></td>
<td>95 % CI</td>
<td>95 % CI</td>
<td>95 % CI</td>
<td>95 % CI</td>
</tr>
<tr>
<td>Baseline</td>
<td>718</td>
<td>93.5 (115/123)</td>
<td>87.6-97.2</td>
<td>86.4 (514/595)</td>
<td>83.4-89.0</td>
</tr>
<tr>
<td>3 hours</td>
<td>515</td>
<td>98.3 (59/60)</td>
<td>91.1-100</td>
<td>85.1 (387/455)</td>
<td>81.4-88.2</td>
</tr>
<tr>
<td>6 hours</td>
<td>310</td>
<td>100 (37/37)</td>
<td>90.5-100</td>
<td>82.4 (225/273)</td>
<td>77.4-86.7</td>
</tr>
</tbody>
</table>
### APACE Study Performance

**99th percentile URL cutoff (14 ng/L) for aid in diagnosis of AMI in females**

<table>
<thead>
<tr>
<th>Females using 14 ng/L cutoff</th>
<th>Sensitivity</th>
<th>Specificity</th>
<th>PPV</th>
<th>NPV</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Time-point</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Base line</td>
<td>%</td>
<td>95 % CI</td>
<td>%</td>
<td>95 % CI</td>
</tr>
<tr>
<td>97.1 (34/35)</td>
<td>85.1-99.9</td>
<td>77.4 (164/212)</td>
<td>71.1-82.8</td>
<td>41.5 (34/82)</td>
</tr>
<tr>
<td>3 hours</td>
<td>100 (17/17)</td>
<td>80.5-100</td>
<td>75.2 (124/165)</td>
<td>67.8-81.5</td>
</tr>
<tr>
<td>6 hours</td>
<td>100 (15/15)</td>
<td>78.2-100</td>
<td>72.3 (68/94)</td>
<td>62.2-81.1</td>
</tr>
</tbody>
</table>

- **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.**
# APACE Study Performance

99th percentile URL cutoff (22 ng/L) for aid in diagnosis of AMI in males

<table>
<thead>
<tr>
<th>Time-point</th>
<th>Sensitivity</th>
<th>Specificity</th>
<th>PPV</th>
<th>NPV</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Base line</strong></td>
<td>90.9 (80/88)</td>
<td>82.9-96.0</td>
<td>85.8-92.2</td>
<td>66.1 (80/121)</td>
</tr>
<tr>
<td></td>
<td>89.3 (342/383)</td>
<td>89.3</td>
<td>95.8-92.2</td>
<td>57.0-74.5</td>
</tr>
<tr>
<td></td>
<td>89.3 (342/383)</td>
<td>89.3</td>
<td>95.8-92.2</td>
<td>97.7 (342/350)</td>
</tr>
<tr>
<td></td>
<td>97.7 (42/43)</td>
<td>87.7-99.9</td>
<td>82.5-90.6</td>
<td>41.0-63.8</td>
</tr>
<tr>
<td></td>
<td>86.9 (252/290)</td>
<td>86.9</td>
<td>82.5-90.6</td>
<td>99.6 (252/253)</td>
</tr>
<tr>
<td></td>
<td>100 (22/22)</td>
<td>84.6-100</td>
<td>80.1-90.8</td>
<td>100 (154/154)</td>
</tr>
<tr>
<td></td>
<td>86.0 (154/179)</td>
<td>86.0</td>
<td>80.1-90.8</td>
<td>97.6-100</td>
</tr>
</tbody>
</table>

3 hours

6 hours
Higher Sensitivity and NPV
Gen 5 vs Other cTn Assays

Comparing Roche’s cTnT Assays to Competitor’s TnI Assays

<table>
<thead>
<tr>
<th></th>
<th>Sensitivity (95% CI)</th>
<th>Specificity (95% CI)</th>
<th>Negative predictive value (NPV)</th>
<th>Positive Predictive value (PPV)</th>
</tr>
</thead>
<tbody>
<tr>
<td>APACE trial (all patients)¹</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Abbott cTnI Architect 99th percentile (0.028 µg/L)</td>
<td>86 (79–.92)</td>
<td>92 (90–94)</td>
<td>97 (95–.98)</td>
<td>69 (61–76)</td>
</tr>
<tr>
<td>Roche cTnT gen 5 99th percentile (14 ng/L)*</td>
<td>95 (90–98)</td>
<td>80 (77–82)</td>
<td>99 (97–100)</td>
<td>50 (43–56)</td>
</tr>
<tr>
<td>Siemens Tnl-Ultra 99th percentile (0.040 µg/L)</td>
<td>89 (82–94)</td>
<td>92 (89–94)</td>
<td>98 (96–99)</td>
<td>68 (60–76)</td>
</tr>
<tr>
<td>Roche TnT gen 4 10% CV (0.03 µg/L)</td>
<td>72 (60–80)</td>
<td>97 (96–98)</td>
<td>94 (92–96)</td>
<td>85 (76–91)</td>
</tr>
</tbody>
</table>

- cTnT gen 5 demonstrated the highest sensitivity (95%) at 99th percentile.
- The high NPV (99%) confirmed that cTnT-hs is a reliable marker to “rule out” AMI.

Reichlin T et al. NEJM 2009; 36(9): 858–67

* This value is considered off-label use because it is below the approved cutoff level of 19 ng/L.
2015 ESC guidelines for the management of acute coronary syndromes in patients presenting without persistent ST-segment elevation

Rapid Rule-Out in the ESC guidelines

Roffi M et al. Eur Heart J. 2015 Aug 29
Doing now what patients need next