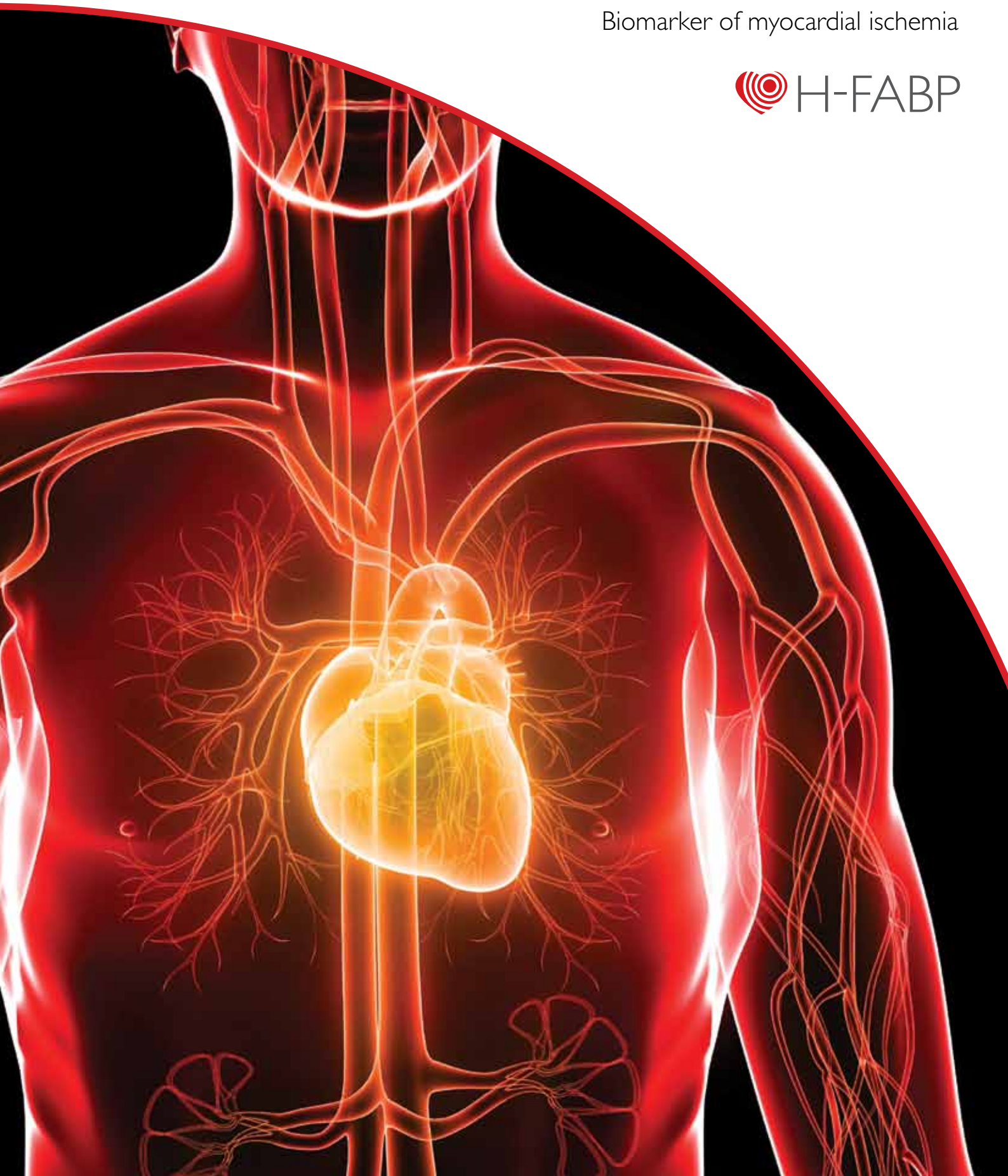
RANDOX
CARDIOLOGY

Heart-type Fatty Acid-Binding Protein

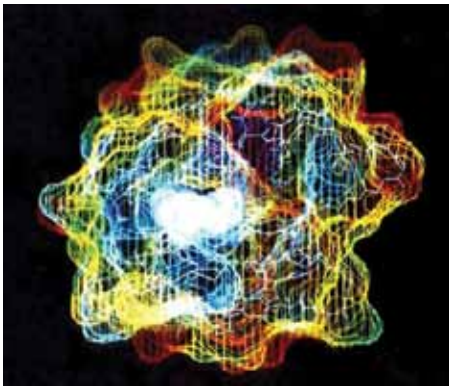
Biomarker of myocardial ischemia

H-FABP

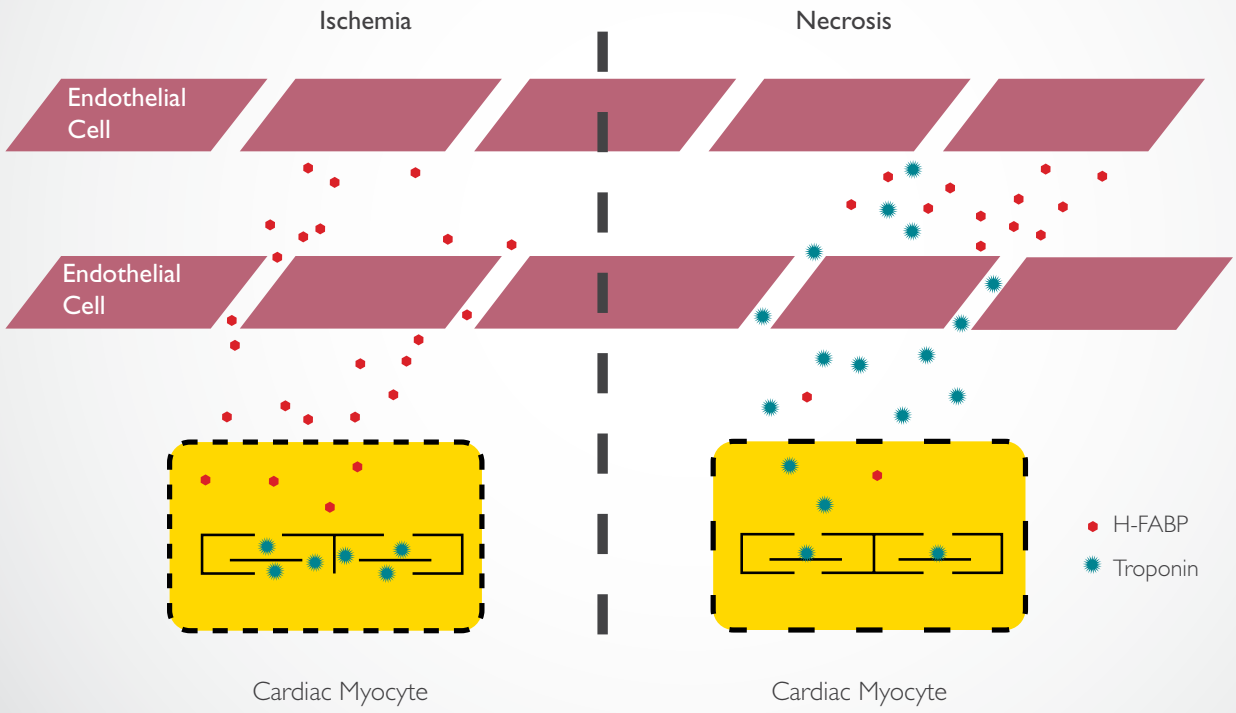


H-FABP: The Protein

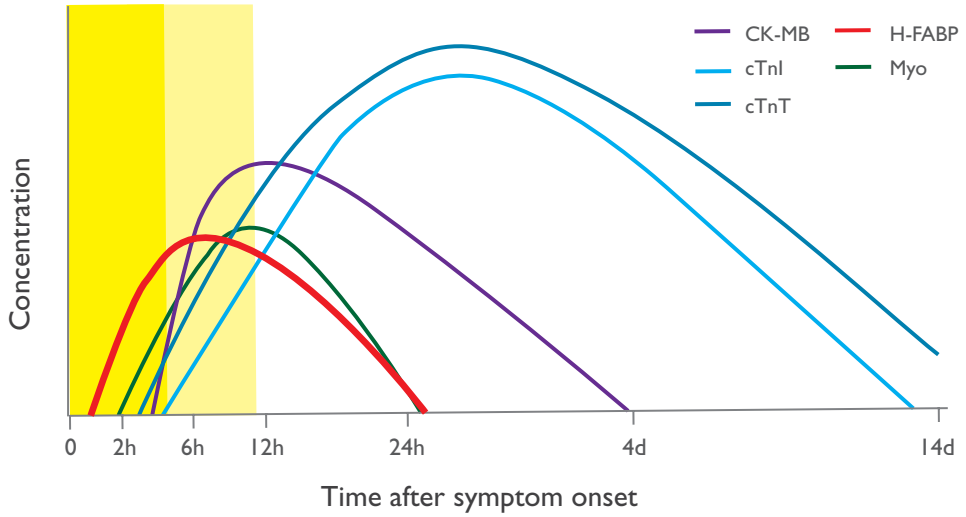
- Heart-type Fatty Acid Binding Protein (H-FABP) is an unbound, low molecular weight protein, located in the cytoplasm of cardiac myocytes.[1]
- The molecular weight is only 15kDa – smaller than Myoglobin (18kDa), Troponin I (22kDa), Troponin T (37dKa) and CK-MB (86kDa).
- The function of H-FABP is in the intracellular uptake of long chain fatty acids in the myocardium.



