Cardiac troponin I measurement: Confluence of clinical need and assay performance

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Emergence of cardiac troponin measurement on the Dimension Vista® Intelligent Lab System as the new standard for acute coronary syndrome

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SIEMENS

Cardiac troponin is the preferred biochemical marker for the diagnosis of myocardial infarction (MI) and risk stratification of suspected acute coronary syndrome (ACS) patients.[1, 2] Due to the high prevalence and high-risk profile of MI and ACS patients, cardiac troponin should be available in virtually every urgent healthcare venue. However, it is important that laboratorians recognize and remind clinicians that "not all troponin assays are created equal".

Over the past decade, cardiac troponin has evolved into one of the most common immunoassay procedures performed in laboratory medicine and, therefore, most in vitro diagnostics companies have developed and marketed assays. Although Point of Care Testing (POCT) platforms are available for measurement of cardiac troponin, data indicate that most (>90%) of these measurements are performed in either the core laboratory or in satellite testing environments by laboratory professionals.[3] Whatever the testing venue, there is clear need for accurate and precise cardiac troponin measurements at low concentrations. Laboratorians have a very important responsibility to assure that the cardiac troponin assay(s) performed at their institution is appropriate for the diagnosis, risk stratification, monitoring, and guiding treatment of patients. To this end, all new technology intended for cardiac troponin measurement must take into account clinical and analytical needs as defined by established standards and guidelines. This article will focus on performance of the cardiac troponin I (cTnI) assay available on Dimension Vista® Intelligent Lab System and how this system meets established needs for these critical patient measurements.

Dimension Vista® Cardiac Troponin Immunoassay: Utilization of sensitive LOCI® technology

The Dimension Vista® Intelligent Lab System is intended for high-volume testing in the core or main laboratory environment. Although the system has the capability of performing many different types of assays, including " classic" chemistries, immunoturbidimetry, nephelometry, etc., the focus here will be the Dimension Vista® immunoassay detection system because cTnI is quantified using this technology. The Dimension Vista® System utilizes a measurement technology termed LOCI®, chemiluminescence via singlet oxygen channeling, as its immunoassay detection system. LOCI® technology involves generation of a high-intensity chemiluminescent signal in a homogeneous assay format to achieve functional sensitivity at very low-analyte concentrations. As illustrated in Figure 1, the experimental strategy for all LOCI® immunoassays utilizes two synthetic beads termed: i) the "Sensibead" and ii) the "Chemibead". The Sensibead contains a photosensitive dye that produces singlet oxygen when excited by incident light having the appropriate wavelength; the Sensibead is coated with streptavidin, which has the property of binding tenaciously to biotinylated entities such as proteins. The Chemibead contains a chemiluminescent dye that can be excited by singlet oxygen. The Chemibead is coated with a binding protein that is specific for the analyte being determined; for example, for the cTnI method, this binding protein is an antibody specific for cTnI. As indicated in Figure 1, LOCI® technology also involves a third reagent; in the cTnI assay, this is a biotinylated antibody that targets a different cTnI epitope than that that targeted by the antibody coating the Chemibead. Chemiluminescence measurements are conducive to sensitive measurements because test solutions rarely have significant background. Thus, the ratio of the analytical signal to background noise is generally very high. Figure 1 shows that in the presence of cTnI, the "business end" of the antibody binds to the cTnI analyte while the biotinylated end is captured by the streptavidin located on the Sensibead's surface. In this same reaction, the cTnI antibody on the surface of the Chemibead binds to another region (epitope) on cTnI and, in this way, a Sensibead/aggregated cTnI-antibody/Chemibead complex is formed (Figure 1). Detection of the cTnI analyte is accomplished on the Dimension Vista® System by illumination of the reaction cell at a wavelength of 680 nm. This light stimulates production of singlet oxygen by the Sensibead's photosensitive dye; the singlet oxygen produced impinges on the bound Chemibead and triggers the chemiluminescence signal (Figure 1). The intensity of the chemiluminescence signal is directly proportional to the amount of the Sensibead/aggregated cTnI-antibody/ Chemibead complex formed in the sample. This straightforward immunoassay technology is conducive to a homogeneous format, i.e. no separation step is required and, therefore, fewer reaction steps are needed.

