Aspects to Consider When Selecting a Troponin Assay: The Quality Performance of Siemens Medical Solutions Diagnostics TnI-Ultra Assay

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The role of troponin assays in the diagnostic assessment of MI gained increased value with the publication of guidelines from the Joint European Society of Cardiology and the American College of Cardiology (ESC/ACC) in the year 2000. Greater awareness was also placed on the standards of quality for troponin assays within clinical laboratories as physicians placed a larger emphasis on their role in the decision-making process. There are numerous factors that effect the quality of troponin assays including precision, equimolar specificity, sensitivity, and the impact of interferences. Antibody selection, assay architecture and the productivity of instrument platforms are also major factors that influence the robustness of troponin testing. All of these factors need to be considered when choosing a Troponin assay.

Diagnosing a myocardial infarction (MI) is confounding, and clinicians must make rapid clinical decisions that may ultimately impact the survival and prognosis of chest pain patients. Traditionally, a myocardial infarction was diagnosed using criteria established by the World Health Organization. This diagnostic algorithm for MI was established in 1959 and modified in 1971.12 WHO criteria indicate that a myocardial infarction can be diagnosed when two of three conditions are met:
1. Chest discomfort that lasts for greater than 20 minutes
2. ECG changes with the development of ST segment elevation or pathological Q waves
3. Elevation of cardiac biomarkers

In this diagnostic algorithm, the diagnosis relied heavily on changes to the ECG and to a lesser extent on the cardiac biomarkers. In addition, the biomarkers of choice at that time were less sensitive and specific than troponin, making diagnosis more difficult to assess.

Over the years, technological advances in precise imaging techniques and the advent of more sensitive and specific serologic biomarkers have necessitated reevaluation of the definition of MI. In 2000, the Joint ESC/ACC Committee indicated that MI should be diagnosed when blood levels of sensitive and specific biomarkers are elevated in the clinical setting of acute ischemia. The ESC/ACC consensus document on the redefinition of MI considers cardiac troponin as the preferred biomarker for myocardial damage due to its capacity to reflect even microscopic zones of myocardial necrosis and its near absolute myocardial specificity. The joint committee suggested that an increased value for cardiac troponin should be defined as a measurement exceeding the 99th percentile of a reference or apparently healthy control group and that acceptable imprecision (coefficient of variation) at the 99th percentile for each assay should be ≤10%. The current ACC/AHA Practice Guidelines also recommend troponin as a single test to efficiently diagnose non-ST elevation myocardial infarction with serial measurements.13

Siemens Medical Solutions Diagnostics has recently launched a new generation Troponin-I assay that meets the ESC/ACC guidelines for precision at the 99th percentile of an apparently healthy population. The novel design of this new assay (Troponin Ultra+) has enabled significant technical enhancements which will assist laboratories in their quest for increased quality standards and physicians in clinical decision making.

As discussed, the precision of troponin assays at the 99th percentile of a reference population is critical to measuring minor myocardial damage. Precision plays a large part in the accuracy of an assay and is a determinant of the test’s ability to separate healthy and diseased populations.

The Siemens ADVIA Centaur® Troponin Ultra Assay is the first “fully-automated” troponin assay that meets the current ESC/ACC criteria for imprecision ≤10%. Precision was established using the CLSI EP15A protocol. A seven member precision panel of patient-based samples consisting of elevated troponin samples diluted into a pool of negative samples was run. Each member of the precision panel was tested in duplicate for twenty days. Two different instruments and two different reagent lots were used to analyze the samples. The total run % CV for each precision panel member was determined and then plotted against dose. Software was used to fit the data to regression analysis, and a dose at 10% CV was calculated from the fitted equation. The total precision profile established by the procedure is shown in Figure 1.13

In addition to meeting the recommendations for precision set forth by the ESC/ACC, the Siemens ADVIA Centaur Troponin Ultra Assay also meets the requirements stated in the current AHA guidelines7 and NACB guidelines8 for the definition of MI.

Figure 1
Siemens ADVIA Centaur Troponin Ultra Assay Precision Profile
**Equimolar Measurement**

Troponin circulates in the serum in both free and complex forms (with cTnC and cTnT). For effective utilization of TnI as a marker of MI, it is critical to measure the total amount of cardiac specific troponin independent of complexation with other cardiac troponins. The complex form of TnI is the predominant molecule that circulates in the bloodstream after MI occurs. Hence, it is vital to recognize both free TnI and TnI complexed with other troponin components when making the differential diagnosis of MI.

The Siemens ADVIA Centaur TnI-Ultra Assay equally measures both free and complexed forms of troponin I, resulting in an effective and reliable assay.

**Antibody Selection**

Sample stability for cTnI depends on the specific epitopes recognized by the antibodies in the cTnI test system. With a typical sandwich immunoassay containing both capture and detection antibodies, maximum cTnI recovery and sample stability are obtained when the capture and detection antibodies recognize adjacent epitopes on an intact proteolytic fragment of the cTnI molecule. The antibodies employed within the Siemens ADVIA Centaur TnI-Ultra Assay are displayed in Figure 2.

