New monoclonal antibodies specific to epitope 24-40 of human cardiac Troponin I

The region 24-40 aar of cardiac troponin I (cTnI) is a perspective target for monoclonal antibodies due to the several reasons. The first 32 aar of the cTnI are not present in skeletal isoforms of the protein. Of the following 8 aar residual (33-40) 3 are also unique to the cardiac isoform, which increases the probability of obtaining of highly-specific antibodies without cross-reaction with the two skeletal isoforms of troponin I. In contrast to the most of the central part of cTnI, the region 24-40 aar is not hindered by the other components of troponin complex and is almost not affected by the action of autoantibodies neither. Moreover, our recent studies have shown that even though cTnI is prone to proteolytic degradation, fragment 24-40 of cTnI molecule is rather stable even at the late times after acute myocardial infarction (AMI). All these features make the antibodies, specific to the 24-40 aar of cTnI, a perspective tool for reliable immunochemical measurements of cTnI concentration.

MONOCLONAL ANTIBODIES SPECIFIC TO THE EPITOPE 24-40 AAR OF CTNI

We have developed two mouse (1017cc and 1039cc of Cat.# 4T21cc) and four rabbit (RecR1, RecR23, RecR33, and RecR85 of Cat.# RC4T21) MAbs specific to epitope 24-40 of cTnI molecule. These MAbs being paired with antibodies specific to other epitopes can be used for the development of sandwich immunoassays with superior sensitivity, limit of detection (LoD) better than 1 ng/L, and high specificity, no cross-reaction to cTnT or to skeletal isoforms of TnI.

That is for the first time Hytest suggests rabbit monoclonal antibodies for the development of high sensitivity cTnI assays. Traditionally mouse monoclonal antibodies were used in cTnI immunoassays, starting from the first commercial assay and until the latest and the most advanced high sensitivity assays. In comparison to murine MAbs, due to the difference in the paratope structure rabbit antibodies generally possess higher affinity to antigen, thus increasing the sensitivity of the assay.

Recommended MAb pairs

New antibodies were tested in CLIA sandwich immunoassay with Hytest anti-cTnI MAbs and the best combinations demonstrating highest sensitivity (LoD between better than 1 ng/L) and effectively recognizing antigen in blood samples from AMI patients could be recommended for the development of the high sensitivity cTnI assays (see Table 1).

Table 1.
Antibody pair recommendations of the aar 24-40 region targeting MAbs for quantitative cTnI sandwich immunoassay.

Capture antibodies	Detection antibodies
RecR1	20C6cc
RecR23	20C6cc
RecR85	20C6cc
RecR33	MF4cc
MF4cc	RecR33
625	1017cc
625	1039cc
19C7	RecR33

Correlation with Abbott ARCHITECT STAT Troponin I and Beckman Coulter ACCESS hsTnI assays

Assay prototypes utilizing new MAbs as capture or detection antibodies are able to detect cTnI in serum and plasma samples from AMI patients with high sensitivity. Assays, utilizing new antibodies, specific to the fragment 24-40, demonstrated good correlation with commercially available Abbott ARCHITECT STAT Troponin I and Beckman Coulter ACCESS hsTnI cTnI assays. The concentration of samples obtained from AMI patients was determined by using two immunoassays utilizing Hytest antibodies and commercially available Abbott (51 samples) and Beckman (38 samples) cTnI assays. The linear correlation of commercial assays and assays utilizing Hytest antibodies was in the range of 0.92 and 0.95 (Figure 1).

Cross-reactivity with skeletal isoforms of cTnI

In high-sensitivity troponin assays, the specificity of the antibodies utilized is of utmost importance as even minor cross-reactivities with skeletal isoforms (slow and fast skeletal TnI) could result in false positives.

We investigated the cross-reactivity of new MAbs being paired with antibodies specific to other epitopes to skeletal isoforms (fast and slow) of troponin I (Figure 2). No cross-reaction to cTnT or to skeletal isoforms of TnI up to 100 µg/l was detected.

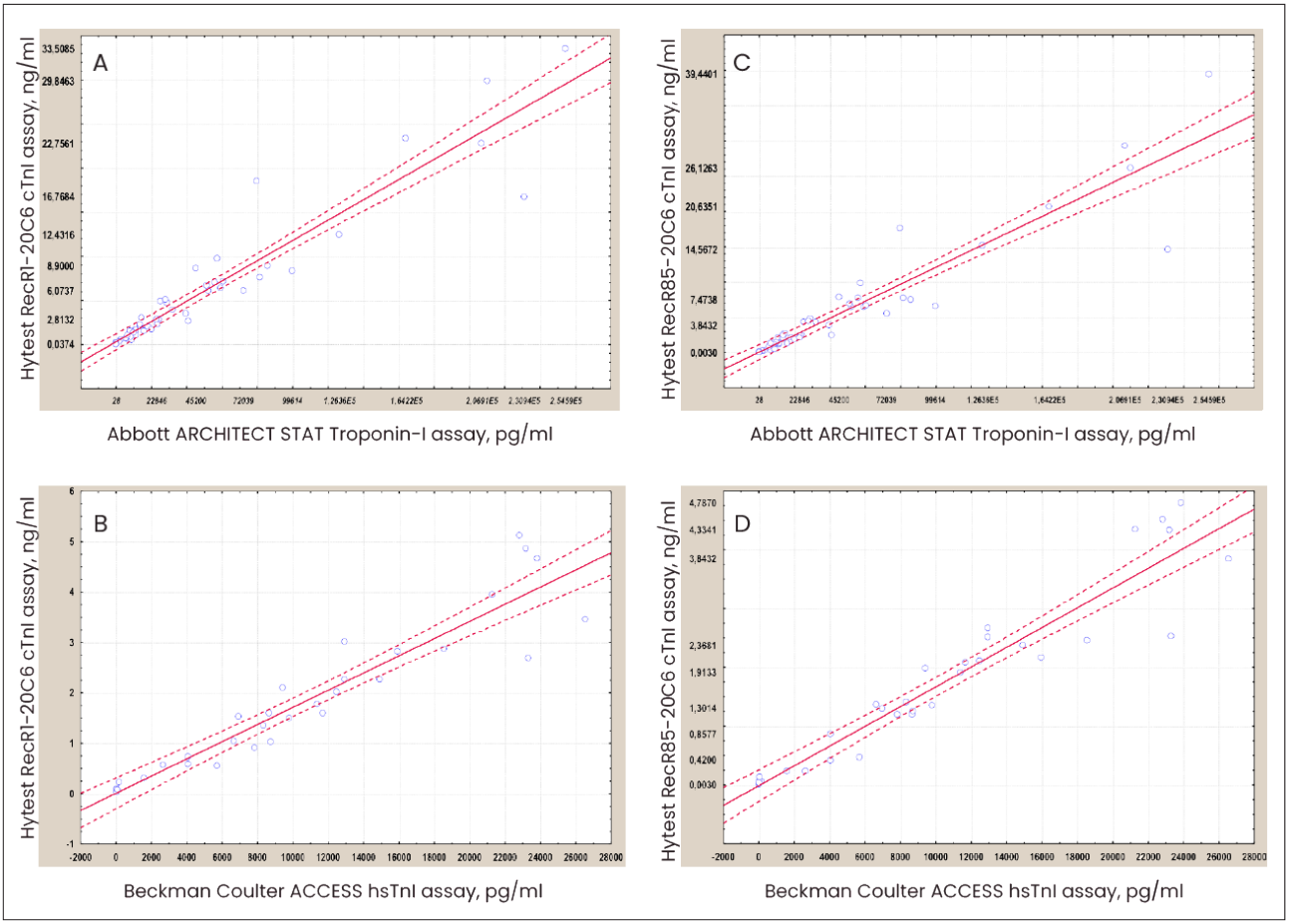

Figure 1.

Correlation of rabbit recombinant MAbs RecR1 and RecR85 with commercially available cTnI assays. *The concentration of 51 (A and C) and 38 cTnI samples (B and D) obtained from AMI patients was determines by using two immunoassays that utilized Hytest antibodies (capture-detection pairs RecR1-20C6 and RecR85-20C6) and two commercially available Abbott ARCHITECT STAT Troponin-I and Beckman Coulter ACCESS hsTnI assays.*

HAMA interference

One of the factors that might influence the specificity of the immunometric detection of analyte in patient sample is the presence of heterophile antibodies including human anti-mouse antibodies (HAMA), that might lead to the emergence of false-positive or (less common) false-negative results. There are several ways to reduce the influence of HAMA on the antibodies of the assay (addition of non-specific mouse antibodies, utilization of antibody fragments or chimeric MAbs in the assay etc). Here we propose an alternative strategy that consists in the usage of the mixed rabbit-mouse pairs of monoclonal antibodies in the sandwich immunoassay. As it is showed in the Figure 3, the usage of rabbit instead of mouse antibody both as a capture or detection MAb completely eliminates the false-positive influence of HAMA that are present in the serum sample.

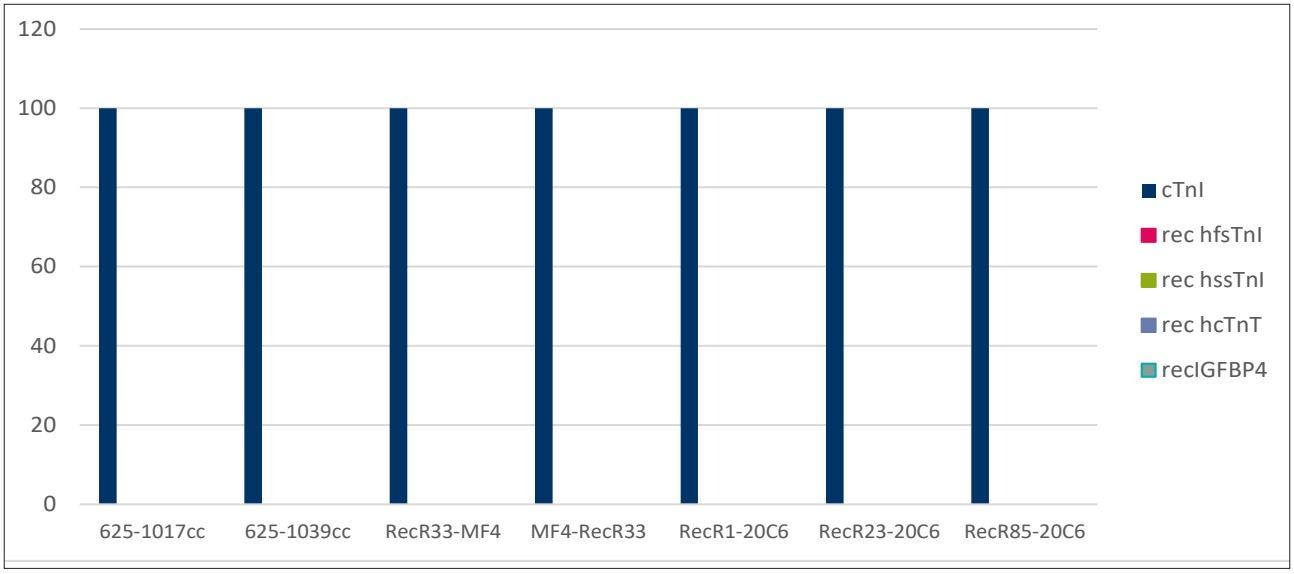

Figure 2.

Absence of cross-reactivity with skeletal isoforms of TnI in assays utilizing the antibodies targeted to the cTnI region of 24-40. *No cross-reaction to cTnT or to skeletal isoforms of TnI up to 100 µg/l were observed.*

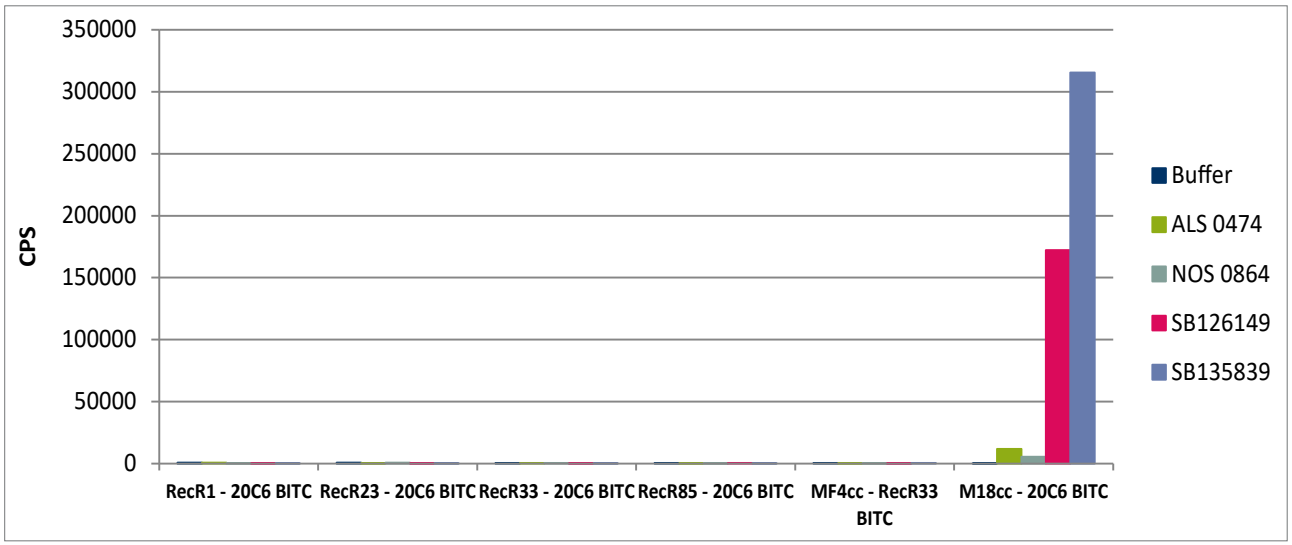

Figure 3.

Rabbit MAbs eliminate the false-positive signal in HAMA-positive serum samples. *M18cc-20C6 – assay utilizing two mouse monoclonal antibodies.*

RABBIT RECOMBINANT MONOCLONAL ANTIBODIES FROM HYTEST

Hytest has developed a new monoclonal antibody technology based on rabbit derived antibodies. One of the company's core strengths is its long experience (25+ years) in hybridoma monoclonal antibody development. The new technology combines the reliability of the hybridoma approach with rapid and flexible gene engineering methods. It is based on the natural immune response of rabbits and includes robust proprietary protocol for cloning of target IgG genes to full-size rabbit antibody backbone.

It is generally considered that rabbit derived antibodies often have higher affinity than mouse derived antibodies. High affinity is especially important in assays where the concentration of the biomarker in sample is very low – such as in the case of cTnI.

Recombinant rabbit MAbs will be available in bulk quantities (gram scale) making them suitable for commercial diagnostic immunoassays.

Combining a recombinant rabbit MAb with a conventional mouse derived MAb in a sandwich type immunoassays also helps to mitigate the effect of heterophile antibodies.

ORDERING INFORMATION

MONOCLONAL ANTIBODIES

Product name	Cat. #	MAb	Subclass	Remarks
Troponin I cardiac	4T21	625	IgG1	EIA, WB, a.a.r. 169-178
	4T21cc	1017cc	IgG1	*In vitro*, EIA, WB, a.a.r. 22-40
		1039cc	IgG1	*In vitro*, EIA, WB, a.a.r. 22-40
		19C7cc	IgG2b	*In vitro*, EIA, WB, a.a.r. 41-49
		MF4cc	IgG1	*In vitro*, EIA, WB, a.a.r. 190-196
	RC4T21	RecR1	IgG	EIA, a.a.r. 24-40, recombinant rabbit antibody
		RecR23	IgG	EIA, a.a.r. 24-40, recombinant rabbit antibody
		RecR33	IgG	EIA, a.a.r. 24-40, recombinant rabbit antibody
		RecR85	IgG	EIA, a.a.r. 24-40, recombinant rabbit antibody
Troponin complex cardiac	4TC2	20C6cc	IgG2b	*In vitro*, EIA

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