Early release of protein after MI

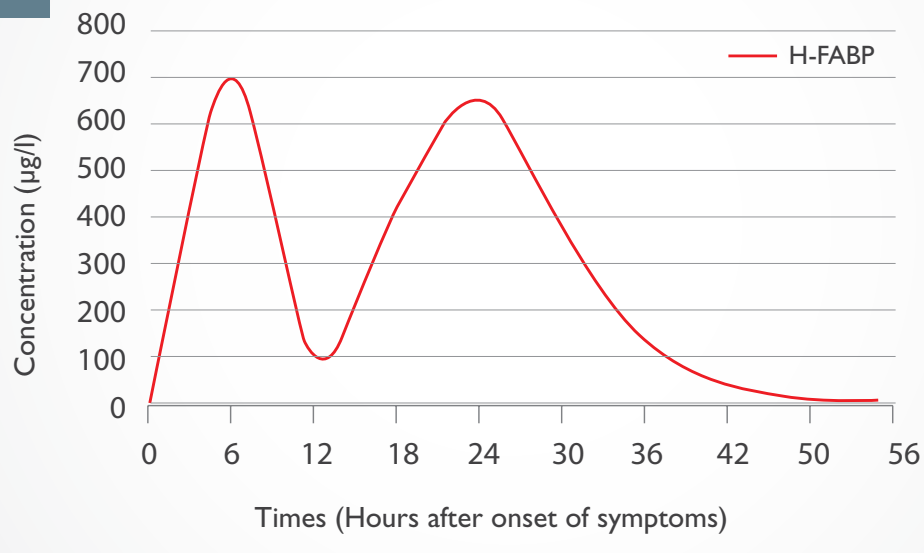


H-FABP: Release Kinetics



- H-FABP is highly specific to the heart – approximately 15-20 times more specific than Myoglobin.[2]
- The normal serum/plasma value is also much lower, compared to Myoglobin.[3]
- Due to the low molecular weight & cytoplasmic location of H-FABP, it is released extremely quickly after an ischemic episode – detectable as early as 30 minutes afterwards.[4,5]
- Furthermore, the rapid return to baseline within 24 hours, offers significant potential utility in patients with suspected reinfarction, instead of CK-MB.[6]

H-FABP in reinfarction [6]