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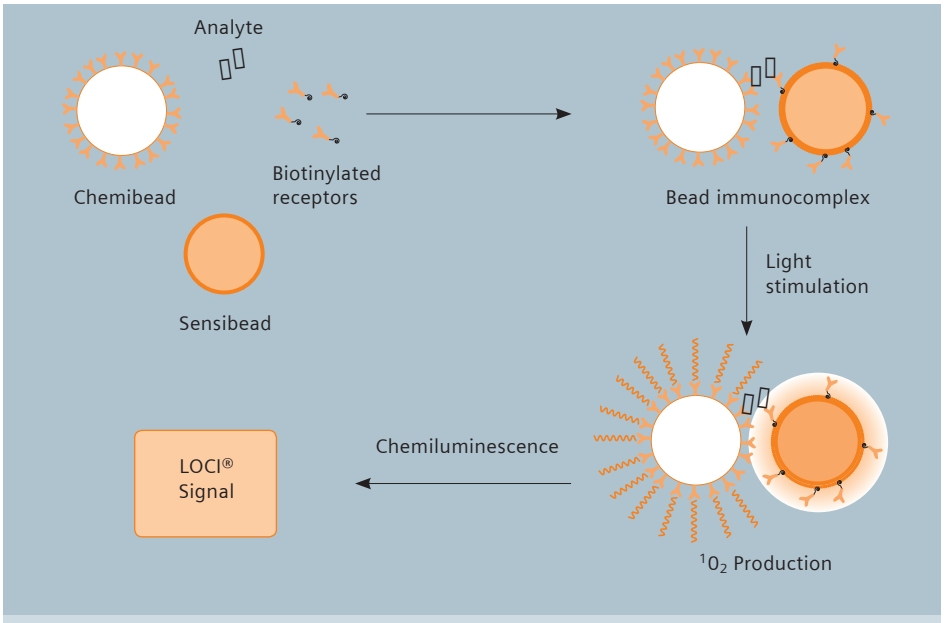


1 The LOCI® process with homogeneous LOCI® immunoassay technology

cTnI availability is important for decision-making in suspected ACS patients as early treatment may reduce myocardial injury.

A pivotal key for the detection of cTnI (or any analyte) using LOCI®-technology is the fact that the Sensibead and Chemibead must be in very close proximity to allow transfer of energy by singlet oxygen and activation of the chemiluminescent dye in the Chemibead. This is because singlet oxygen is relatively unstable in aqueous solution; therefore, if the Sensibead and Chemibead are not bridged together by the aggregated complex, the singlet oxygen produced by the Sensibead degrades before reaching any Chemibead and there is zero chemiluminescence signal. For this reason, the background signal is low and the resulting signal-to-noise ratio is very favorable. Interference from non-specific binding must always be considered in immunoassays. This is one reason why testing drugs and other substances that may be present in patient samples is important. The non-specific binding phenomenon is minimized with LOCI® technology because both the Sensibead and Chemibead are covered by a blocking layer. As discussed earlier, the nature of LOCI®-technology is that bridging of both beads must occur for a transfer of energy from singlet oxygen, so non-specific binding is unlikely.