**Maximized Specificity**

The specificity of TnI assays is essential to minimize false positives due to cross reactivity with skeletal TnI, cTnI, cTnC, CK-MB, actin, myosin, and myoglobin. This is of particular importance when there is a combination of skeletal and cardiac muscle injury.

The Siemens ADVIA Centaur TnI-Ultra Assay was tested against a variety of substances that have potential for cross reactivity. None of these substances were shown to significantly interfere with Siemens ADVIA Centaur TnI-Ultra Assay as shown in Table 1.

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**Table 1**

<table>
<thead>
<tr>
<th>Cross-Reactant</th>
<th>Amount (ng/mL)</th>
<th>% Cross Reactivity</th>
</tr>
</thead>
<tbody>
<tr>
<td>Cardiac Troponin T</td>
<td>1000</td>
<td>ND</td>
</tr>
<tr>
<td>Skeletal Troponin I</td>
<td>1000</td>
<td>&lt;0.007</td>
</tr>
<tr>
<td>Tropomyosin</td>
<td>1000</td>
<td>ND</td>
</tr>
<tr>
<td>Actin</td>
<td>1000</td>
<td>&lt;0.005</td>
</tr>
<tr>
<td>Troponin C</td>
<td>1000</td>
<td>&lt;0.005</td>
</tr>
<tr>
<td>Myosin Light Chain</td>
<td>1000</td>
<td>ND</td>
</tr>
<tr>
<td>Myoglobin</td>
<td>1000</td>
<td>ND</td>
</tr>
<tr>
<td>CK-MB</td>
<td>1000</td>
<td>ND</td>
</tr>
</tbody>
</table>

ND=Not Detectable.

Interference testing was determined according to CLSI Document EP7-A.
Minimum Response to Heterophiles

Heterophile antibodies are developed by humans in response to exposure to specific animal or animal species immunoglobulins. The most well known heterophiles include human anti-mouse antibodies (HAMAs) and Rheumatoid Factors (RFs). HAMAs are circulating human antibodies developed against mouse antigens. Over the past years, many laboratories have seen an increase in the incidence of HAMAs due to the use of mouse monoclonal antibodies for therapeutic and imaging purposes. Circulating HAMAs in patient serum and/or plasma have been shown to exhibit nonspecific binding to analytical antibodies used in immunoassay test formats producing false positive, or, less frequently, false negative results. RFs may behave like HAMAs and exhibit nonspecific binding to analytical antibodies used in immunoassay test formats. Serum RFs are known to be IgM-isotype antibodies, with specificity against the Fc fragment of human IgG. Rheumatoid factor interference can be significant, especially in Rheumatoid arthritis patients.

Specific features were built into the Siemens ADVIA Centaur Tri-Ultra Assay to guard it from heterophilic interference. While both capture antibodies are mouse antibodies, the detection antibody is goat. This minimizes HAMA interaction by preventing the formation of a mouse-goat bridge. In addition, the buffers of the Solid Phase and LITE reagents have four efficient blocking agents that act to further prevent the interference of HAMAs.

Assay Architecture and Platform Technology

The Siemens ADVIA Centaur Tri-Ultra Assay is a three site sandwich immunoassay that yields excellent analytical sensitivity and precision. In this assay, two biotin-labeled mouse monoclonal anti-troponin antibodies, a high quantum yield acridinium ester-goat polyclonal anti-troponin complex and non-magnetic latex particles are incubated with the patient sample for 2.5 minutes. At that point, streptavidin-labeled magnetic latex particles are added and the reaction is allowed to incubate for 5 more minutes before being washed with a buffer. Acid and base are then added, exposed to light and the reaction is read and quantified by the ADVIA Centaur analyzer. With this particular architectural format, the capture efficiency of troponin is maximized, leading to excellent analytical sensitivity. The minimum detectable concentration of the Siemens ADVIA Centaur Tri-Ultra Assay is 0.006 ng/mL, well below the 99th percentile level of 0.04 ng/mL. A 10% CV is achieved at 0.03 ng/mL.