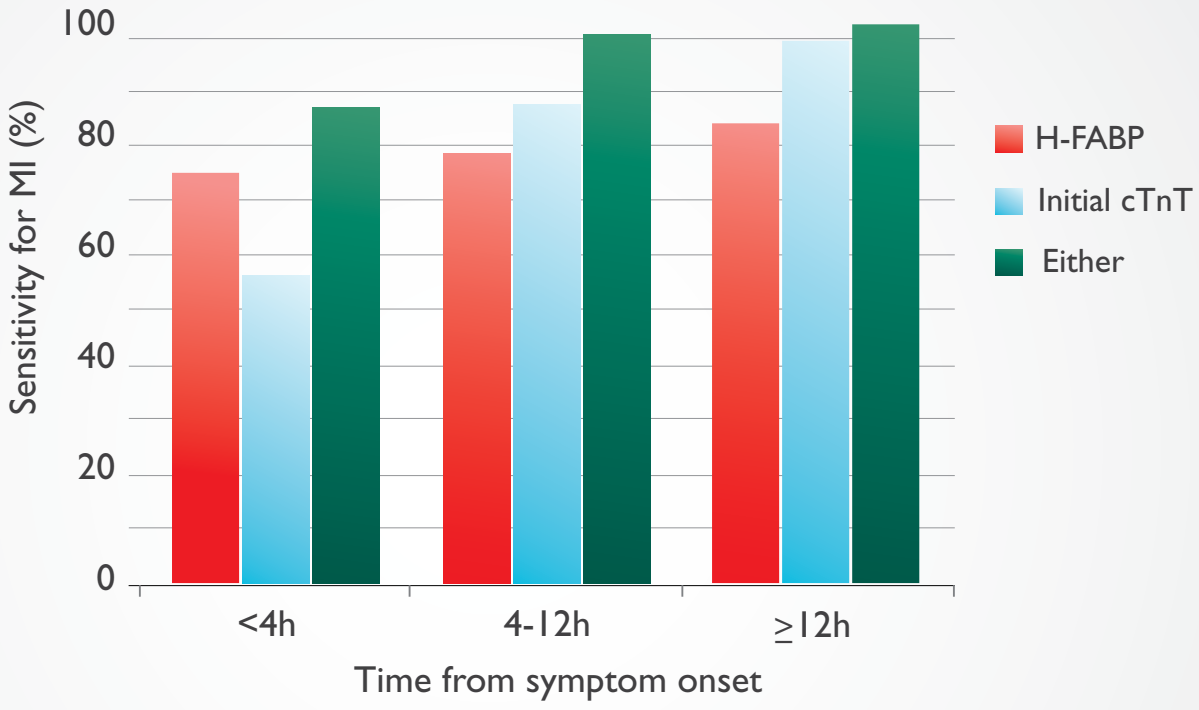
Diagnostic Value in ACS

- "Combining H-FABP and cTnT…provided a significant improvement in sensitivity for patients presenting within 4 and 12 hours" [7]
- "Using the combination approach consistently improved the NPV, negative likelihood ratio, and the risk ratio" [7]

"Multi-marker measurement of H-FABP and cTnI is 20% more sensitive than Troponin alone" [8]

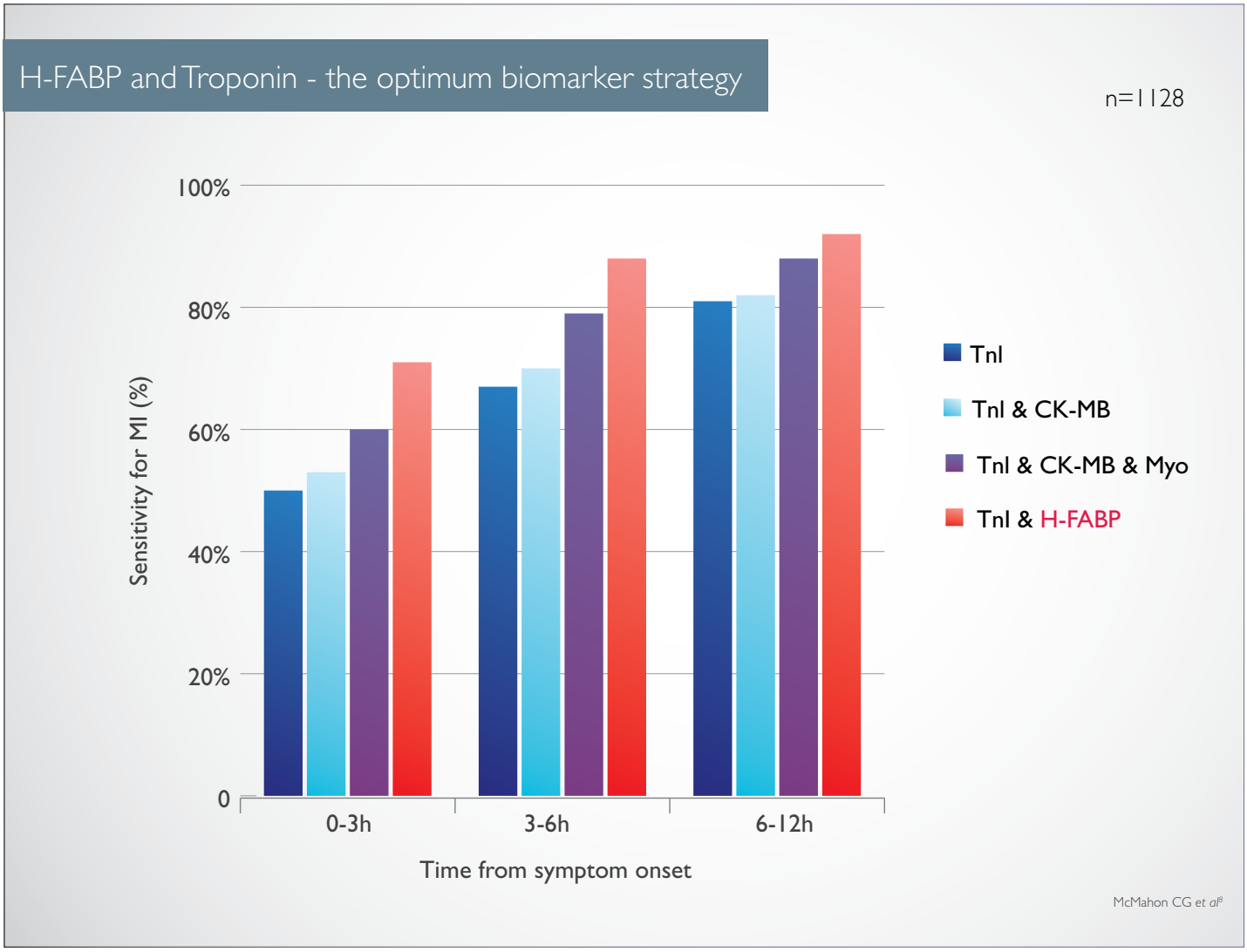
H-FABP - A highly sensitive early ischemic marker

n=664



McCann CT *et al*[7]