Acute coronary syndrome (ACS):
The target patient cohort for cardiac troponin measurement

ACS is continuum of disease spanning from unstable angina to frank MI with a large area of myocardial cell death.
ACS patients typically present with a constellation of clinical symptoms caused by acute myocardial ischemia.[4, 5] These patients have a high risk for cardiac death or ischemic complications. Patients with ACS must be identified among the estimated 6 to 9 million patients with non-traumatic chest discomfort and vague symptoms listed in Figure 2 who present for emergency evaluation annually in the US.6 ACS is the biggest killer in the western world, leading to the mortality of nearly 500,000 individuals annually in the US alone.[7]
Patients with ACS are subdivided into two major categories based on the 12-lead electrocardiogram (ECG) at presentation; i) those with new ST-segment elevation on the ECG are categorized as acute ST-elevation myocardial infarction (STEMI) and ii) those who present with ST-segment depression, T-wave changes, or no ECG abnormalities are termed non-ST elevation ACS (NSTE ACS). NSTE ACS encompasses both unstable angina and non-ST elevation myocardial infarction (NSTEMI). This terminology evolved along clinical lines based upon a major divergence in the therapeutic approach to STEMI versus NSTE ACS. Unstable angina and NSTEMI are considered to be closely-related conditions sharing a common pathogenesis and clinical presentation but differing in severity.[4]

NSTE MI is distinguished from NSTE unstable angina by ischemia sufficiently severe in intensity and duration to cause cell death (myocyte necrosis), recognized by the detection of biomarkers of myocardial injury.[8] ACS is a complex syndrome with a heterogeneous etiology;[9] the most common cause is atherosclerotic coronary artery disease with a component of unstable coronary plaque. Erosion or rupture of the plaque lesion exposes the highly pro-coagulant contents of the

Symptoms of an acute coronary syndrome (heart attack) may include one or more of the following:
Uncomfortable pressure, fullness, squeezing, or pain in the center of the chest lasting more than a few minutes.
Pain spreading to the shoulders, neck, or arms. The pain may be mild to intense. May feel like pressure, tightness, burning, or heavy weight.
Pain located in the chest, upper abdomen, back, neck, jaw, or inside the arms or shoulders.
Chest discomfort with lightheadedness, fainting, sweating, nausea, or shortness of breath. Anxiety, nervousness, and/or cold, sweaty skin.
Paleness or pallor.
Increased or irregular heart rate.
Feeling of impending doom.

2 Symptoms of ACS
Modified from www.healthcentral.com/heart-disease/patient-guide-44510-6 (accessed 08.2007).

atheroma core to circulating platelets and coagulation proteins, resulting in formation of an intra-coronary thrombus.[10–12] Other less common causes of ACS include i) progressive mechanical obstruction; ii) inflammation; iii) secondary unstable angina (e.g. due to severe anemia or hyperthyroidism), and iv) dynamic obstruction (coronary vasoconstriction).[13] Antithrombotic and antiplatelet therapies aimed at halting the propagation or recurrence of coronary thrombus are key to the management of most ACS patients.[4, 5, 14]

Commensurate with the heterogeneous pathobiology of ACS, the risk of subsequent death and/or recurrent ischemic events also varies widely. As a result, effective risk stratification and targeting of therapy is a focus of contemporary clinical management of this condition.[15,16] In addition, among patients with definite ACS, early treatment may reduce the extent of myocardial injury; therefore, rapid diagnosis and initiation of therapy is also a central tenet of management.[4]

It follows that the objectives of the initial evaluation of patients with non-traumatic chest pain are two-fold:

1) to assess the probability that the patient's symptoms are related to acute coronary ischemia; and 2) to assess the patient's risk of recurrent cardiac events, including death and recurrent ischemia.[4]

In conjunction with the clinical history, physical examination, and interpretation of the ECG, cardiac biomarkers are valuable in achieving both of these objectives. cTnI is an essential part of acute care for the NSTE ACS patient. Largely due to the vague and variable presentation, the identification and disposition of ACS patients in the emergency department (ED) is one of the most difficult challenges facing caregivers. Admission of patients with a low probability ACS often leads to excessive hospital costs.[6]

A strategy that is too liberal with regard to ED discharges may lead to higher numbers of high-risk patients released with unstable angina or MI. Inappropriate discharge of ED patients who have MI has been estimated to occur in 2 to 5% of patients and is the single most common cause of malpractice lawsuits against ED physicians.[17] Laboratorians have a central role in providing critical information for the diagnosis, risk evaluation, and management of ACS patients; how to best deliver this information is presented in guidelines from the NACB.[18]

