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White Paper

Antibody Selection Criteria for Cardiac Troponin I Assays

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Introduction

Cardiac troponins are considered the most sensitive and specific biochemical markers for the detection of myocardial damage. The redefinition of an acute myocardial infarction (AMI) by the European Society of Cardiology and American College of Cardiology (ESC/ACC) recommends that an increased level of cardiac troponin should be defined as a measurement above the 99th percentile value of the reference group.[1] Moreover, the ESC/ACC recommendation requires that the total imprecision at 99th percentile decision limit be 10% or less (Table 1).

These new standards of performance for cardiac troponin assays require new approaches to immunoassay design in order to achieve quantitative and precise measurement of extremely low levels of troponin found in minor or early myocardial damage. The ADVIA Centaur® TnI-Ultra™ immunoassay from Siemens Healthcare Diagnostics incorporates new assay architecture, antibodies and detection technology to achieve and surpass these new performance standards.

Antibody Selection

Antibody selection is central to meeting the new performance goals as defined by the ESC/ACC. Selection criteria must take into account not only the specificity of cardiac troponin I binding, but also binding affinities which determine detection limits and assay time. Cross-reactivities to other cardiac and skeletal troponins must be insignificant, especially to skeletal troponin I. The TnI-Ultra assay has insignificant cross reactivity to skeletal muscle, cardiac muscle or other cardiovascular biomarkers (Table 2).

It is critical to measure the total amount of cardiac specific troponin I in a patient sample for maximum analytical sensitivity. Since cardiac troponin I (cTnI) exists as free and as complexed forms with cardiac troponin C (cTnC) and, to a lesser extent, with cardiac Troponin T (cTnT), it is important to select antibodies that bind cTnI epitopes which are expressed independently of complexation with other cardiac troponins. The ability to bind free and complexed forms of cTnI is also important in situations where EDTA plasma is the sample type used. The association constant between cTnI and cTnC is stronger in the presence of Ca^{2+}. EDTA chelates Ca^{2+} resulting in an increase in the proportion of free cTnI in the sample. Additionally, the proportion of free and complexed cTnI is modulated to some extent by the degree of cTnI and cTnC proteolysis.

Antibody selection must account for how cTnI epitopes expression is influenced by heparin binding to central regions of the molecule. This well-known phenomena results in a bias of cTnI recoveries between serum and heparin plasma samples found among commercial cTnI assays. Minimizing the bias between serum and heparin is important not only to ensure the flexibility of specimen choice in the laboratory, but also to reduce the risk of suboptimal cTnI recovery for patients undergoing heparin therapy. For any patient undergoing anti-coagulant therapy, plasma is the preferred sample type for cTnI assays since plasma allows for a more rapid time to result and avoids the problems with incomplete

Table 1: Performance of the ADVIA Centaur TnI-Ultra Assay [2]

Minimum Detectable Concentration	0.006 ng/mL
99th percentile	0.04 ng/mL
10% Total CV	0.03 ng/mL

Table 2: Cross-Reactivity Studies with the ADVIA Centaur TnI-Ultra Assay[2]

Cross-Reactant	Amount (ng/mL)	% Cross-Reactivity
Cardiac Troponin T	1000	ND
Skeletal Troponin I	1000	<0.007
Tropomyosin	1000	ND
Actin	1000	<0.005
Troponin C	1000	<0.005
Myosin Light Chain	1000	ND
Myoglobin	1000	ND
CK-MB	1000	ND

ND=Not Detectable.
Interference testing was determined according to CLSI Document EP7-A.

clotting of serum associated with this patient population. The average bias between heparin plasma and serum is 1%, while the average bias between EDTA plasma and serum is 4% utilizing theADVIA Centaur TnI-Ultra assay.[2]

Careful antibody selection is essential to ensure recovery of cTnI after proteolysis both by proteases present in necrotic myocardium and in the patient's plasma. The extent of degradation varies between individual patients. The manifestation of cTnI proteolysis leads to the apparent differences in sample stability between commercially available cTnI methods and stability differences between samples. 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 contiguous epitopes on an intact protolytic fragment of the cTnI molecule. Antibody selection for the ADVIA Centaur TnI-Ultra assay employed this selection strategy (Figure 1).

Finally, antibodies utilized for cTnI testing, should bind cTnI independently of other known heterogeneities of the cTnI molecule such as phosphorylation and redox state.

Reagent Architecture

The ADVIA Centaur TnI-Ultra method employs an unique reagent architecture that employs dual capture antibodies instead of the typical single capture formats used by other commercial assays. The dual capture format allows for the use of antibodies with the preferred binding characteristics described above. Siemens Healthcare Diagnostics' dual capture architecture yields a lower limit of cTnI detection because of the increased capture efficiency brought about by using two antibodies instead of one.

Figure 1: ADVIA Centaur TnI-Ultra Epitope Recognition

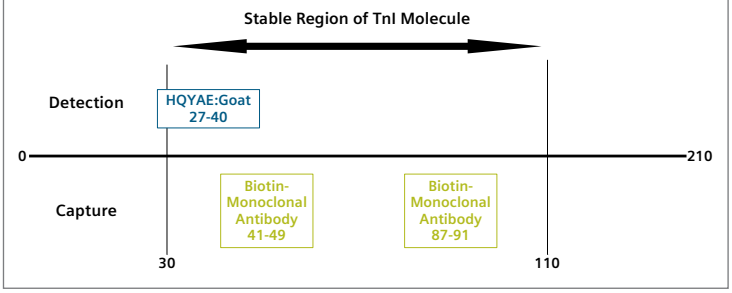
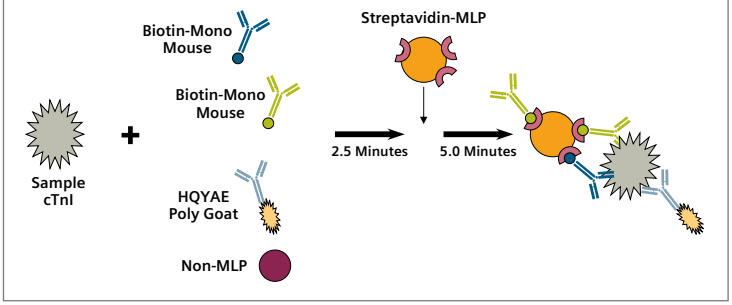


Figure 2: ADVIA Centaur TnI-Ultra Assay Architecture



The polyclonal detection antibody is affinity purified and recognizes a cTnI epitope which is cognate with both capture antibodies. Moreover, the proprietary High Quantum Yield Acridinium ester used to conjugate the detection antibody boosts the chemiluminescent output by four-fold. As a result of these innovations the ADVIA Centaur TnI-Ultra assay is one of the most precise and sensitive cTnI methods on the market today (Figure 2).

Conclusions

The ADVIA Centaur TnI-Ultra assays meets and exceeds the recent recommendations of the ESC/ACC, opening a new analytical window for cTnI testing. New antibodies and reagent architecture enhance sample stability and recovery, reduce bias between serum and plasma samples, lower minimum levels of cTnI detection, and maximize assay precision.

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References:

1. Alpert J. for the Joint European Society of Cardiology/American College of Cardiology Committee for the Redefinition of Myocardial Infarction. J Am Coll Cardiol. 2000;36:959-69.
2. ADVIA Centaur Assay Manual – TnI-Ultra, part # 02794339, Rev A, 2006-01, Siemens Medical Solutions Diagnostics, Tarrytown, NY.

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