H-FABP and Troponin - the optimum biomarker strategy

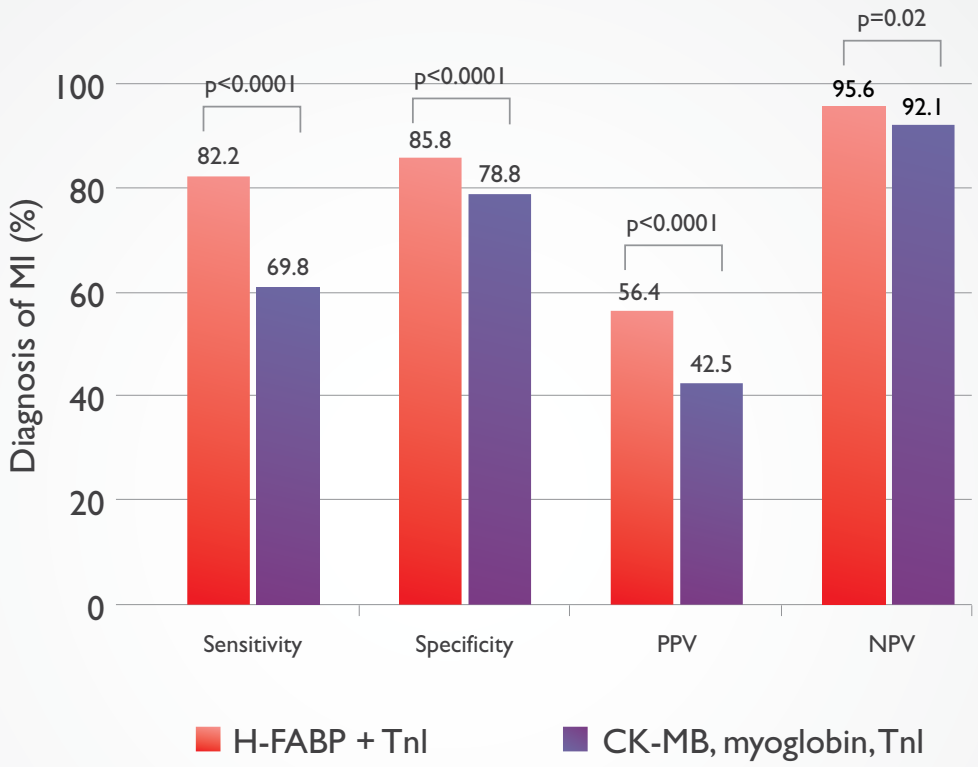


- "In the early hours after chest pain onset (CPO), H-FABP offered superior diagnostic sensitivity for AMI than Troponin" [8]
- The optimal combination of biomarkers across all timepoints was Troponin I and H-FABP [8]

Elevated H-FABP is a significant predictor of death or MI up to 1 year[15]

H-FABP and Troponin - the optimum biomarker strategy

n=705



Body R *et al*[9]

- Even based on samples taken immediately after hospital admission (<24h after CPO), the combination of H-FABP & Troponin I was superior to the triple marker strategy across the measures of sensitivity, specificity, PPV & NPV.[9]

H-FABP and Troponin enables early MI rule out

n=1128

	NPV for MI (%)			
Time post pain	0-3h	3-6h	6-12h	12-24h
Individual markers				
H-FABP	93	97	98	99
cTnI	92	95	97	99
2 marker combinations				
H-FABP + cTnI	94	98	99	100

McMahon CG et al[8]

- "The combination of H-FABP & TnI can be used effectively as a rule-out test to exclude AMI within 6 hours of pain onset [8]
- H-FABP & TnI offers a NPV of 98% at 3-6 hours after symptom onset [8]
- Measuring H-FABP with TnI also appears to identify at-risk patients with ACS more effectively than a single TnI assay [8]

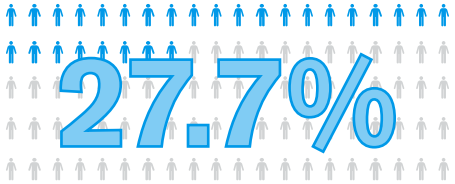
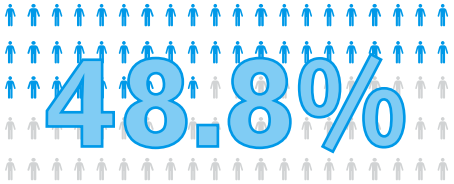
"H-FABP allows for more accurate risk stratification of low-medium risk chest pain patients"[8]

Diagnostic value of H-FABP combined with hsTnT

- In a recent study at two Emergency Departments in the UK, 1171 patients with suspected cardiac chest pain had single blood samples taken on presentation (0h) to assess the potential value of the combination of H-FABP, hsTnT & ECG to reliably rule-out AMI based on a single sample on presentation.[10]

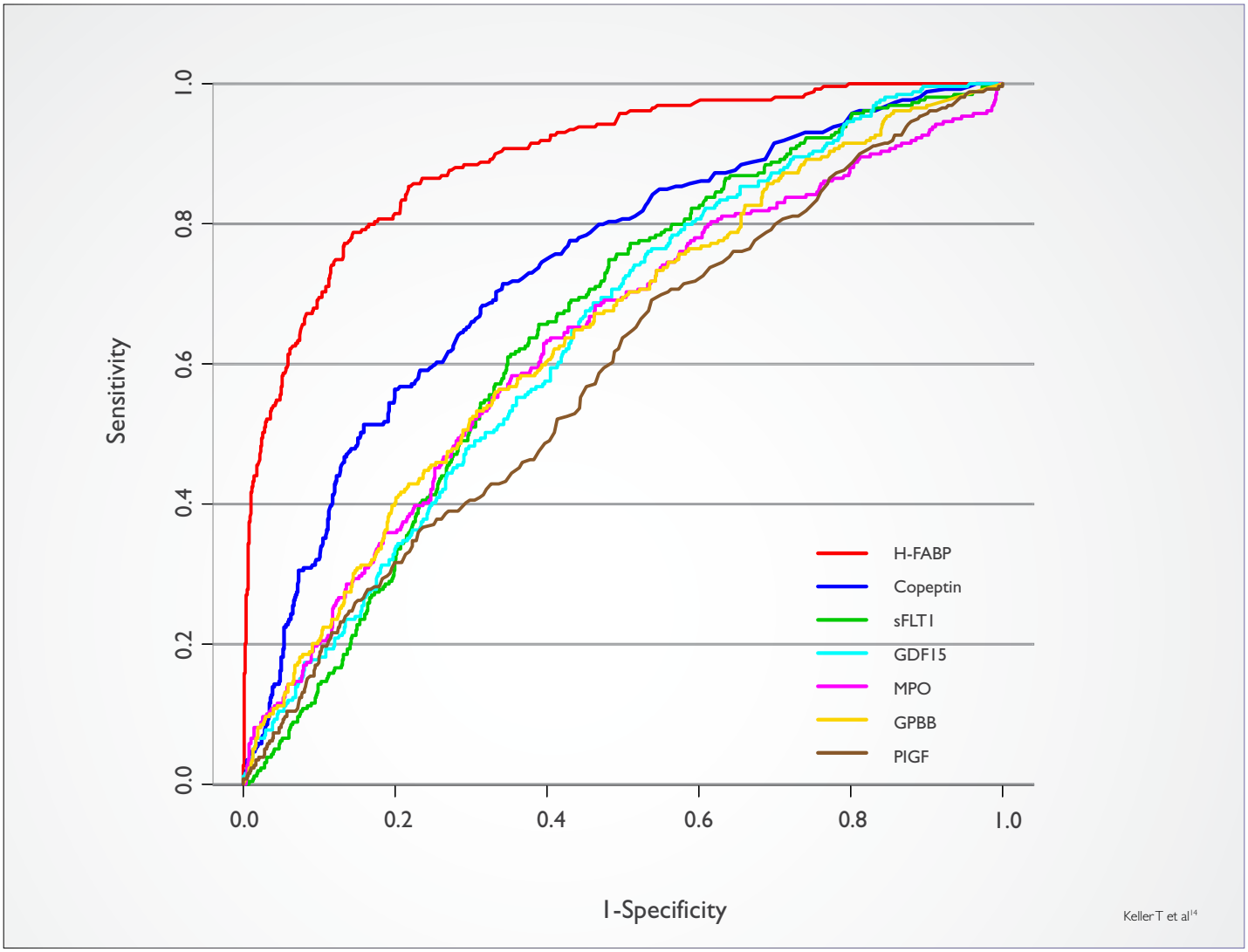
Protocol	Diagnostic cut-offs	Sensitivity	Specificity	PPV	NPV
H-FABP & hsTnT & ECG	95th centile = 2.5 ng/ml 99th centile = 14ng/l ECG negative	**99.1%**	59.3%	35%	**99.7%**

This protocol on presentation resulted in a sensitivity for AMI of 99.1%, and a NPV of 99.7% - and could have enabled AMI to be excluded in 48.8% of patients.

Percentage of symtomatic patients excludable for AMI according to diagnostic protocol		
		
ASPECT Study (n=3582)[11]	**Manchester Study hsTnT (n=703)[12]**	**Manchester & Stockport Study (n=1171)[10]**
TnI, CK-MB & Myo at 0h + 2h Plus ECG & TIMI scoring	hsTnT at 0h using assay LoD as cut-off (3ng/l)	hsTnT, H-FABP & ECG at 0h
9.8% of patients excluded for AMI at 2h	**27.7% of patients excluded for AMI at 0h**	**48.8% of patients excluded for AMI at 0h**

- In addition, an economic analysis was undertaken on a subset of this cohort (n=473). The results showed that applying this protocol in this subset would have enabled AMI to be excluded in 44.3% of patients, reduced the mean length of stay (4.1 days vs 5.3 days), and would have resulted in a projected saving of £327 per presenting patient.[13]

Diagnostic Value compared with Novel ACS Biomarkers

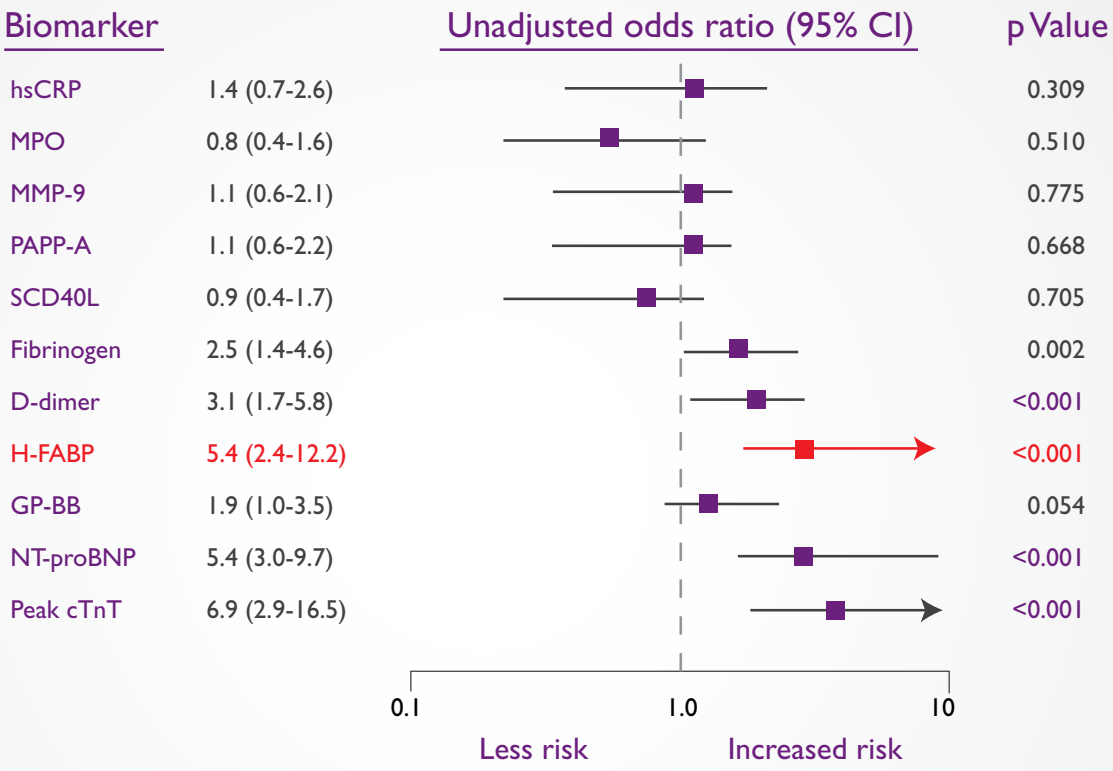


- H-FABP has been shown to offer superior diagnostic performance for AMI compared to a range of novel ACS biomarkers (based on ROC analysis). This is based on measurement on admission to a chest pain unit, then at 3 and 6 hours later.[4]

Prognostic Value in ACS

H-FABP - Presence equals increased risk

n=664



McCann CT *et al*[15]

- Elevated H-FABP is a significant predictor of death or MI up to 1 year [15]
- H-FABP provides additional prognostic information, independent of Troponin T, ECG and clinical examination [15]

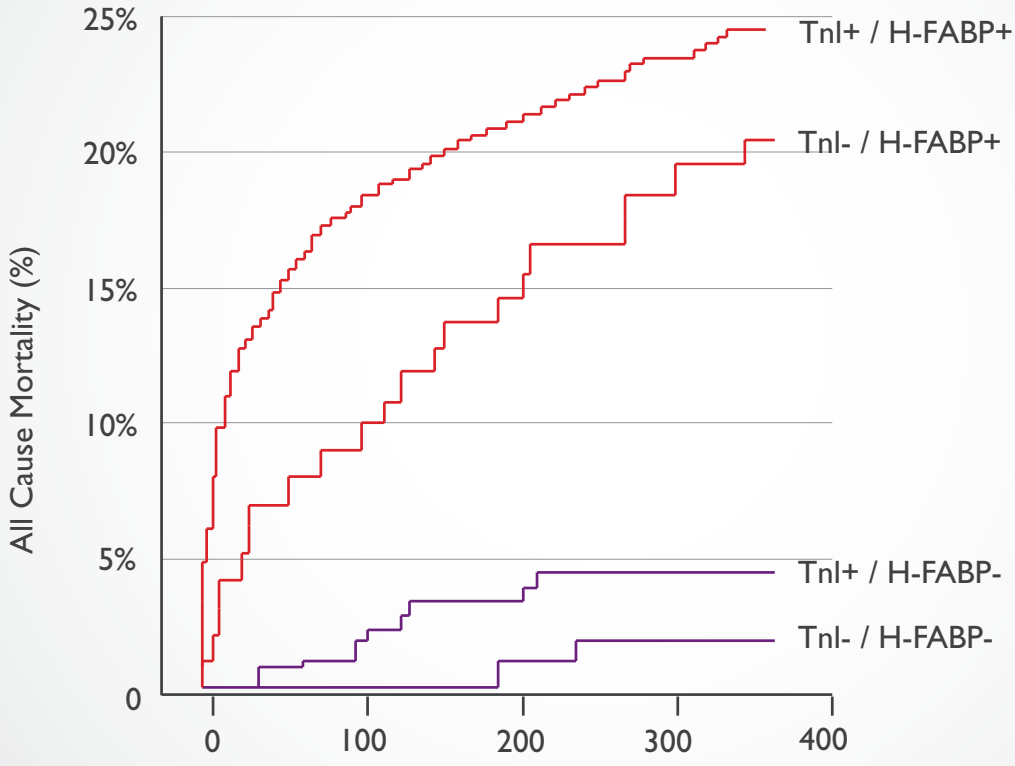
Prognostic value in Troponin - Negative patients

- H-FABP allows identification of high risk patients across the full range of TnI concentrations[16]
- Negative test result for both TnI and H-FABP was associated with 0% mortality at 6 months[16]

Raised concentrations of H-FABP are strongly predictive of mortality after ACS[16]

H-FABP predicts mortality after ACS

n=1448



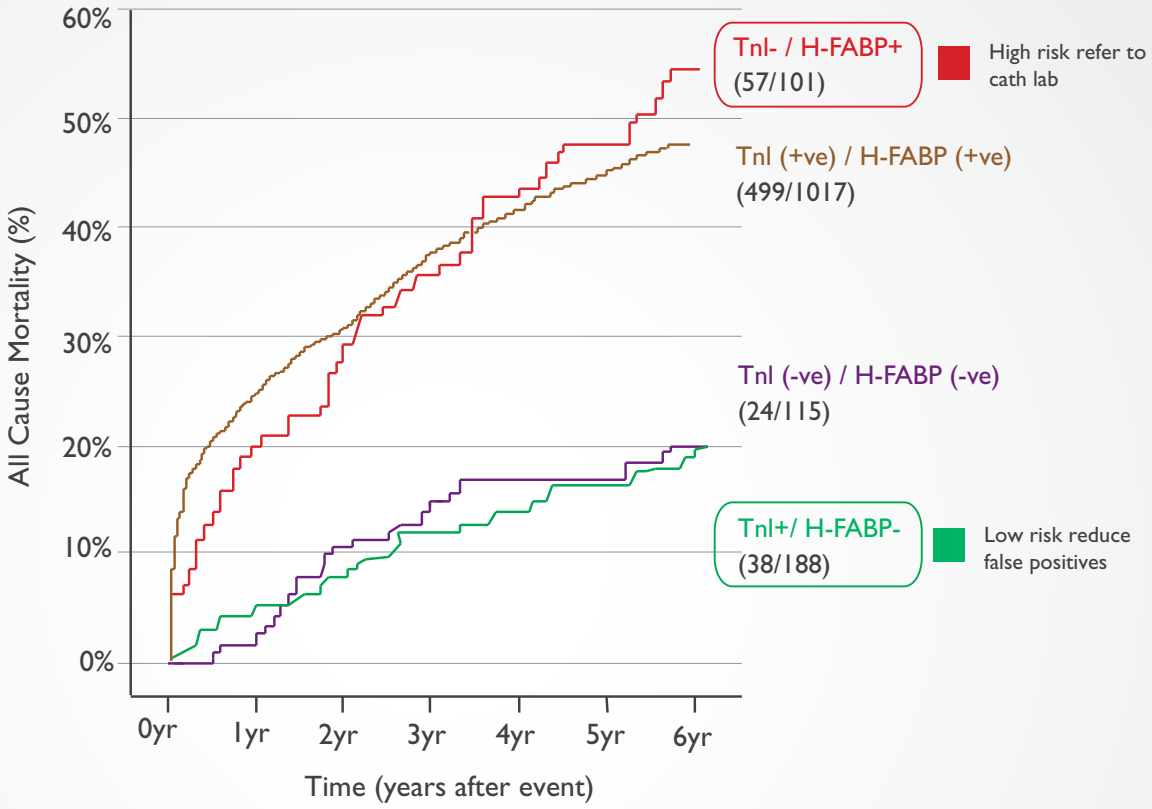
Based on a single blood sample at 12-24 hrs after ACS system onset

Kilcullen N *et al*[16]

Prognostic value in Troponin - Negative patients

- H-FABP taken 12-24 hours after admission, can identify Troponin-negative ACS patients who are at long-term risk of death[17]
- H-FABP identifies a very high risk group of patients who warrant further investigation & possible intervention[17]
- Conversely, some ACS patients undergo angiography based on a false positive Troponin level. The addition of H-FABP to Troponin measurement could avoid unnecessary investigations caused by false positives[17]

H-FABP predicts mortality after ACS



Based on a single blood sample at 12-24 hrs after ACS system onset

Pearson IR *et al*[18]

Prognostic value in Troponin - Negative patients

Hazard Ratios for Death or MI Stratified by H-FABP results among Troponin-Negative patients			
Quartile	Unadjusted	HR(95% CI)	p Value
1	H-FABP 0.15-3.26 µg/l	1.00	<0.001
2	H-FABP 3.27-6.48 µg/l	3.46 (1.69-7.10)	<0.001
3	H-FABP 6.49-12.77µg/l	11.20 (4.95-25.36)	<0.001
4	H-FABP 12.78-151.0 µg/l	16.64 (2.21-125.51)	0.006
	Adjusted for Age and Serum Creatinine	Adjusted HR (95% CI)	pValue
1	H-FABP 0.15-3.26 µg/l	1.00	0.01
2	H-FABP 3.27-6.48 µg/l	1.55 (0.72-3.36)	0.26
3	H-FABP 6.49-12.77µg/l	3.12 (1.11-8.76)	0.03
4	H-FABP 12.78-151.0 µg/l	16.67 (2.19-127.06)	0.007

Viswanathan K *et al*[17]

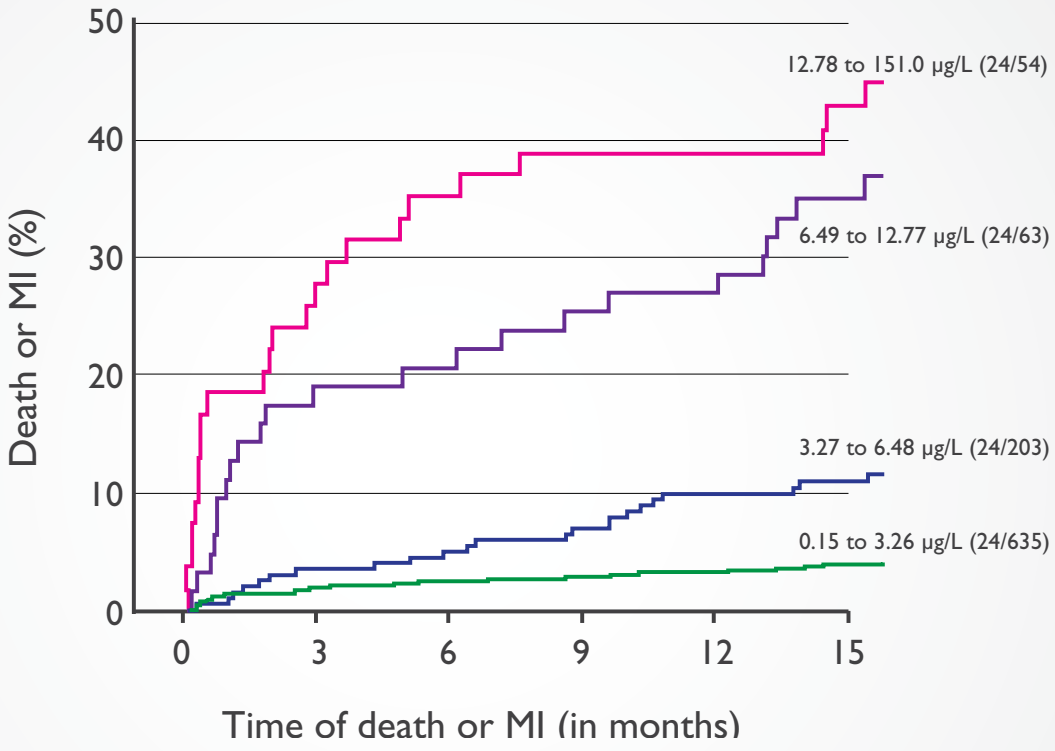
- Study excluded patients with STEMI [17]
- This shows increasing risk in quartile 2, 3, and 4 as compared with quartile 1, with a very significantly increased event rate in patients with H-FABP concentrations above 6.48 ng/ml [17]
- The long-term prognostic value of H-FABP in troponin-negative patients is independent of age and serum creatinine [17]

Over 79% of patients in the cohort were negative TnI at 12-24 hours after symptom onset[17]

Value of a fully quantitative H-FABP assay

Increased H-FABP concentrations confers increased risk

n= 955



Viswanathan K et al[17]

- Patients with H-FABP concentrations >6.48µg/L had significantly increased risk of adverse events[17]
- Among Troponin negative patients, the cut-off of 6.48µg/L identified patients at very high risk of adverse outcomes independent of patient age and serum creatinine[17]