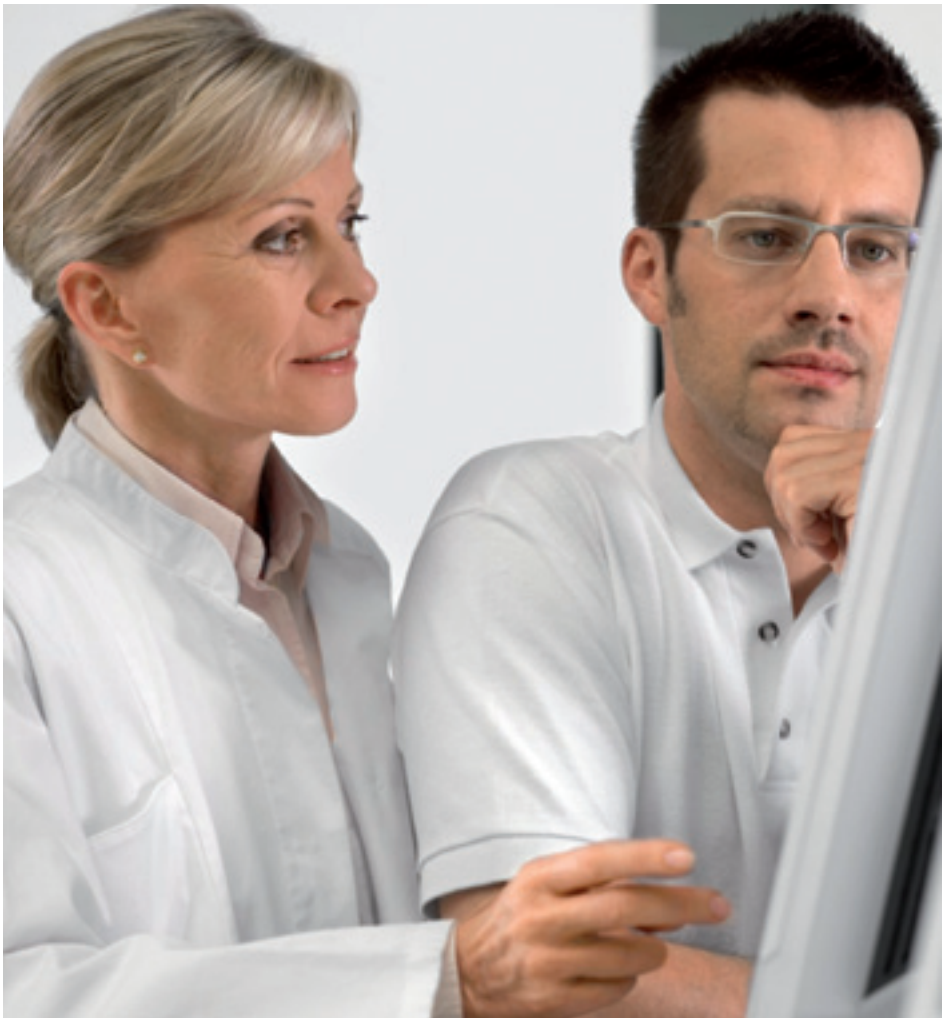
Guidelines for cardiac troponin: Decision point for ACS