This level of sensitivity and precision is of particular relevance in the assessment of chest pain patients presenting to the Emergency Department or Physician’s Office, where many times the level of myocardial injury as detected by a troponin assay may be extremely low. The Siemens ADVIA Centaur Tri-Ultra Assay incorporates the use of nonmagnetic latex particles in the 3rd well of the ReadyPack® to reduce any non-specific binding at the very low end of the linear range that could lead to noise in the test system, yielding more accurate and reliable results. The assay also incorporates an enhanced solid phase and chemiluminescent detection material. The magnetic latex particle and high quantum yield acridinium ester both provide improved low end precision of the assay. The features and benefits of the Siemens ADVIA Centaur Tri-Ultra Assay are described in Table 2 on the following page.
Table 2

<table>
<thead>
<tr>
<th>Feature</th>
<th>Siemens TnI-Ultra Assay</th>
<th>Assay Benefits</th>
</tr>
</thead>
<tbody>
<tr>
<td>Architecture</td>
<td>Three-site Sandwich Immunoassay</td>
<td>Enhances the opportunity for the antibodies to bind to very small amounts of troponin-I in the specimen, allowing for greater analytical sensitivity.</td>
</tr>
<tr>
<td>Auxiliary Reagent</td>
<td>Non-magnetic Latex Particles</td>
<td>Latex Particles Decrease non-specific binding, thereby reducing the potential for background noise in the test system. This leads to more accurate results.</td>
</tr>
<tr>
<td>Solid Phase</td>
<td>Magnetic Latex Particles</td>
<td>Provides stability with the reaction, yielding excellent precision across the linear range of the assay.</td>
</tr>
<tr>
<td>Chemiluminescent Material</td>
<td>High Quantum Yield Acridinium Ester</td>
<td>Each acridinium ester provides very high levels of light units, enhancing the flash and enabling detection of even minute concentrations of troponin-I in the sample.</td>
</tr>
<tr>
<td>Wash Solution</td>
<td>Wash 1</td>
<td>Provides excellent precision throughout the entire linear range of the assay.</td>
</tr>
</tbody>
</table>

Flexibility and Productivity

In most situations, analyzer characteristics also play a role in your decision as to where troponin testing is performed. The Siemens ADVIA Centaur TnI-Ultra Assay is available on the ADVIA Centaur, ADVIA Centaur® CP and ADVIA IMS' immunoassay analyzers. All three assays utilize the same 99th percentile value (0.04 ng/mL) eliminating the need for rebaselining as patient testing moves from the Emergency Dept. laboratory to the Core Laboratory, or from hospital to hospital within the same network. These analyzers all offer simple-to-use STAT sampling features ensuring that test results are reported in 20 minutes or less, easily meeting the NACB recommendation of 30 minutes.1

Coupled with the maximum productivity of the ADVIA Centaur, ADVIA Centaur CP and ADVIA IMS systems, the Siemens ADVIA Centaur TnI-Ultra Assay provides rapid results and a high level of analytical sensitivity and precision.

- 99th percentile at 0.04 ng/mL, 10% CV at 0.03 ng/mL or less.
- Additon of EDTA plasma as a recommended sample type allowing physicians to draw 1 tube of blood for BNP and TnI-Ultra testing (ADVIA Centaur and ADVIA Centaur CP only).
- Lithium Heparin plasma and serum are also validated sample types.
- No operator intervention required for auto-repeat, dilute and reflex testing.
- Offered in conjunction with other Cardiovascular Disease assays (CK-MB, Myoglobin, Homocysteine and BNP).
Siemens — A Leader In Immunoassay and Cardiovascular Testing

The launch of the new Siemens ADVIA Centaur Tri-Ultra Assay is another milestone in Siemens’ commitment to cardiovascular disease testing. This new addition to our comprehensive cardiac test menu adds to our long list of “firsts” in the Cardiovascular testing arena.

- First company to commercially develop the chemiluminescent technology that is now used widely in the diagnostic industry.
- First to launch a “fully-automated” BNP assay.
- First company to launch a “fully-automated” troponin assay that meets the ESC/AACC guidelines for imprecision of ≤10% at the 99th percentile of a reference population.

Siemens also has a long history in participating in outcomes based on clinical trials.

- The Siemens cTnl Assay was utilized in the TIMI 11B and TIMI 18 trials. The data from the TIMI 11B trial provided Siemens with FDA clearance for a claim for risk stratification of patients with non-ST segment elevation acute coronary syndromes.
- Siemens ADVIA Centaur BNP Assay was utilized in a prospective substudy of 4497 patients with non-ST-elevation or ST-elevation ACS who were enrolled in phase Z of the A to Z trial conducted in 41 countries/322 acute care hospitals. This study assisted in the FDA cleared indication for prediction of survival and likelihood of future heart failure in ACS patients.
- Siemens ADVIA Centaur BNP Assay was tested in 4162 post-ACS patients as part of the PROVE IT-TIMI 22 study. In this analysis, patients were randomized to either intensive or moderate STATIN therapy and monitored for heart failure (HF) hospitalization for a 24 month period. Assignment of BNP levels into quartiles showed a significant reduction in HF hospitalization for those on the most intensive STATIN therapy.

Siemens is committed to expanding its portfolio of laboratory testing for use in the assessment and management of cardiovascular diseases. The design and performance of the Siemens ADVIA Centaur Tri-Ultra Assay provide enhanced precision and improved sensitivity for greater confidence in result reporting and clinical decision-making.

References: