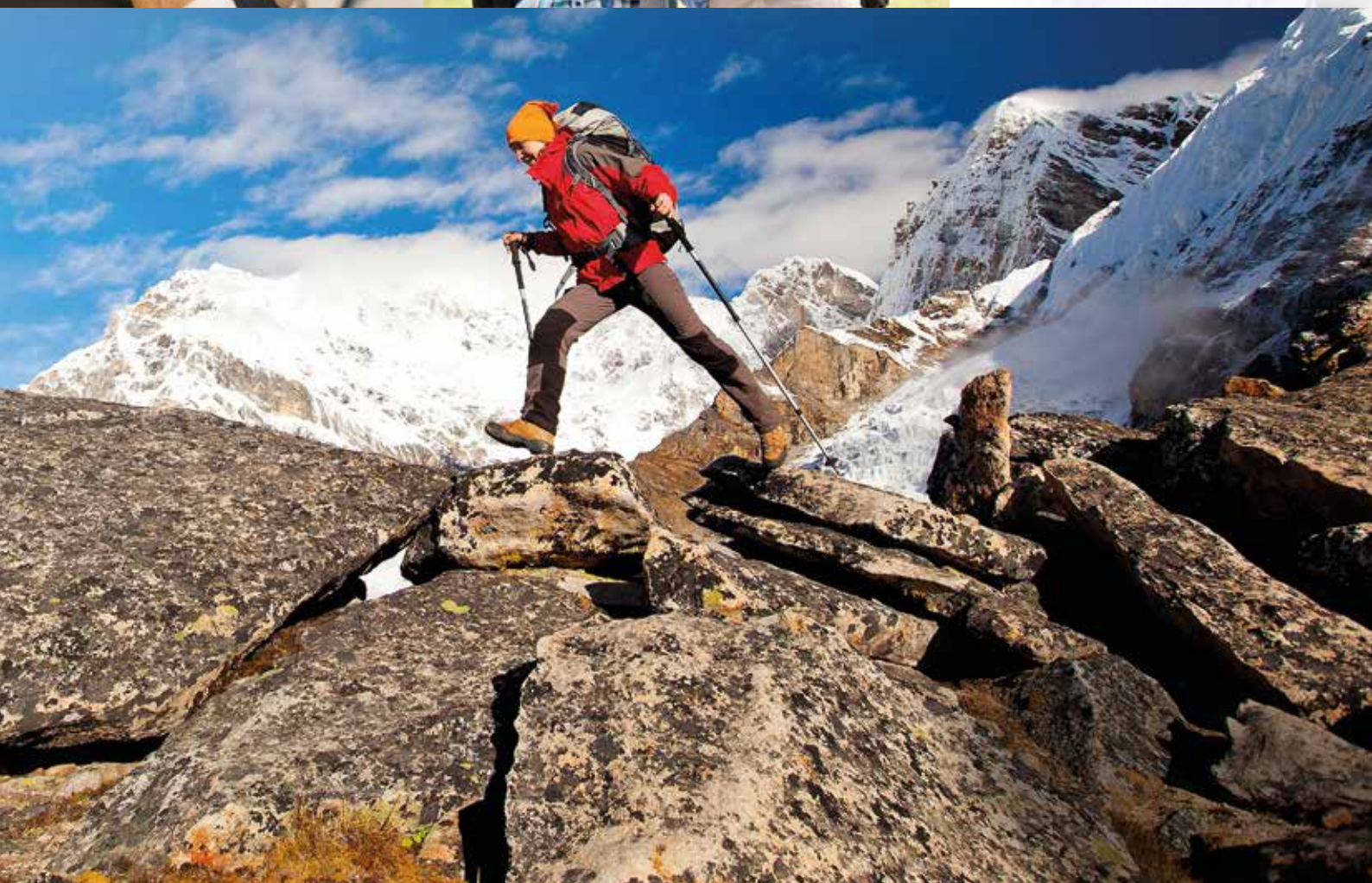
HyTest

Cardiac Markers

Antibodies and antigens



Introduction – cardiac biomarkers and diagnostics

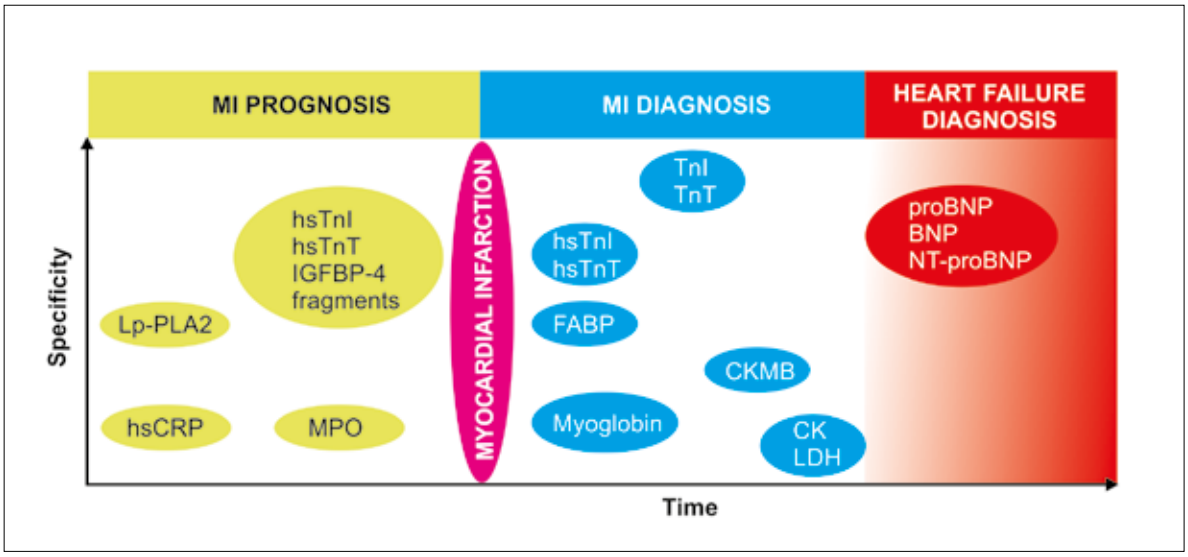
Cardiovascular diseases (CVDs) are a group of disorders of the heart and blood vessels. CVDs are the leading cause of death globally; it is estimated that approximately 30% of all deaths are caused by CVDs.

First markers for cardiac diseases diagnostics were described already in the late 1950s and early 1960s when it was shown that measurements of lactate dehydrogenase (LDH), creatine kinase (CK) or aspartate aminotransferase (ASAT) enzymatic activities could be used in diagnosis of acute myocardial infarction (AMI). However, these assays had low specificity and sensitivity. First improvement was the development of immunological assays using polyclonal antibodies. In the early 1980s the first monoclonal antibodies were brought to the market marking a major leap in the development of immunoassays.

Since those days cardiac diseases diagnostics has gone through a significant evolution. Enzymatic assays and myoglobin assays have been replaced with cardiac troponins (I and T) and their high sensitivity versions are becoming the leading paradigm in AMI diagnostics. At the same time, markers for heart failure diagnosis have been adopted into routine use in most clinical laboratories. Within the recent years, more emphasis has been put on the development of markers which could be used for CVD prevention and risk assessment.

HyTest has been at the fore front of development of reagents for CVDs' diagnostics. During the past 20 years our scientists have authored or co-authored more than 30 articles published in peer reviewed scientific journals. This investment in scientific work has helped us to develop raw materials used by world's leading diagnostics companies. HyTest scientists have also worked actively in IFCC and AACC standardization committees. In 2004, HyTest's cardiac troponin complex material was chosen as a raw material for the international troponin standard.

We believe that reliable raw materials for diagnostics can be produced only if the design is based on solid understanding of the behavior of the biomarker, the disease state and most importantly, the needs of the industry using the raw materials.



Markers of myocardial infarction and heart failure. This schematic representation shows how they differ in timing and in specificity.

Selected cardiac marker articles from HyTest scientists

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Semenov AG, et al. Processing of pro-brain natriuretic peptide is suppressed by O-glycosylation in the region close to the cleavage site. Clin. Chem. 2009, 55(3): 489-498.

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Cardiac troponin I (cTnI)

CLINICAL UTILITY

- **Acute myocardial infarction (AMI)**
- **Unstable angina**
- **AMI prognosis**
- **Cardiac muscle injury and cell death**

Cardiac troponin I is currently considered to be the gold standard biomarker test for myocardial infarction. Moreover, cTnI measurements by a new generation of high-sensitivity cTnI assays could be helpful for long-term risk stratification of different patient groups, including patients with heart failure or stable coronary artery disease.

At HyTest, we have intensively studied troponin I for over 20 years. Based on this research, we constantly aim to develop improved antibodies to be used in the immunoassays that are needed for accurate cardiac disease diagnostics. We have generated and tested several thousand monoclonal antibodies specific to different regions of the cTnI molecule and have tested numerous different MAb combinations in order to find the best pairs for a precise and sensitive cTnI immunoassay.

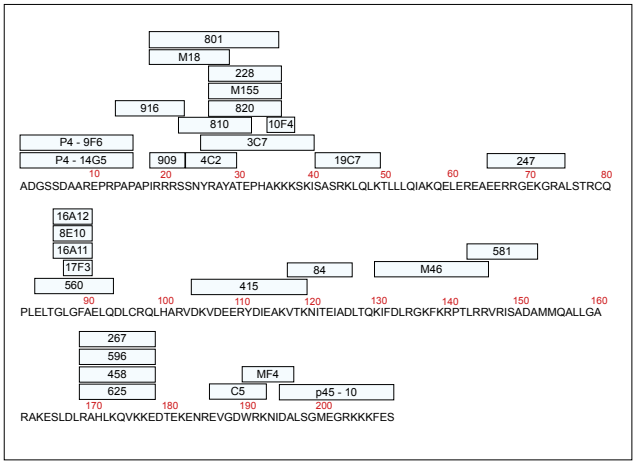


Figure 1. Epitope mapping of HyTest anti-cTnI monoclonal antibodies. We offer more than 30 specially selected antibodies specific to various epitopes along the cTnI molecule.

Factors influencing epitope recognition by antibodies

The most common reason for discrepancy in the cTnI assay measurements is the difference in the epitope specificity of the antibodies used in various assays. Due to several possible posttranslational modifications of the cTnI molecule found in patients' blood and the presence of autoantibodies in some clinical samples, it is critical to carefully validate the performance of antibodies in order to achieve reliable, quantitative detection of cTnI in blood samples.

> At HyTest, we have intensively studied troponin I for over 20 years.

When designing a sensitive and precise immunoassay, it is important to consider the effect of all the factors influencing biomarker detection. The assay should not be affected by partial proteolytic degradation of cTnI molecule, oxidation, reduction, phosphorylation, complex formation with TnC, or the presence of heparin in the samples (Katrukha, 2003). This helps to minimize bias in the assay. Factors that influence cTnI measurements are schematically presented in Figure 2 and examples of the effects of these interfering factors on analyte detection are shown in Figures 3 and 4.

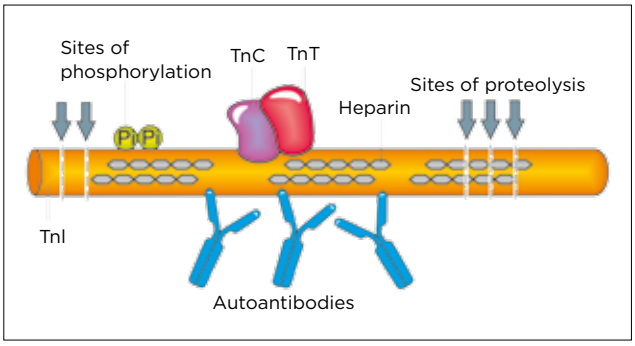


Figure 2. Factors influencing cTnI immunodetection.

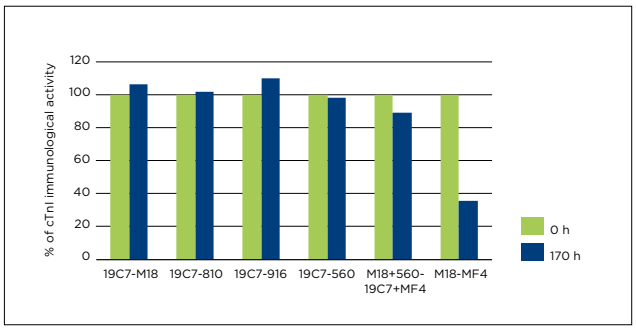


Figure 3. Effect of proteolytic degradation. Best two-site combinations of cTnI antibodies specific to the stable part of cTnI molecule tested with troponin complex before (green columns) and after (blue columns) incubation for 170 hours with a mixture of endogenous proteases from human cardiac tissue. Control assay M18-MF4 is sensitive to cTnI proteolytic degradation.

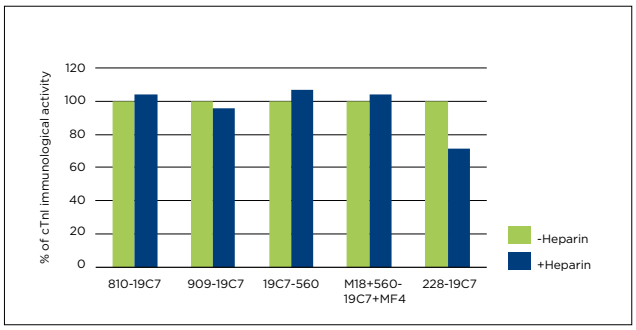


Figure 4. Effect of heparin. cTnI concentration was measured in the absence (green columns) or presence (blue columns) of 5 IU/ml heparin. Antibody 228 in the assay 228-19C7 is sensitive to the presence of heparin in the sample.

Antibodies specific to different parts of the molecule are sensitive to interfering factors in different degrees. For instance, it is well known that purified cTnI is highly susceptible to proteolytic degradation. However, in a troponin complex the central part of the cTnI closely interacts with TnC which protects cTnI from endogenous proteases. Consequently, the epitopes located in the central part of the cTnI are significantly more stable than the epitopes located at the terminal parts of the molecule. On the other hand, not every antibody specific to the central part of the molecule can recognize cTnI in a patient's blood because TnC covers some of the epitopes located in that region.

Antibodies for high-sensitivity cTnI immunoassays

In an immunoassay, the limit of detection is dependent on many features — platform, label, incubation time, buffers used, and many others. However, the most critical is the affinity of antibodies that are used for the assay design. Today HyTest antibodies are successfully utilized for the development of a new generation of high sensitivity cTnI assays. An example of a highly sensitive assay is shown in Figure 5.

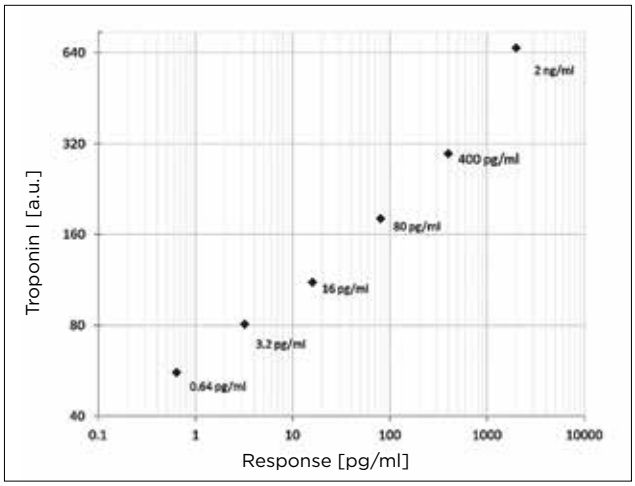


Figure 5. Highly sensitive quantitation of cTnI (down to 0.64 pg/ml) using MAbs 801 and 19C7.[1] With LamdaGen's plasmonic ELISA platform utilizing MAbs 801 and 19C7 from HyTest. The limit of detection for cTnI was 0.64 pg/ml. The dose response curve was obtained by spiking cTnI (Cat.# 8T62) into 87% fetal bovine serum. Each data point represents the average of five independent measurements. *Reprinted with permission from LamdaGen Corp.*

[1]OES™ quantification of cTnI in whole serum. Application Note, 2013. LamdaGen Corporation. www.LamdaGen.com

NEW! Chimeric cTnI antibodies

Heterophile antibodies arise when people are exposed to different animals or products derived from animals. As far as immunodiagnostics is concerned, the problem is most commonly associated with human anti-mouse antibodies (HAMA) due to the fact that most diagnostics assays use mouse derived antibodies. HAMA might cause both false negative and false positive results that could lead to delays in making the correct diagnosis. Troponin assays are particularly susceptible to HAMA due to low cut-off value requirements and because the levels of cTnI even in the plasma of AMI patients are very low.

A powerful tool to solve the issue with HAMA in diagnostics tests is the use of chimeric or fully humanized antibodies. We have now converted two of our cTnI antibodies, MAbs 19C7 and 16A11, to chimeric proteins by changing the antibody constant regions from mouse to human derived sequences. The chimeric cTnI antibodies RecChim19C7 and RecChim16A11 consist of the original mouse derived variable regions that are responsible for antigen specificity and human derived constant regions of IgG1 isotype (see Figure 6).

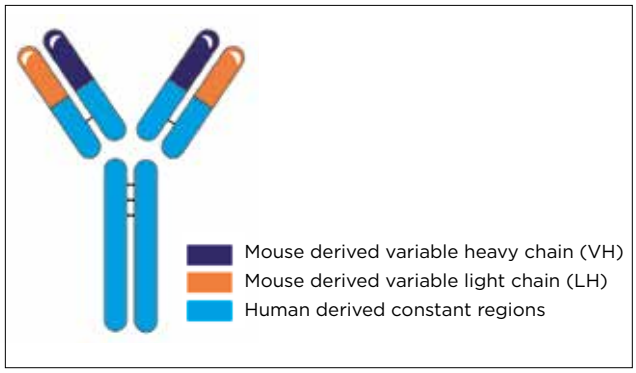


Figure 6. Schematic illustration of HyTest chimeric cTnI antibodies.

Chimeric antibodies prevent the HAMA effect

The performance of different combinations of chimeric and native antibodies was tested using HAMA containing serum samples that were obtained from acute myocardial patients in order to verify that the chimeric antibodies are not sensitive to the HAMA effect (see Figure 7).

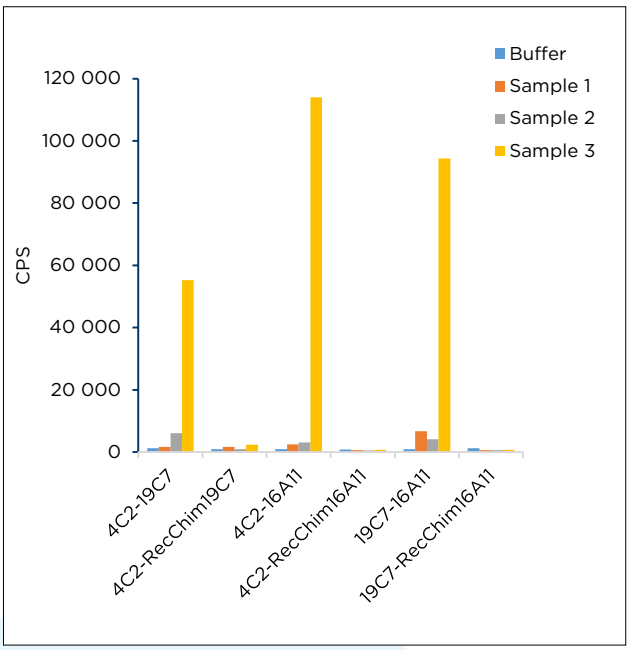


Figure 7. Chimeric antibodies mitigate the HAMA effect. The performance of chimeric and native 19C7 and 16A11 in the presence of HAMA was tested with three serum samples with varying HAMA concentrations: 807 ng/ml in Sample 1, 1388 ng/ml in Sample 2 and 6220 ng/ml in Sample 3. As a control, buffer without serum was used. Antibody pairs compared are indicated in the picture.

HyTest troponin complex selected as reference material

cTnI, which is extremely unstable in its free form, demonstrates significantly better stability in complex with TnC or in ternary cTnI-cTnT-TnC complex (not shown). These two forms of the protein are preferable as material for standard and calibrator preparation. In the troponin complex supplied by HyTest, cTnI is presented in the same form as it can be detected in the blood of AMI patients. Purification of the troponin complex is performed in mild conditions without treatment with urea containing buffers (as is usually done when preparing individual troponin components). The concentration is precisely determined for each of the three components of the complex.

In 2004, HyTest's troponin I-T-C complex was selected by the American Association for Clinical Chemistry Standardization Subcommittee to be used by assay manufacturers as reference material in troponin I assays. The certified reference material (SRM 2921) is available only from the National Institute of Standards and Technology.

> The certified reference material SRM 2921 based on HyTest's troponin complex is available from the National Institute of Standards and Technology (NIST).

MONOCLONAL ANTIBODIES

Cat.#	Product	Tested applications
4T21*	Monoclonal mouse anti-cardiac troponin I (cTnI)	Enzyme immunoassays Western blotting Immunoprecipitation Immunohistochemistry Immunoaffinity purification
4T21cc*	Monoclonal mouse anti-cardiac troponin I (cTnI), in vitro	Enzyme immunoassays Western blotting
RC4T21*	Recombinant chimeric anti-cTnI MAb	
4T45	Monoclonal mouse anti-cardiac troponin I (cTnI), phosphorylated form	Enzyme immunoassays Western blotting
4T46	Monoclonal mouse anti- cardiac troponin I (cTnI), dephosphorylated form	Enzyme immunoassays Western blotting
4TC2	Monoclonal mouse anti-human native cardiac troponin complex	Enzyme immunoassays
4T20*	Monoclonal mouse anti-skeletal muscle troponin I (skTnI)	Enzyme immunoassays Western blotting

* Several MAbs available under one catalogue number. Please see www.hytest.fi.

POLYCLONAL ANTIBODY

Cat.#	Product	Host	Tested applications
4T21/2	Polyclonal anti-cardiac troponin I (cTnI)	Goat	Immunoassays

ANTIGENS

Cat.#	Product	Source	Purity
8T53	Troponin I cardiac, human	Human cardiac muscle	>98%
8RTI7	Troponin I cardiac, human, recombinant	Recombinant	>95%
8T53dp	Troponin I cardiac, dephosphorylated	Human cardiac muscle	>95%
8T53ph	Troponin I cardiac, phosphorylated	Human cardiac muscle	>95%
8IC63	Troponin complex (I-C)	Human cardiac muscle	N/A
8T62	Troponin complex (ITC), human	Human cardiac muscle	N/A
8T62a	Troponin complex (ITC), artificial	Human cardiac muscle	N/A
8T25	Human skeletal TnI	Human skeletal muscle	>95%

Note. Animal specific antigens are also available. For more information please visit www.hytest.fi.

SERUM AND OTHER PRODUCTS

Cat.#	Product	Source/Remarks
8TFS	cTnI free serum	Pooled normal human serum
K01	Troponin I Diversity Kit	Different forms of human cTnI
8T60	Troponin I Calibrator set	Troponin complex in normal human serum

Cardiac troponin T (cTnT)

CLINICAL UTILITY

- **Acute myocardial infarction (AMI)**
- **Unstable angina**
- **AMI prognosis**
- **Cardiac muscle injury and cell death**

Cardiac troponin T (cTnT), along with cardiac troponin I (cTnI) is accepted as a "Golden marker" for myocardial infarction (MI) diagnosis. Both biomarkers are released into circulation with same kinetics and either of them can be used for the diagnosis of MI when tested using contemporary or point of care instruments. Moreover, cTnT and cTnI measurements by high-sensitivity assays could be helpful for long-term risk stratification of different patient groups with cardiac disease and/or for early rule-out or rule-in patients in Emergency Departments.

High-sensitivity cTnT assay prototypes

Assay prototypes utilizing our newly developed anti-cTnT monoclonal antibodies demonstrate a good linearity and superior sensitivity. The limit of detection (LoD) of both assays was better than 0.3 ng/l. A typical calibration curve for a purified cTnT in low concentration (0.14-100 ng/l) is presented in Figure 8.

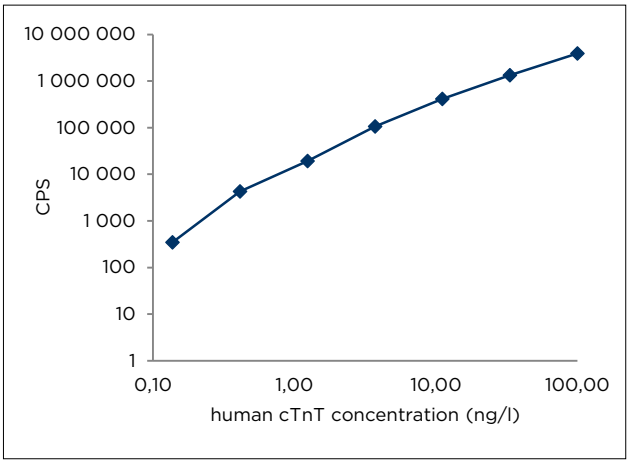


Figure 8. Calibration curve for a MAb combination 329-406. Purified native human cTnT (Cat.# 8T13) was used as the antigen.

AMI blood testing and correlation with a commercially available hs-cTnT assay

We studied the correlation of the two prototype immunoassays to a commercially available hs-cTnT assay by analyzing 38 serum samples from AMI patients. Figure 9 shows that there is a good correlation between cTnT values obtained with our assay prototypes and the commercially available hs-cTnT assay. The new cTnT MAbs allow for the development of highly sensitive immunoassays for the detection of cTnT in the blood of AMI patients with high specificity.

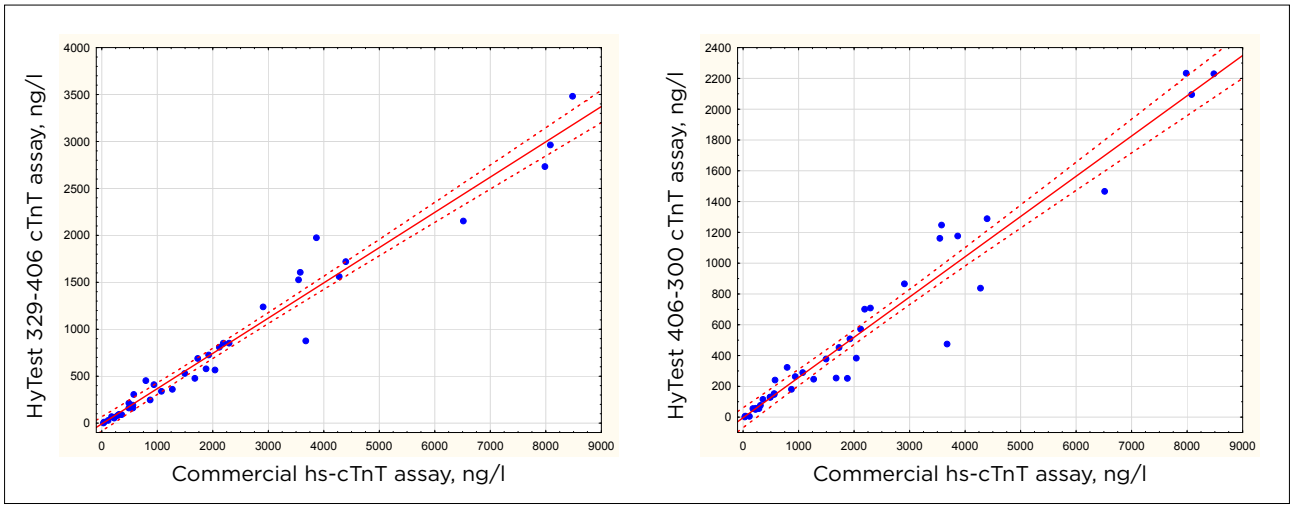


Figure 9. HyTest immunoassays show good correlation to a commercially available hs-cTnT assay. Concentration of cTnT in 38 serum samples obtained from AMI patients was determined by using two immunoassays utilizing HyTest antibodies (capture-detection pairs 329-406 and 406-300) and a commercially available hs-cTnT assay.

MONOCLONAL ANTIBODIES

Cat.#	Product	Tested applications
4T19*§	Monoclonal mouse anti-cardiac troponin T (cTnT)	Western blotting Affinity purification Immunohistochemistry Immunoprecipitation
4T19cc*	Monoclonal mouse anti-cardiac troponin T (cTnT), in vitro	Enzyme immunoassays

* Several MAbs available under one catalogue number. Please see www.hytest.fi.
§ Recommended for research purposes.

POLYCLONAL ANTIBODY

Cat.#	Product	Host	Tested applications
4T19/2	Polyclonal anti-cardiac troponin T (cTnT)	Goat	Enzyme immunoassays Western blotting Immunohistochemistry Immunoprecipitation

ANTIGENS

Cat.#	Product	Source	Purity
8T13	Human cardiac TnT	Human cardiac muscle	>98%
8RTT5	Human cardiac TnT, recombinant	Recombinant	>95%
8T24	Human skeletal TnT	Human skeletal muscle	>95%

Note. Animal specific antigens are also available. For more information please visit www.hytest.fi.

Human proBNP and its derivatives NT-proBNP and BNP

CLINICAL UTILITY

- **Identification or exclusion of heart failure (HF)**
- **Assessment of the severity of HF**
- **Prognosis of the disease development**
- **Monitoring of drug therapy in the presence of HF**

Pro-B-type natriuretic peptide (proBNP) derivatives BNP and NT-proBNP are established biomarkers in heart failure (HF) diagnostics (Figure 10). The concentrations of BNP and NT-proBNP in blood increase rapidly as a consequence of cardiac wall stretch and correlate with the severity of the disease. Analysis of BNP and NT-proBNP levels are used e.g. for exclusion of heart failure, risk stratification and as a prognostic marker of heart failure.

Information obtained from BNP and NT-proBNP studies conducted within the last few years has greatly improved our understanding of the properties and processing of proBNP. This, in turn, makes it easier to design immunoassays which reliably and quantitatively detect the biomarkers from clinical samples.

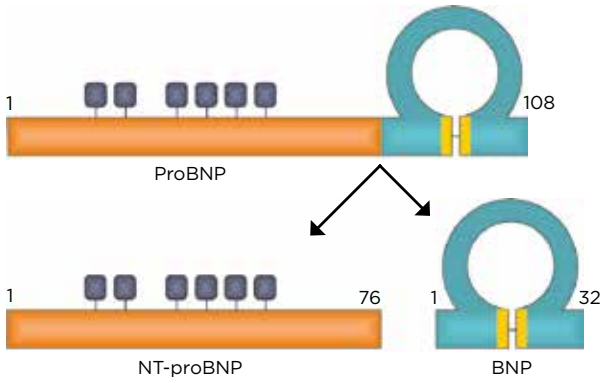


Figure 10. Schematic representation of proBNP processing. ProBNP is processed in a convertase-dependent reaction into NT-proBNP and BNP. BNP has biological activity whereas the role of NT-proBNP is unknown.

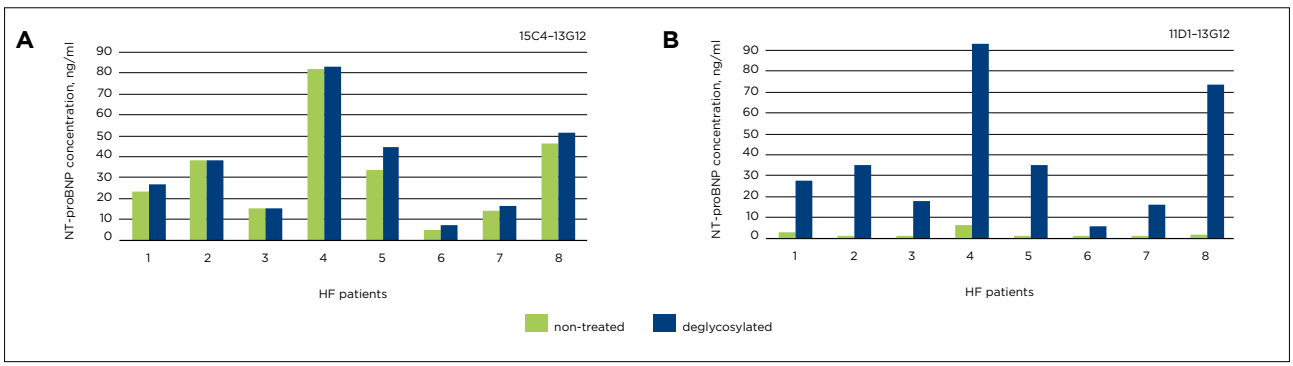


Figure 11. Immunoreactivity of endogenous NT-proBNP before and after deglycosylation. Concentration of endogenous NT-proBNP before (green columns) and after (blue columns) deglycosylation was measured in 8 HF patient samples by sandwich immunoassays. (A) MAbs 15C4$_{63-71}$ and 13G12$_{13-24}$ are specific to the N- or C-terminal parts of the molecule which are not glycosylated. (B) The capture MAb (11D1$_{31-39}$), recognizes an epitope located at the central region. This region of endogenous NT-proBNP becomes available for antibody recognition only after deglycosylation.

NT-proBNP detection is affected by glycosylation

Our studies have revealed that the majority of antibodies specific to the central part of the NT-proBNP molecule scarcely detect the antigen in human blood samples. We found that this is due to glycosylation of the central part of endogenous NT-proBNP. Glycosylation changes the epitope availability for antibody recognition, subsequently decreasing reliable quantitation of NT-proBNP with such antibodies (Figure 11 and Seferian et al., 2008). For precise NT-proBNP measurements in human blood, we recommend using a pair of antibodies specific to the N- and C-terminal parts of the NT-proBNP molecule.

BNP is an unstable molecule

BNP belongs to a family of peptide hormones that all have a 17 amino acid ring structure with a disulfide bond between two cysteine residues. BNP is an unstable molecule and it contains several protease cleavage sites. Especially the N-terminus is highly susceptible to degradation. The ring structure is relatively stable. In order to reliably detect BNP, it is recommended to choose antibodies that are less sensitive to proteolytic degradation of BNP.

Novel BNP immunoassay – SES-BNP™

We have developed a novel type of immunoassay for BNP. This Single Epitope Sandwich (SES) assay improves the precision and sensitivity of BNP measurements. In our proprietary SES-BNP™ assay the capture antibody (MAb 24C5) is specific to a stable ring part of the BNP molecule. The detection antibody (MAb Ab-BNP2) is specific to the complex formed by the capture antibody and BNP (or proBNP; Figure 12).

Benefits

- Equally recognizes proBNP+BNP and their truncated forms
- Enables extremely high sensitivity (1 pg/ml)
- Targeted at a stable epitope 11-17 a.a.r. of BNP

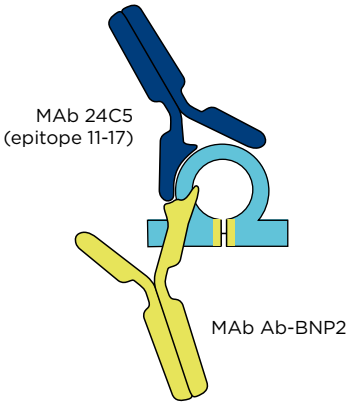


Figure 12. The SES-BNP assay principle. The capture antibody is specific to a stable ring part of BNP. The detection antibody recognizes only the complex formed by the capture antibody and BNP.

SES-BNP™
For more information about SES-BNP technology please visit www.hytest.fi/literature/ses-bnp or contact us at hytest@hytest.fi.

MONOCLONAL ANTIBODIES

Cat.#	Product	Tested applications
4BNP2*	Monoclonal mouse anti-human brain natriuretic peptide (BNP)	Enzyme immunoassays Western blotting
4BNP2cc*	Monoclonal mouse anti-human brain natriuretic peptide (BNP), in vitro	Enzyme immunoassays Western blotting
4BFab5	Monoclonal mouse anti-immune complex (24C5-BNP/proBNP)	Enzyme immunoassays
4BFab5cc	Monoclonal mouse anti-immune complex (24C5-BNP/proBNP), in vitro	Enzyme immunoassays
4NT1*	Monoclonal mouse anti-human N-terminal proBNP (NT-proBNP)	Enzyme immunoassays Western blotting

* Several MAbs available under one catalogue number. Please see www.hytest.fi.

ANTIGEN

Cat.#	Product	Source	Purity
8NT2	NT-proBNP, recombinant	Recombinant	>95%
8PRO9	ProBNP, recombinant	Recombinant	>95%
8GOB2	ProBNP, glycosylated, recombinant	Recombinant	>95%

DEPLETED PLASMA

Cat.#	Product	Source/Remarks
8BFP	BNP and NT-proBNP free plasma	Pooled normal human plasma

Lipoprotein-associated phospholipase A2 (Lp-PLA2)

CLINICAL UTILITY

- **Prognostic marker of adverse cardiac-related events**

Lipoprotein-associated phospholipase A2 (Lp-LA2, which is also known as the platelet-activating factor acetyl hydrolase) is a Ca^{2+}-independent phospholipase that circulates in the bloodstream in the form of a complex with lipoprotein particles (Stafforini, 2009; 2015). Lp-PLA2 levels have been shown to predict adverse cardiac-related events in both patients with stable coronary artery disease (Brilakis et al., 2005) and in a healthy adult population (Ballantyne et al., 2004). The increase in Lp-PLA2 levels can predict the development of incident peripheral arterial disease in humans (Garg et al., 2016).

Recent guidelines from four major international societies, which include the European Society of Cardiology, the American College of Cardiology, the American Heart Association and the American Society of Endocrinology, have included Lp-PLA2 among the biomarkers whose measurement could be useful for risk stratification of asymptomatic adult patients.

Recombinant human Lp-PLA2

HyTest provides recombinant human Lp-PLA2 (recLp-PLA2) that is expressed in a mammalian cell line. The protein contains 6×His tag on its C-terminus linked with a GG spacer.

Upon serial dilutions, recombinant Lp-PLA2 and endogenous Lp-PLA2 in normal human serum showed the same pattern of signal decrease in sandwich fluoroimmunoassays employing the MAb combination PL42cc–PL46cc (see Figure 13). This demonstrates that the immunochemical properties of recombinant Lp-PLA2 are similar to those of native Lp-PLA2.

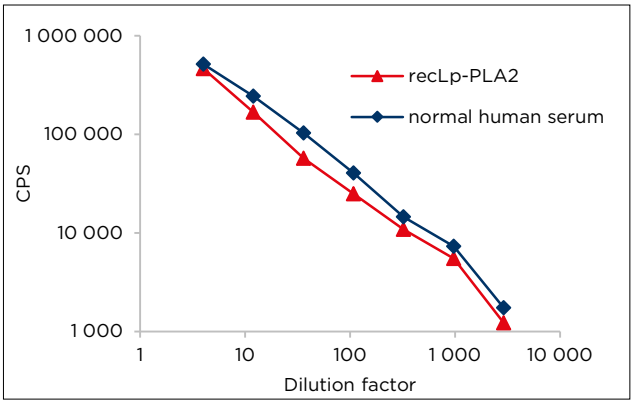


Figure 13. Dilutional linearity study. Dilutional linearity study of recombinant Lp-PLA2 and native Lp-PLA2 (normal human serum from an apparently healthy volunteer) studied using the MAb combination PL42cc–PL46cc. The initial concentration of the recombinant human Lp-PLA2 was 111 ng/ml.

Measuring patient samples

We provide several different monoclonal antibodies specific to human Lp-PLA2 that allow the development of quantitative immunoassays for detecting endogenous Lp-PLA2 in serum samples. When compared to a commercially available ELISA kit, the HyTest antibodies detected Lp-PLA2 in a manner that was very similar or slightly different to the commercial kit (see Figure 14).

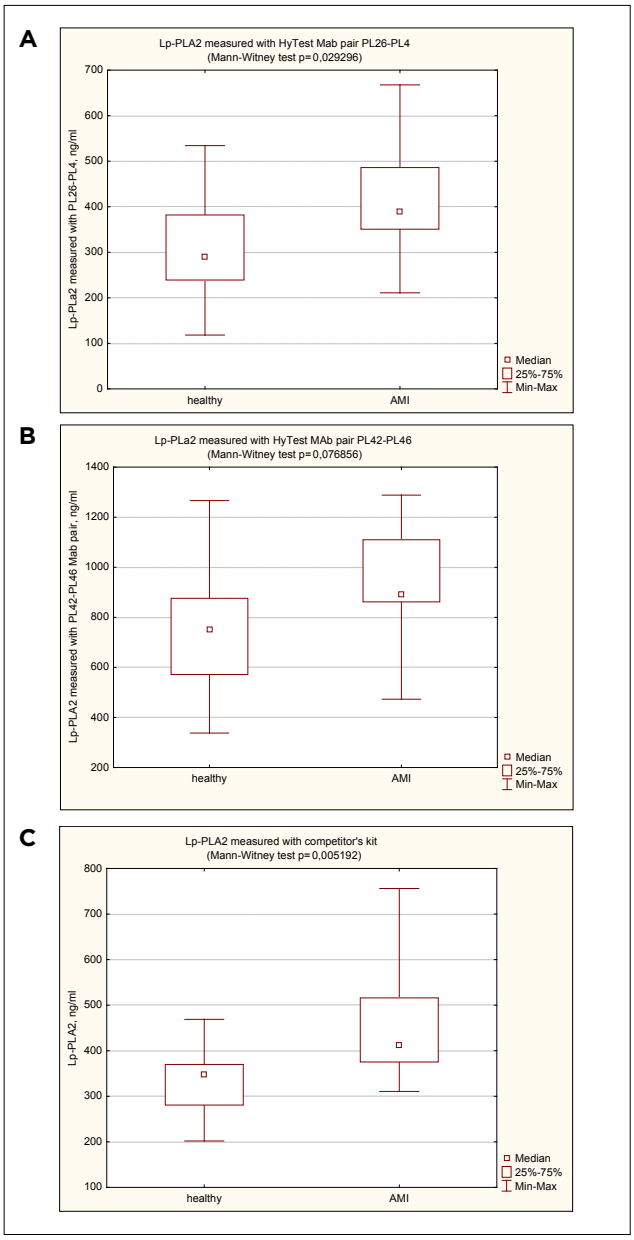


Figure 14. Detection of native Lp-PLA2 in serum samples. Lp-PLA2 was detected in serum of acute myocardial infarction patients and healthy volunteers using the HyTest fluoroimmunoassay with the MAb pair PL26cc–PL4cc (A) and PL42cc–PL46cc (B) or by using a commercially available ELISA kit (C). Serum samples were diluted 1:30 with an assay buffer (A and B) or according to the manufacturer's instructions (C). Samples were incubated for 2.5 hours at 37°C (A and B) or for 3 hours at room temperature (C).

MONOCLONAL ANTIBODIES

Cat.#	Product	Tested applications
4LA7cc*	Monoclonal mouse anti-human lipoprotein-associated phospholipase A2 (Lp-PLA2), in vitro	Enzyme immunoassays Western blotting

* Several MAbs available under one catalogue number. Please see www.hytest.fi.

ANTIGEN

Cat.#	Product	Source	Purity
8PL7	Recombinant human lipoprotein-associated phospholipase A2 (Lp-PLA2)	Recombinant	>75%

Pregnancy Associated Plasma Protein A (PAPP-A)

CLINICAL UTILITY

- **Acute myocardial infarction**
- **Acute coronary syndrome**
- **Unstable angina**
- **Down syndrome**

The pregnancy-associated plasma protein-A (PAPP-A) has been used as a biochemical marker for Down syndrome in the first trimester of pregnancy for a long time. In addition to this, several studies show that PAPP-A is a promising marker for cardiac diseases.

PAPP-A can be found in blood in two different forms: as a heterotetrameric complex (htPAPP-A) and as a homodimeric complex (dPAPP-A) (Figure 15). Of these, the dimeric form is the one associated with cardiac diseases. dPAPP-A is abundantly expressed in unstable coronary atherosclerotic plaques (Bayes-Genis et al., 2001).

It has been demonstrated that the level of dPAPP-A in the blood is significantly elevated in patients with unstable angina or acute myocardial infarction, in comparison to patients with stable angina and control subjects (Heeschen et al., 2005, Hájek et al., 2008). In addition, dPAPP-A has also been shown to be a strong independent marker of risk stratification for patients with acute coronary syndrome (ACS) (Qin et al., 2002). In their literature search report, Richard Body and Craig Ferguson (2006) concluded that PAPP-A is a promising biomarker for unstable coronary disease and that it also could have great potential as a prognostic marker as part of a multimarker strategy.

dPAPP-A specific sandwich immunoassay

Our dPAPP-A specific monoclonal antibodies enable the development of immunoassays that are suitable for selective quantitative measurements of dPAPP-A in human blood, even in the presence of ht-PAPP-A (Figures 16 and 17).

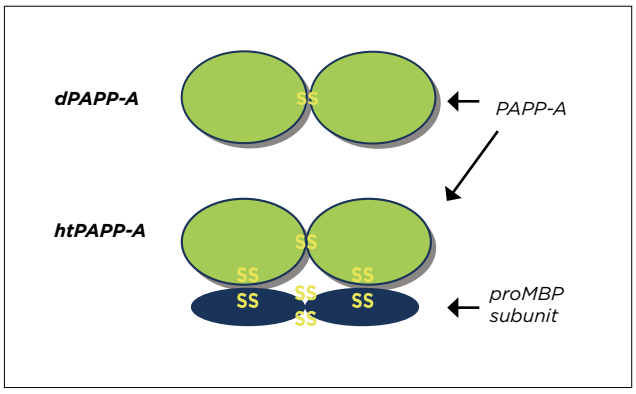


Figure 15. Structure of PAPP-A. PAPP-A is a metalloprotease that belongs to the metzincin superfamily of zinc peptidases. Homodimeric dPAPP-A consists of two 200 kDa PAPP-A subunits covalently linked with a disulfide bond. Heterotetrameric htPAPP-A includes two PAPP-A subunits and two 50-90 kDa subunits of the preform of the eosinophil major basic protein (proMBP), all covalently linked with disulfide bonds. It has been shown that proMBP has inhibitory properties against the protease activity of PAPP-A.

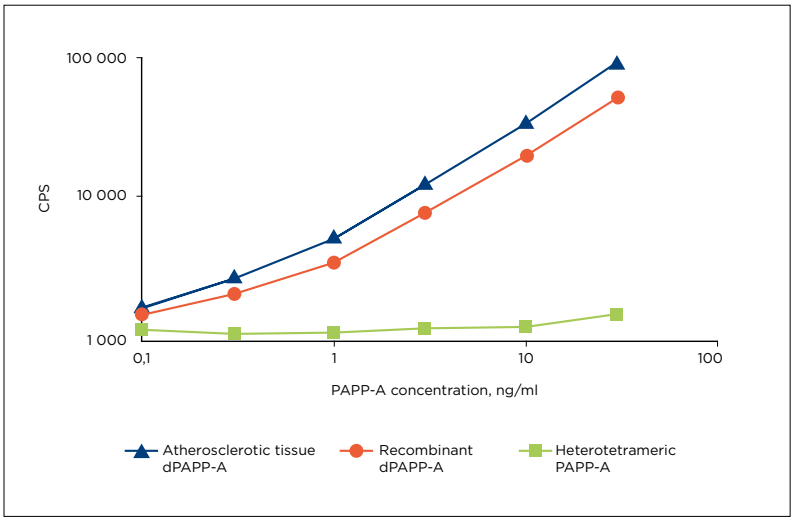


Figure 16. Calibration curves for dPAPP-A specific immunoassay. The detection limit of this assay was < 0.3 ng/ml. dPAPP-A specific MAbs do not detect the heterotetrameric form. Capture MAb: PAPP2 (Cat.# 4PD4) Detection MAb: 7A6 (Cat.# 4P41; labeled with Eu^{3+} chelate)
Incubation volume: 100 µl
Incubation time: 30 min at room temperature

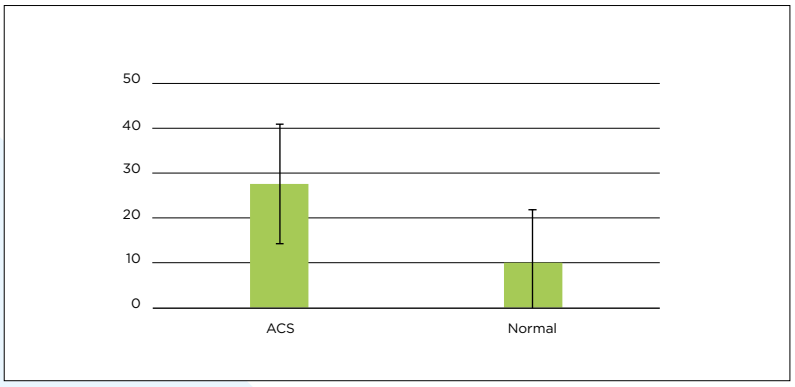


Figure 17. dPAPP-A measured in clinical samples. Concentration of dPAPP-A in plasma samples of 43 ACS patients (ACS) and 34 non-ACS patients control group (Normal) measured by PAPP52 – PAPP30 sandwich immunoassay (mean+/-SD). Capture MAb: PAPP52 (Cat.# 4P41) Detection MAb: PAPP30 (Cat.# 4PD4; labeled with Eu^{3+} chelate)
Incubation volume: 100 µl.
Incubation time: 30 min at room temperature.

The level of dPAPPA in the blood is significantly elevated in patients with unstable angina or acute myocardial infarction.

MONOCLONAL ANTIBODIES

Cat.#	Product	Tested applications
4P41*	Monoclonal mouse anti-human pregnancy-associated plasma protein A (PAPP-A)	Enzyme immunoassays Western blotting
4PD4*	Monoclonal mouse anti-human dimeric form of pregnancy-associated plasma protein A (dPAPP-A)	Enzyme immunoassays

* Several MAbs available under one catalogue number. Please see www.hytest.fi.

ANTIGEN

Cat.#	Product	Source	Purity
8P64	PAPP-A, heterotetrameric form (htPAPP-A)	Pooled retroplacental blood	>85%
8P97	PAPP-A, homodimeric form (dPAPP-A), recombinant	Recombinant	>90%

Insulin-like growth factor binding protein-4 (IGFBP-4) and its fragments

CLINICAL UTILITY

- **Prediction of major adverse cardiac events**
- **Acute myocardial infarction**
- **Acute coronary syndrome**
- **Unstable angina**

IGFBP-4 has been shown to be a substrate for dPAPP-A (Figure 18). dPAPP-A is a promising marker for predicting plaque rupture which, in turn, may lead to acute thrombosis. However, measuring dPAPP-A concentrations reliably is challenging due to many reasons (Terkelsen et al., 2009, Tertti et al., 2009). Our studies indicate that quantifying N- and C-terminal IGFBP-4 fragments (NT-IGFBP-4 and CT-IGFBP-4, respectively) that are the result of dPAPP-A cleavage instead of dPAPP-A could be used as an indirect but more reliable method for obtaining information about dPAPP-A concentration and, consequently, for predicting the rupture of vulnerable plaques (Postnikov et al., 2012).

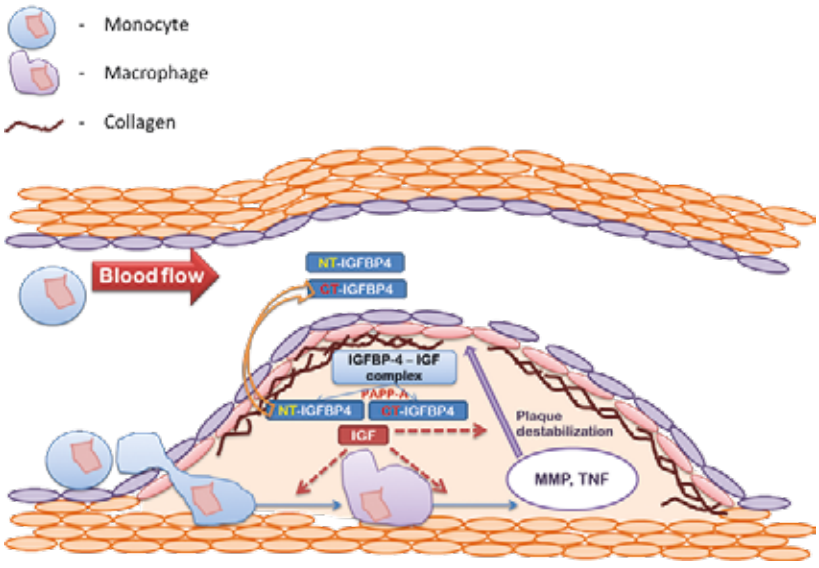


Figure 18. Schematic representation of dPAPP-A dependent cleavage of IGFBP-4 in unstable atherosclerotic plaque. dPAPP-A cleaves IGFBP-4 (preferably complexed with IGF) into NT-IGFBP4 and CT-IGFBP-4 fragments. As a result, IGF is released and activated. IGFBP-4 fragments are released into circulation and can be detected from blood.

Immunoassays for quantifying NT-IGFBP4 and CT-IGFBP4

In order to quantify IGFBP-4-fragments, we have generated MAbs specific to epitopes available for MAb binding only after the proteolytic cleavage of IGFBP-4 by dPAPP-A (Figure 19). Cross-reactivity of these neoepitope-specific MAbs with full-length IGFBP-4 is negligible (1.4% or less), thus allowing for specific quantitation of cleaved fragments regardless of the presence of non-cleaved IGFBP-4.

> We have generated MAbs specific to epitopes available for MAb binding only after the proteolytic cleavage of IGFBP-4 by dPAPP-A.

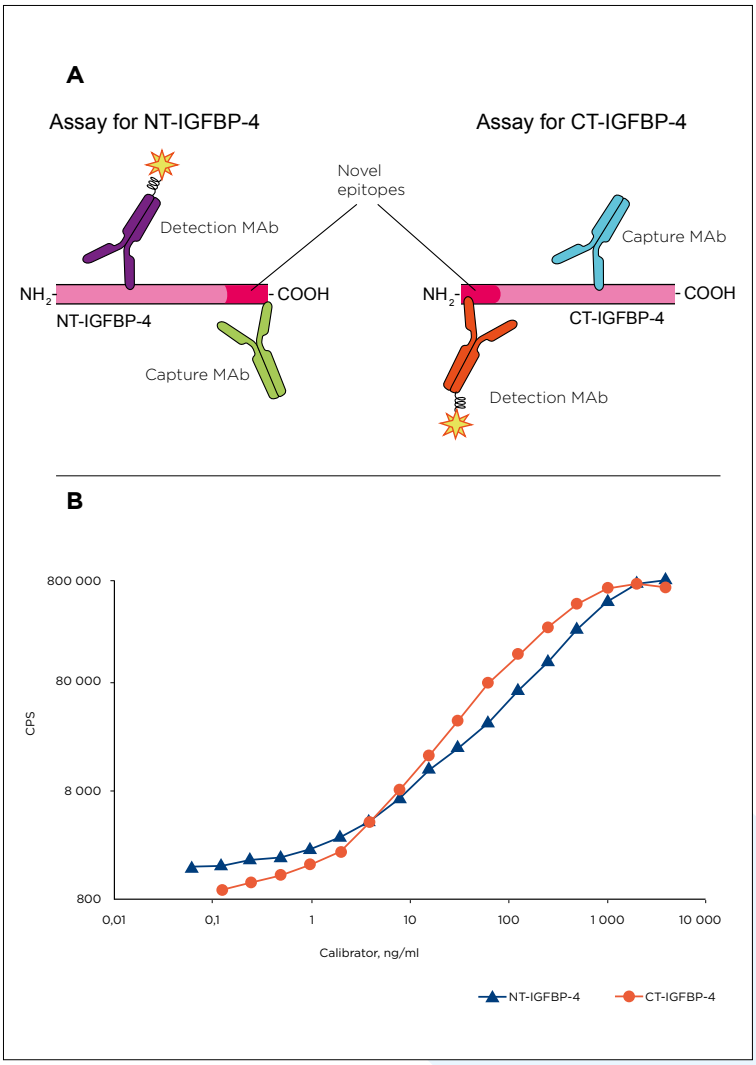


Figure 19. Sandwich immunoassays for detecting NT- and CT-IGFBP-4. (A) Schematic representation of the assays and capture/detection MAbs chosen for each assay. (B) Representative immunoassays with purified recombinant NT- and CT-IGFBP-4 fragments.

MONOCLONAL ANTIBODIES

Cat.#	Product	Tested applications
4IGF4*	Monoclonal mouse anti-Insulin-like growth factor binding protein 4 (IGFBP-4)	Enzyme immunoassays

* Several MAbs available under one catalogue number. Please see www.hytest.fi.

ANTIGEN

Cat.#	Product	Source	Purity
8IGF4	IGFBP-4, human, recombinant	Recombinant	>90%
8NFB4	NT-IGFBP-4, human, recombinant	Recombinant	>95%
8CIG4	CT-IGFBP-4, human, recombinant	Recombinant	>95%

C-reactive protein (CRP)

CLINICAL UTILITY

- **Prediction of future cardiovascular risk**
- **Inflammation**

C-reactive protein (CRP) is one of the so called acute phase proteins. Its concentration in blood increases rapidly as a response to inflammation.

In recent years, more information has been obtained about the possible role of inflammation in contributing to the development of serious health issues such as diabetes or the development of cardiovascular diseases. These studies show that elevated basal levels of CRP indicate increased risk for cardiac diseases, thus making CRP a promising biomarker for predicting future development of a heart disease. Many epidemiologic studies have indicated that CRP is a strong independent predictor of future cardiovascular events, including myocardial infarction, ischemic stroke, peripheral vascular disease, and sudden cardiac death without a known cardiovascular disease (as reviewed by Clearfield, 2005).

High sensitivity CRP (hsCRP) immunoassays

In 2003, the Centers for Disease Control and Prevention (CDC) and the American Heart Association (AHA) issued a statement that identified CRP as the inflammatory marker best suited for use in current clinical practice to assess cardiovascular risk (Ridker, 2003). While the CRP level in blood can rapidly increase to tens or even hundreds of milligrams per liter during an acute inflammation, it is the basal level of blood CRP that has more clinical significance when predicting future cardiac diseases (Scirica et al., 2007; Koenig et al., 2008). This is why present day hsCRP assays are aimed at nanogram per milliliter (ng/ml) CRP level distinction (Figure 20).

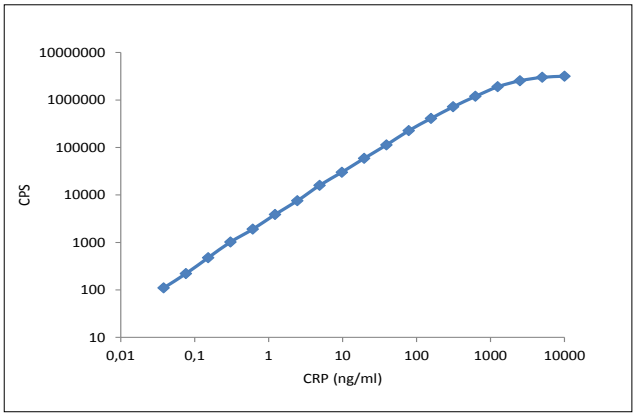


Figure 20. Immunodetection of CRP standard in a sandwich immunoassay by MAb pair C2-C6. MAb C2 is biotinylated, MAb C6 is labeled with stable Eu^{3+} chelate. The mixture of antibodies and antigen samples (100 µl) was incubated for 10 min at room temperature in streptavidin coated plates.

Antibodies for hsCRP immunoassays

Anti-CRP antibodies developed by HyTest have been utilized in several immunoassays achieving excellent sensitivity and a broad linear detection range (Meyer et al., 2007; Shiesh et al., 2006; Sin et al., 2006). These antibody combinations could be used for the development of hsCRP assays for different diagnostic platforms. In addition, for the convenience of our customers, we have monoclonal antibodies with different affinity (Figure 21 and Table 1), thus enabling them to be used in different types of immunoassays.

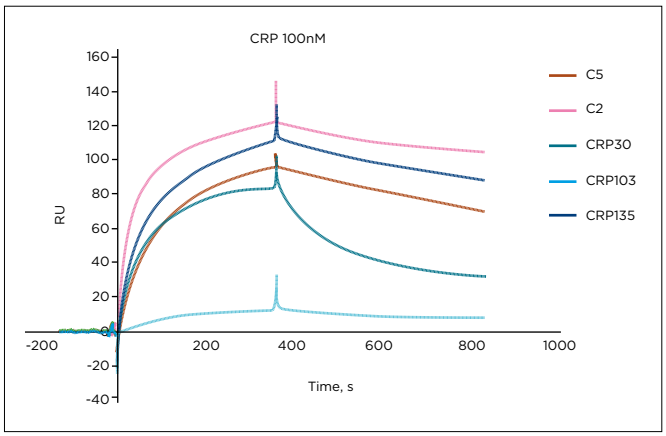


Figure 21. Biacore X sensograms of five different anti-CRP MAbs. 100 nM native CRP was exposed to the chip-immobilized MAbs in HBS-EP buffer (0.01 M HEPES, 0.15 M NaCl, 3 mM EDTA, 0.005% polysorbate 20, pH 7.4).

Table 1. Affinity constants for selected anti-CRP MAbs.

	Kd (M)
C2	1.93×10^{-9}
C5	1.7×10^{-8}
CRP30	4.3×10^{-8}
CRP103	5.2×10^{-8}
CRP135	4.4×10^{-9}

MONOCLONAL ANTIBODIES

Cat.#	Product	Tested applications
4C28*	Monoclonal mouse anti-human C-reactive protein (high sensitivity CRP)	Enzyme immunoassays (also high sensitivity) Western blotting Turbidimetric assays Immunohistochemistry Immunoaffinity purification
4C28cc	Monoclonal mouse anti-human C-reactive protein (high sensitivity CRP), *in vitro*	Enzyme immunoassays (high sensitivity)

* Several MAbs available under one catalogue number. Please see www.hytest.fi.

ANTIGEN

Cat.#	Product	Source	Purity
8C72	C-reactive protein	Human pleural/ascetic fluid or plasma	>95%

DEPLETED SERUM

Cat.#	Product	Source/Remarks
8CFS	C-reactive protein free serum	Pooled normal human serum

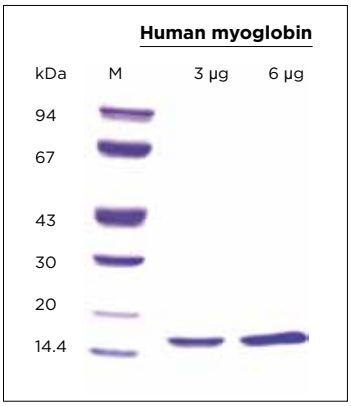
Myoglobin

CLINICAL UTILITY

- **Myocardial damage**
- **Acute myocardial infarction**

Myoglobin has been used as a marker of myocardial damage for almost six decades. It is commonly used in clinical practice as an early marker of AMI (Penttilä et al., 2002). However, due to the high concentration of myoglobin in skeletal muscle tissues, even minor skeletal muscle injury increases the myoglobin levels in blood (van Nieuwenhoven et al., 1995). Therefore, myoglobin alone is not considered to be a reliable and sufficient marker in AMI diagnosis. Instead, it should be used together with cTnI or cTnT analysis as part of a multi-marker strategy.

Figure 22. SDS-PAGE of human myoglobin. M is molecular weight standard (Pharmacia).



MONOCLONAL ANTIBODIES

Cat.#	Product	Tested applications
4M23*	Monoclonal mouse anti-human cardiac myoglobin	Enzyme immunoassays

* Several MAbs available under one catalogue number. Please see www.hytest.fi.

ANTIGEN

Cat.#	Product	Source	Purity
8M50	Myoglobin	Human cardiac muscle	>95%

DEPLETED SERUM

Cat.#	Product	Source/Remarks
8MFS	Myoglobin free serum	Pooled normal human serum

Myeloperoxidase (MPO)

CLINICAL UTILITY

- **Acute coronary syndrome**
- **Coronary artery disease**
- **Cardiovascular disease risk stratification**
- **Prediction of long term incident major adverse cardiac events**

Myeloperoxidase (MPO) is a peroxidase enzyme that is abundantly secreted by activated leukocytes (neutrophils) during an inflammation reaction. Within the last decade, multiple studies have indicated that MPO is a promising cardiac marker. Brennan et al. (2003) showed that unlike troponins, CK-MB and CRP, MPO facilitates the identification of patients that are at risk for cardiac events in the absence of myocardial necrosis. It was also demonstrated that an increased level of MPO in a patient's blood serves as a risk marker for atherosclerosis (Nambi, 2005) and coronary artery disease (Zhang et al., 2001).

Our anti-MPO antibodies have been tested with clinical samples.

Sandwich immunoassay for quantitative MPO detection

All our MAbs have been screened to provide sensitive and specific detection of endogenous MPO with good kinetics. In addition, we have tested our most sensitive two site MAb combinations with blood samples containing high titers of MPO autoantibodies and we have selected MAb combinations that are less sensitive to autoantibodies.

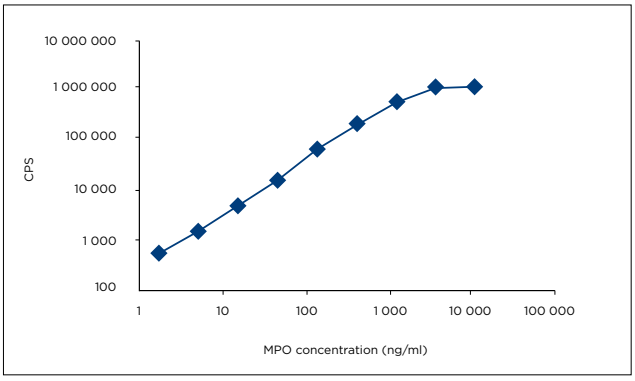


Figure 23. Calibration curve for a MPO sandwich immunoassay. MAbs 16E3 and 18B7 were used as capture and detection antibodies, respectively. Native purified human MPO was used as the antigen.

MONOCLONAL ANTIBODIES

Cat.#	Product	Tested applications
4M43*	Monoclonal mouse anti-human myeloperoxidase (MPO)	Enzyme immunoassays Western blotting

* Several MAbs available under one catalogue number. Please see www.hytest.fi.

ANTIGEN

Cat.#	Product	Source	Purity
8M80	Myeloperoxidase	Human leukocyte mass	>90%

DEPLETED SERUM

Cat.#	Product	Source/Remarks
8MPFS	Myeloperoxidase free serum	Pooled normal human serum

Fatty acid binding protein (FABP)

CLINICAL UTILITY

- **Acute coronary syndrome**
- **Myocardial injury**

Fatty acid-binding proteins (FABPs) are a group of small cytoplasmic proteins that are abundant in tissues with active fatty acid metabolism. This includes the heart (Storch and Thumser, 2000). The heart-type fatty acid binding protein (H-FABP) is an early marker of myocardial injury and is widely applied in emergency triage of patients with acute coronary syndromes (Alhadi and Fox, 2004). H-FABP is considerably more cardio-specific than myoglobin, another early AMI marker. However, it is less cardio-specific than troponins due to the fact that some H-FABP is also expressed in skeletal muscle tissues.

Various MAb pairs can be used for H-FABP measurement from blood samples of AMI patients

Our anti-FABP antibodies allow for the development of quantitative, highly sensitive immunoassays for the detection of H-FABP (Figure 24). All MAbs have been tested with clinical samples.

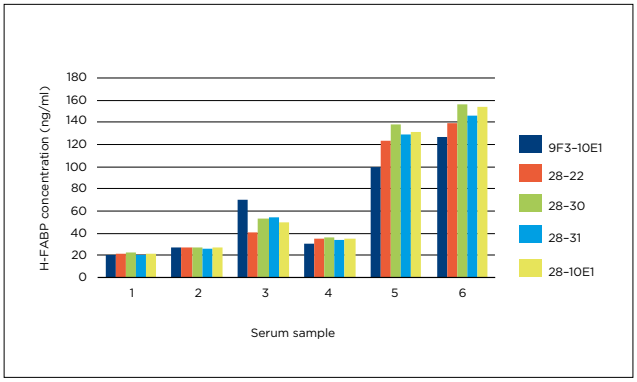


Figure 24. Detection of H-FABP in serum samples of AMI patients. Comparison of H-FABP measurements in sera of six AMI patients using different antibody combinations in sandwich immunoassays.

MONOCLONAL ANTIBODIES

Cat.#	Product	Tested applications
4F29*	Monoclonal mouse anti-human fatty acid binding protein (FABP)	Enzyme immunoassays Western blotting

* Several MAbs available under one catalogue number. Please see www.hytest.fi.

ANTIGEN

Cat.#	Product	Source	Purity
8F65	Fatty acid binding protein	Human cardiac muscle	>95%

DEPLETED SERUM

Cat.#	Product	Source/Remarks
8FFS	Fatty acid binding protein free serum	Pooled normal human serum

Other markers of cardiovascular disease

Troponin C (TnC)

Troponin C (TnC) is the Ca^{2+}-binding subunit of the troponin complex. In human muscle cells, it exists in two different isoforms, fast and slow. In myocardium, TnC is presented by the slow skeletal isoform.

TnC forms high affinity complexes with cTnI. It was demonstrated that cTnI is presented mainly as a complex with TnC in the blood stream of AMI patients (Katrukha et al., 1997). In the binary cTnI-TnC complex, TnC protects cTnI from protease cleavage. Therefore, TnC can be used as a natural stabilizer of cTnI in water solutions (Katrukha et al., 1998).

MONOCLONAL ANTIBODIES

Cat.#	Product	Tested applications
4T27*	Monoclonal mouse anti-troponin C (TnC)	Enzyme immunoassays Western blotting
4T27cc	Monoclonal mouse anti-troponin C (TnC), *in vitro*	Enzyme immunoassays Western blotting
4TC2*	Monoclonal mouse anti-human native cardiac troponin complex	Enzyme immunoassays

* Several MAbs available under one catalogue number. Please see www.hytest.fi.

ANTIGENS

Cat.#	Product	Source	Purity
8T57	Troponin C, human	Human cardiac muscle	>98%
8RSC4	Recombinant human slow skeletal/cardiac troponin C (TnC)	Recombinant	>95%
8RKC3	Recombinant human troponin C skeletal muscle, isoform 2	Recombinant	>95%

Note. Animal specific antigens are also available. For more information please visit www.hytest.fi.

D-dimer and high molecular weight fibrin degradation products

Elevated levels of D-dimer are found in the blood of patients with pulmonary embolism, deep vein thrombosis and atherosclerosis. D-dimer diagnostic tests are widely used to exclude the diagnosis of deep vein thrombosis. In addition, an increased amount of D-dimer in blood is believed to be a reliable marker of the pathological coagulation that underlies the pathogenesis of most cardiovascular diseases.

MONOCLONAL ANTIBODIES

Cat.#	Product	Tested applications
4D30*	Monoclonal mouse anti-D-dimer	Enzyme immunoassays Western blotting

* Several MAbs available under one catalogue number. Please see www.hytest.fi.

ANTIGENS

Cat.#	Product	Source	Purity
8D70	D-dimer	Human plasma	>90%

Soluble CD40 ligand (sCD40L)

CD40 ligand (CD40L) is a member of the tumor necrosis factor (TNF) family and is expressed on the surface of CD4+ T-cells, basophiles, platelets and mast cells. The binding of CD40L to its receptor CD40 mediates various inflammatory processes in cells. Soluble CD40 ligand (sCD40L) is formed upon the cleavage of CD40L. This soluble form has been shown to act as a cytokine. Some studies suggest that an increased level of sCD40L in blood might correlate with acute and chronic heart failure and the severity of the disease.

MONOCLONAL ANTIBODIES

Cat.#	Product	Tested applications
4CD40*	Monoclonal mouse anti-soluble CD40 ligand (sCD40L)	Enzyme immunoassays Western blotting

* Several MAbs available under one catalogue number. Please see www.hytest.fi.

Cystatin C

Cystatin C is a small protease inhibitor that is constantly expressed and secreted by most nucleated cells. In clinical practice, it is a well-described serum marker of renal failure. In addition, due to the link between impaired renal function and cardiovascular risk and diseases, cystatin C is also investigated for its putative role as a cardiac marker.

MONOCLONAL ANTIBODIES

Cat.#	Product	Tested applications
4CC1*	Monoclonal mouse anti-cystatin C	Enzyme immunoassays Western blotting

* Several MAbs available under one catalogue number. Please see www.hytest.fi.

POLYCLONAL ANTIBODY

Cat.#	Product	Host	Tested applications
PCC2	Polyclonal anti-cystatin C	Sheep	Enzyme immunoassays Western blotting Immunoprecipitation

ANTIGENS

Cat.#	Product	Source	Purity
8CY5	Cystatin C, human, recombinant	Recombinant	>95%
8CN4	Cystatin C, human	Pooled human serum, available only in research amounts	>95%

DEPLETED SERUM

Cat.#	Product	Source/Remarks
8CCFS	Cystatin C free serum	Pooled normal human serum

Human serum albumin (HSA)

Albumin is produced by liver cells and it is the main protein found in human plasma. Albumin has several different functions. For example: regulating the filtration and adsorption of fluid across capillary walls and transporting different substances in blood. The concentration of albumin is often measured from either blood or urine, and it can be used as a marker of dehydration, malnutrition, liver or kidney disease and also as a marker for cardiovascular diseases.

MONOCLONAL ANTIBODIES

Cat.#	Product	Tested applications
4T24*	Monoclonal mouse anti-human serum albumin	Enzyme immunoassays Western blotting

* Several MAbs available under one catalogue number. Please see www.hytest.fi.

Procalcitonin (PCT)

Procalcitonin (PCT) is a small protein that is synthesized by the C-cells in thyroid glands. In cells, PCT is further cleaved into three molecules: N-terminal fragment (N-terminal PCT), calcitonin and katacalcin.

PCT is considered to be the main marker of disorders that are accompanied by systemic inflammation and sepsis. In addition to sepsis and infection, the level of PCT can increase e.g. as a result of surgery, polytrauma, heat shock, burn injuries or cardiogenic shock. Monitoring PCT levels after cardiac surgery or heart transplantation helps to differentiate an acute graft rejection from bacterial or fungal infections.

MONOCLONAL ANTIBODIES

Cat.#	Product	Tested applications
4C10*	Monoclonal mouse anti-human calcitonin	Enzyme immunoassays Western blotting
4C10cc	Monoclonal mouse anti-human calcitonin, *in vitro*	Enzyme immunoassays
4PC47*	Monoclonal mouse anti-human procalcitonin (PCT)	Enzyme immunoassays Western blotting

* Several MAbs available under one catalogue number. Please see www.hytest.fi.

POLYCLONAL ANTIBODY

Cat.#	Product	Host	Tested applications
PPC3	Polyclonal anti-procalcitonin (PCT)	Goat	Enzyme immunoassays

ANTIGENS

Cat.#	Product	Source	Purity
8PC5	Procalcitonin, tag-free, recombinant	Recombinant	>95%

Glycogen phosphorylase isoenzyme BB (GPBB)

Glycogen phosphorylase isoenzyme BB (GPBB) plays an important role in glycogen turnover. BB isoform is synthesized by cardiac and brain tissues. GPBB is considered to be an early marker of myocardial cell death and its release kinetics closely resemble those of myoglobin and FABP. GPBB could be a useful marker in the diagnosis of acute coronary syndrome and unstable angina.

MONOCLONAL ANTIBODIES

Cat.#	Product	Tested applications
4GP31*	Monoclonal mouse anti-glycogen phosphorylase isoenzyme BB (GPBB)	Enzyme immunoassays Western blotting

* Several MAbs available under one catalogue number. Please see www.hytest.fi.

ANTIGENS

Cat.#	Product	Source	Purity
8G67	Glycogen phosphorylase BB isoenzyme	Human cardiac muscle	>95%

Serum amyloid A (SAA)

Serum amyloid A proteins form a family of apolipoproteins that are mostly associated with high density lipoprotein (HDL). The acute phase SAA proteins SAA1 and SAA2 are secreted into the blood following interleukin-6 stimulation in response to infection, inflammation, injury or stress. Acute phase proteins are intensively studied as potential markers that can predict the development of cardiovascular diseases and SAA has proven to be a good candidate for a cardiac biomarker.

MONOCLONAL ANTIBODIES

Cat.#	Product	Tested applications
4SA11*	Monoclonal mouse anti-serum amyloid A (SAA)	Enzyme immunoassays Western blotting
4VS4*	Monoclonal mouse anti-serum amyloid A (SAA), animal	Enzyme immunoassays Western blotting

* Several MAbs available under one catalogue number. Please see www.hytest.fi.

Retinol-binding protein 4 (RBP4)

Retinol-binding protein 4 (RBP4) belongs to the lipocalin family of proteins and functions as a carrier protein for vitamin A in serum. RBP4 is shown to play an important role in insulin resistance. Recently, several studies have suggested that RBP4 levels in blood may be associated with cardiovascular diseases as well as metabolic syndrome.

MONOCLONAL ANTIBODIES

Cat.#	Product	Tested applications
4RB2*	Monoclonal mouse anti-human retinol-binding protein 4 (RBP4)	Enzyme immunoassays Western blotting

* Several MAbs available under one catalogue number. Please see www.hytest.fi.

ANTIGENS

Cat.#	Product	Source	Purity
8RF9	Retinol-binding protein 4 from human plasma, free form	Pooled human plasma	>95%
8RP7	Retinol-binding protein 4 from human plasma, complexed with transthyretin	Pooled human plasma	>95%

Soluble lectin-like oxidized LDL receptor (sLOX-1)

Soluble lectin-like oxidized LDL receptor (sLOX-1) is produced by the proteolytic cleavage of the extracellular domain of LOX-1. LOX-1 is a transmembrane protein that is for example found on the cell surface of endothelial cells and smooth muscle cells. The serum level of sLOX-1 is increased in atherosclerotic conditions and with inflammation. A few studies suggest that sLOX-1 could serve as a biomarker for plaque rupture and that it might have clinical value in diagnosing atherosclerosis-related diseases.

MONOCLONAL ANTIBODIES

Cat.#	Product	Specificity	Tested applications
4LOX1*	Monoclonal mouse anti-sLOX-1	Human recombinant sLOX-1_{58-273}	Enzyme immunoassays Western blotting

* Several MAbs available under one catalogue number. Please see www.hytest.fi.

Adiponectin (Adn)

Adiponectin is an abundant protein hormone that belongs to the so-called adipokines family. It is expressed mostly by adipocytes and it is an important regulator of lipid and glucose metabolism. It is established that adiponectin is an insulin-sensitizing hormone with anti-diabetic, anti-inflammatory and anti-atherogenic properties. Adiponectin levels in serum have been shown to correlate with various life-style related diseases, including atherosclerosis and heart failure.

MONOCLONAL ANTIBODIES

Cat.#	Product	Tested applications
2AN6*	Monoclonal mouse anti-human adiponectin	Enzyme immunoassays Western blotting

* Several MAbs available under one catalogue number. Please see www.hytest.fi.

ANTIGENS

Cat.#	Product	Source	Purity
8AN7	Adiponectin, human	Pooled human plasma	>95%

S100 protein

S100 proteins are acidic, calcium-binding proteins with a molecular weight of 10-12 kDa. Over 20 different members of this family have been identified in humans. These proteins form homo- and heterodimers and they appear to be involved in diverse cellular processes such as in cell growth and differentiation or the inflammatory response. Some family members (S100B and S100A1) have been studied for their role in cardiac diseases.

MONOCLONAL ANTIBODIES

Cat.#	Product	Tested applications
4S37*	Monoclonal mouse anti-human S100 protein	Enzyme immunoassays Western blotting

* Several MAbs available under one catalogue number. Please see www.hytest.fi.

ANTIGENS

Cat.#	Product	Source	Purity
8S9h	S100BB homodimer and S100A1B heterodimer, human	Human brain	>95%
8S9b	S100BB homodimer and S100A1B heterodimer, bovine	Bovine brain	>95%
8S9-2h	S100BB homodimer, human	Human brain	>95%
8S9-2b	S100BB homodimer, bovine	Bovine brain	>95%

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Patents and trademarks

Immunoassay Kit for Quantification of BNP and proBNP (US 9,145,459)

Detection of Cardiac Muscle Necrosis by Immunoassay and Appropriate Antibodies (EP 0965043, FI 104857)

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Immunoassay for Quantification of an Unstable Antigen Selected from BNP and proBNP (US 9,034,591, US 9,034,592, JP 5686593, CN 101842707, CA 268391, EP 2135087)

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