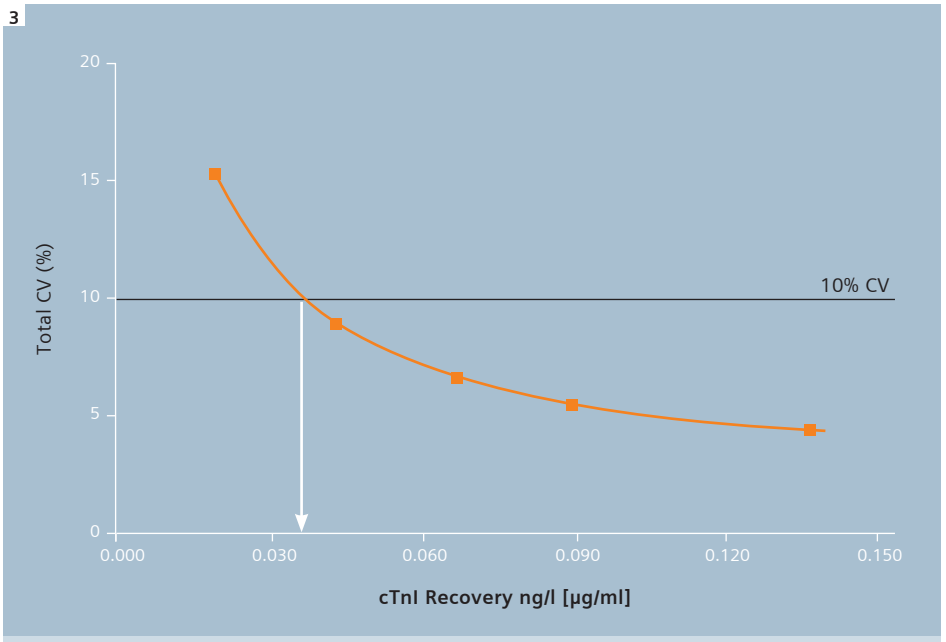
The National Academy of Clinical Biochemistry (NACB) recently disseminated laboratory medicine practice guidelines (LMPG) for ACS in the cardiology journal Circulation,[1] the laboratory medicine journal Clinical Chemistry,[2] Point-of-Care journal,[18] and on the NACB website www.aacc.org/AACC/members/nacb/.

These clinical, analytical, and logistics guidelines recommend strongly that cardiac troponin should be measured in all patients who present with symptoms consistent with ACS. Guidance for the interpretation of cardiac troponin values in the presence of a clinical history suggestive of ACS indicating a high-risk profile and diagnosis of MI and the imprecision specifications for cardiac troponin are as follows:[1, 2]

1) Maximal concentration of cardiac troponin exceeding the 99th percentile of values (with optimal precision defined by total CV of 10%) for a reference control group on at least one occasion during the first 24 hours after the clinical event. Observation of a rise and/or fall in values is useful in discriminating the timing of injury.

2) Assays for cardiac biomarkers should improve towards a total imprecision (%CV) of ≤10% at the 99th percentile reference limit.





3 **Diagram of the LOCI® troponin I assay** functional sensitivity experiment showing the cTnI concentration corresponding to the 10% coefficient of variation (CV)

Historically, the notion of defining a very low cardiac troponin concentration at the 99th percentile of a reference control population was first recommended as part of the European Society for Cardiology/American College of Cardiology consensus committee convened for the redefinition of myocardial infarction.[19] When this document was published in 2000, none of the commercial cardiac troponin methods could meet this rather stringent criterion. Subsequently, it was demonstrated that the Stratus® CS Acute Care™ System and several other technologies were close to this criterion,[20] and a later study demonstrated that the Stratus® CS instrument was in fact able to achieve a 10% CV that is lower than the 99th percentile of a reference control population.[21] In short, one decision limit, the 99th percentile, is recommended as the optimum cutoff for cTnI for risk assessment of symptomatic patients and MI diagnosis.

Compliance and guidelines of the Dimension Vista® cardiac troponin I method

Normal reference interval of cardiac troponin I

The initial step for validation of an assay on a novel platform is establishment of the normal reference interval. This was accomplished for the cTnI assay on the Dimension Vista® Intelligent Lab System using 150 samples from apparently healthy individuals. This group of normals consisted of 41 women and 109 men; the age range was 18 to 65 years. The range of cTnI results for this reference cohort was 0.000 to 0.045 ng/ml; the group's 99th percentile for evaluating compliance with the NACB guideline was <0.045 ng/ml for both serum and plasma samples.

cTnI concentration at the 10% CV

Characterizing the functional sensitivity of the Dimension Vista® System was accomplished by determining the total imprecision of cTnI measurements using a panel of five patient pools having low cardiac troponin concentrations. In accordance with Clinical Laboratory Standards Institute (CLSI; formerly NCCLS) guidance, each patient pool was measured in duplicate, at two times each day separated by at least four hours, for 20 days. Total imprecision was determined by ANOVA, as specified by the EP-9 A2

protocol. Figure 3 shows the results of this study; the data from this study showed the 10% CV is <0.040 ng/ml. Clearly the 10% CV of <0.040 ng/ml is less than the 99th percentile of a reference control population and, therefore, the Dimension Vista® cTnI method is compliant with the NACB guidelines.

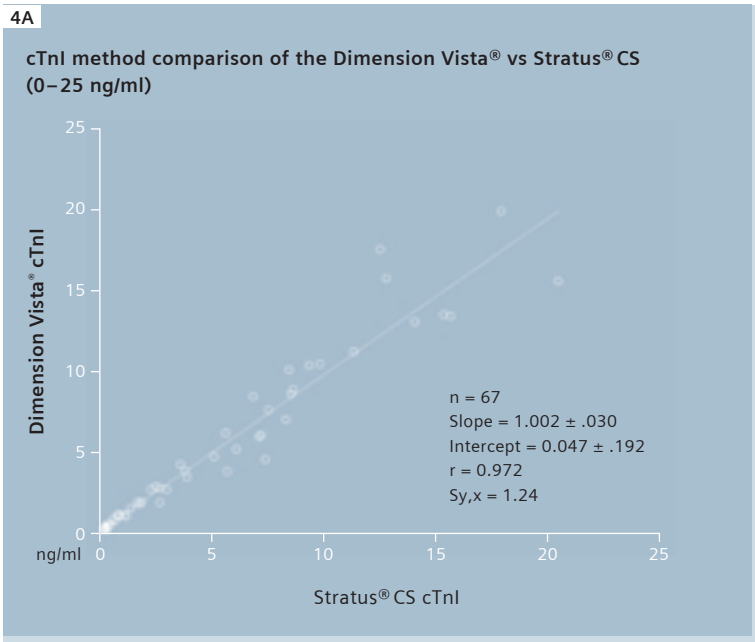
Designation of "high sensitivity" cardiac troponin assays

This guidance is important because even small increases in cardiac troponin provide important information for patient outcomes.[22] However there was no way to readily distinguish cardiac troponin methods that are compliant from those that are not. Like cTnI, there are other analytes that are frequently measured in the clinical laboratory where excellent precision at low concentrations is clinically important. Two examples are thyroid stimulating hormone (TSH) and C-reactive protein (CRP). Highly precise methods for TSH and CRP are designated "third-generation TSH" and "high-sensitivity (hs) CRP" so that users can immediately identify that the assays are characterized as having excellent precision at low concentrations in compliance with guidelines for their measurements. One idea will be to create a designation for cTnI assays that achieve imprecision of <10% at the 99th percentile reference as "high-sensitivity" cardiac troponin assays. The Dimension Vista® troponin I assay has demonstrated this functional sensitivity and therefore may well pass muster for designation as a "high-sensitivity troponin assay".

Required turnaround time (TAT) for cardiac troponin

cTnI availability is important for decision-making in suspected ACS patients as early treatment may reduce myocardial injury. Therefore, there is a "need for speed" for turnaround of this important marker for rapid diagnosis and initiation of therapy to improve patient outcomes.[5] Frequently, the laboratory considers the TAT from acknowledgement of receipt in the laboratory to the time of reporting. However, NACB guidelines concerning the logistics of cTnI testing defines the TAT as the time from blood collection to the reporting of results.[18] These guidelines indicate that the laboratory should perform cardiac marker testing with a turnaround time of 1 hour, optimally 30 minutes, or less. Operationally, achieving such a turnaround time can be quite complicated. To help with this challenge, the NACB guidelines recommend that the specimen of choice for analysis of biochemical markers of cardiac injury is plasma or anticoagulated whole blood to facilitate a more rapid turnaround time for testing.[7] Although there is some difference of opinion, the NACB guidelines recommend that institutions which cannot consistently deliver cardiac marker turnaround times of approximately one hour should implement Point of Care (POC) testing devices.[18] Further, the guidelines note that the performance specifications and characteristics of the POC and central laboratory troponin assays must not differ.[18]

A benefit of the LOCI® technology utilized on the Dimension Vista® Intelligent Lab System is that it provides for a rapid result; the on-instrument TAT is under 10 minutes and the sample size less than 20 microliters. Also, heparinized plasma is an appropriate sample for the Dimension Vista® System so specimens for testing can be centrifuged immediately and placed on the instrument. Therefore, if laboratory medicine and the ED work together and develop a system to minimize the pre-analytical aspects of sample collection and specimen transport to the testing area, a TAT of <1 hour is certainly feasible, and the more ambitious performance target of <30 minutes is also achievable, In some institutions the preanalytical challenges preclude central lab testing in a timely fashion, i.e. <1 hour. Many of these institutions



4 **Correlation between Dimension Vista® Troponin I and Stratus® CS Acute Care™ Troponin I (Unpublished data)**

opt for a satellite laboratory located in or near the ED area to provide cTnI results.

In this case, cTnI measurement with a Stratus® CS instrument may work ideally as this cTnI assay meets the ESC/ACC Committee precision requirements for high sensitivity and that is in harmony with the Dimension Vista® system (Figure 4).

Analytical interferences in cardiac troponin assays

The NACB analytical guidelines state that before introduction into clinical practice, cardiac biomarker assays must be characterized with respect to potential interferences, including rheumatoid factors, human anti-mouse antibodies, and heterophile antibodies.[2] It is also critical that various drugs and medications are examined for interference. The impact of many common medications, as well as hemolysis, lipemia, and icterus on the Dimension Vista® cTnI method was tested, and no interference was observed. Additionally, no interference was found for samples containing rheumatoid factors, human anti-mouse antibodies, and heterophile antibodies.

Future

This article focused on performance of the cTnI method on the Dimension Vista® Intelligent Lab System and compliance with NACB guidelines for cardiac troponin measurement. The sensitivity of the LOCI® technology used for cTnI measurement is conducive to meeting the specifications listed in the NACB Clinical and Analytical guidelines for biochemical cardiac marker testing in suspected ACS patients. Although Figure 5 lists the features specified above for the

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1. Correlation with other Siemens instruments	Agreement (harmony) with Dimension® RxL and Stratus® CS Systems: Slope = 1.02 y-intercept = 0.003 ng/ml Correlation coefficient = 0.993
2. Functional sensitivity	99th percentile of Reference Control Population: <0.045 ng/ml 10% Total Imprecision determined according to CLSI P9-A2: <0.040 ng/ml
3. Turnaround time	<10 minutes on analyzer
4. Specimen types	Serum and heparinized plasma Specimen volume 20 µl
5. Interference studies showed no significant effect for	Hemolysis, lipemia, icterus Over 50 physiological substances and common medications Rheumatoid factors, human anti-mouse antibodies, and heterophile antibodies

There is a 'need for speed' for turnaround of cardiac troponin for rapid diagnosis and initiation of therapy to improve patient outcomes.

Dimension Vista® cTnI Method, there are clearly other important characteristics that were not discussed. A comprehensive description of the characteristics for the cTnI and other methods on the Dimension Vista® Intelligent Lab System can be obtained by examining the Siemens website. Note that the Dimension Vista® System is intended for use in providing high-volume testing in a main (or core) laboratory environment; however, it is important to note that the vast majority of cardiac marker testing is performed in this environment. Overall, the Dimension Vista® Intelligent Lab System is compliant with the current guideline standards for management of ACS patients.

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