This demonstrates a clear need for a fully quanitative H-FABP assay

Prognostic Value in Unstable Angina

H-FABP - The first true global ACS biomarker

	All Cause Mortality		
	Unstable Angina	NSTEMI	STEMI
H-FABP negative (≤5.8µg/l)	2.1% (2)	4.8% (9)	0% (0)
H-FABP positive (>5.8µg/l)	**22.9% (19)**	**26.1% (189)**	**23.0% (77)**
p Value	0.006	0.004	_*
ECG	X	X	✓
Troponin	X	✓	✓
H-FABP	✓	✓	✓

Kilcullen N et al[16]

- "This demonstrated the additive value of H-FABP, particularly for ACS subtypes such as Unstable Angina, traditionally considered to be associated with a low long-term risk" [16]

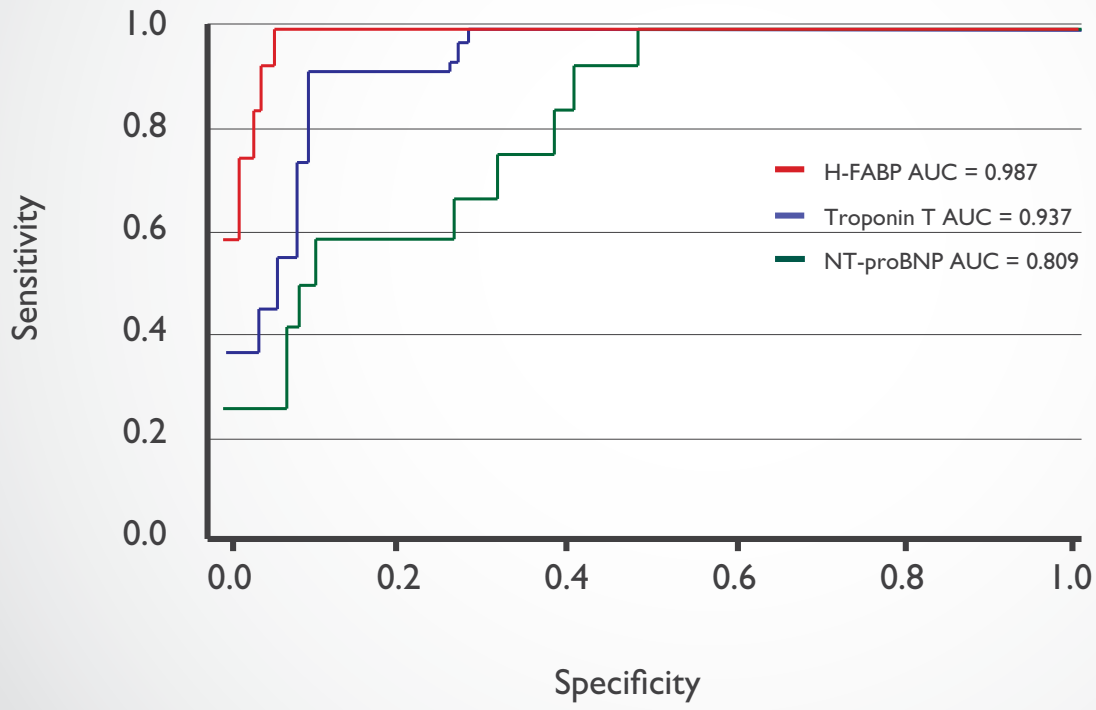
Value in Pulmonary Embolism

- Elevated H-FABP on admission is a very reliable predictor of outcome in patients with PE, with a prognostic value superior to cTnT & NT-proBNP[19]
- None of the patients with initially normal H-FABP levels had a complicated 30-day outcome or died of PE-related causes[19]
- H-FABP will hopefully improve prediction of patient risk & optimise treatment strategies by promptly identifying candidates for urgent medical (thrombolysis), surgical, or catheter-based recanalisation[19]

H-FABP provides a NPV of 100% and PPV of 41%[11]

Prognostic value in hypotensive high risk patients

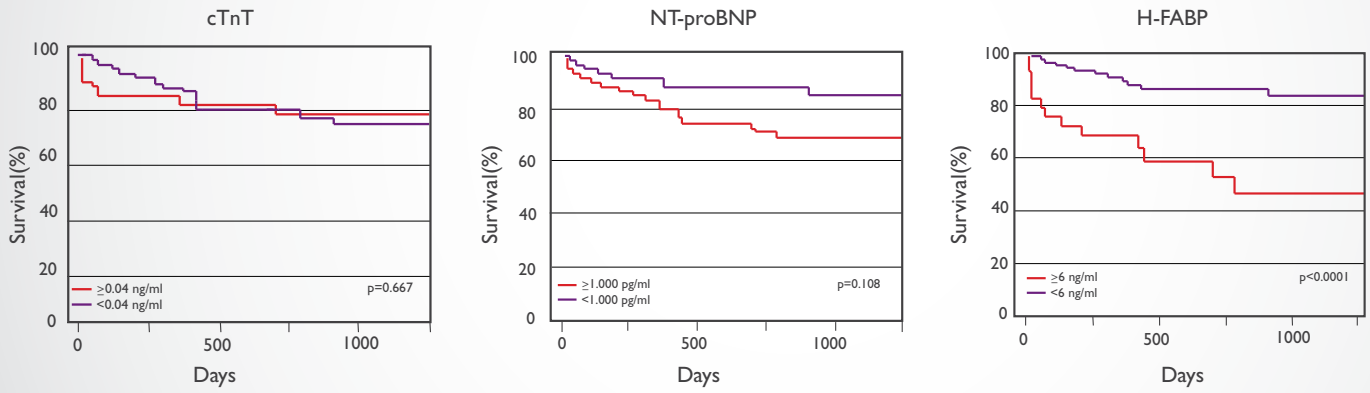
n=107



Puls M et al[17]

Prognostic value in normotensive low-medium risk patients

n=126



Dellas C et al[20]

Prediction of complicated 30 day outcome

Parameter	OR (95% CI)	p Value
Heart Rate ≥94 beats/min	10.6 (1.3-87.2)	0.029
H-FABP ≥6 ng/ml	**36.6 (4.3-3.08)**	**0.001**
cTnT ≥0.04 ng/ml	3.3 (0.4-13.1)	0.087
RV dysfunction (echocardiography)	2.8 (0.6-12.3)	0.178

Dellas C et al[20]

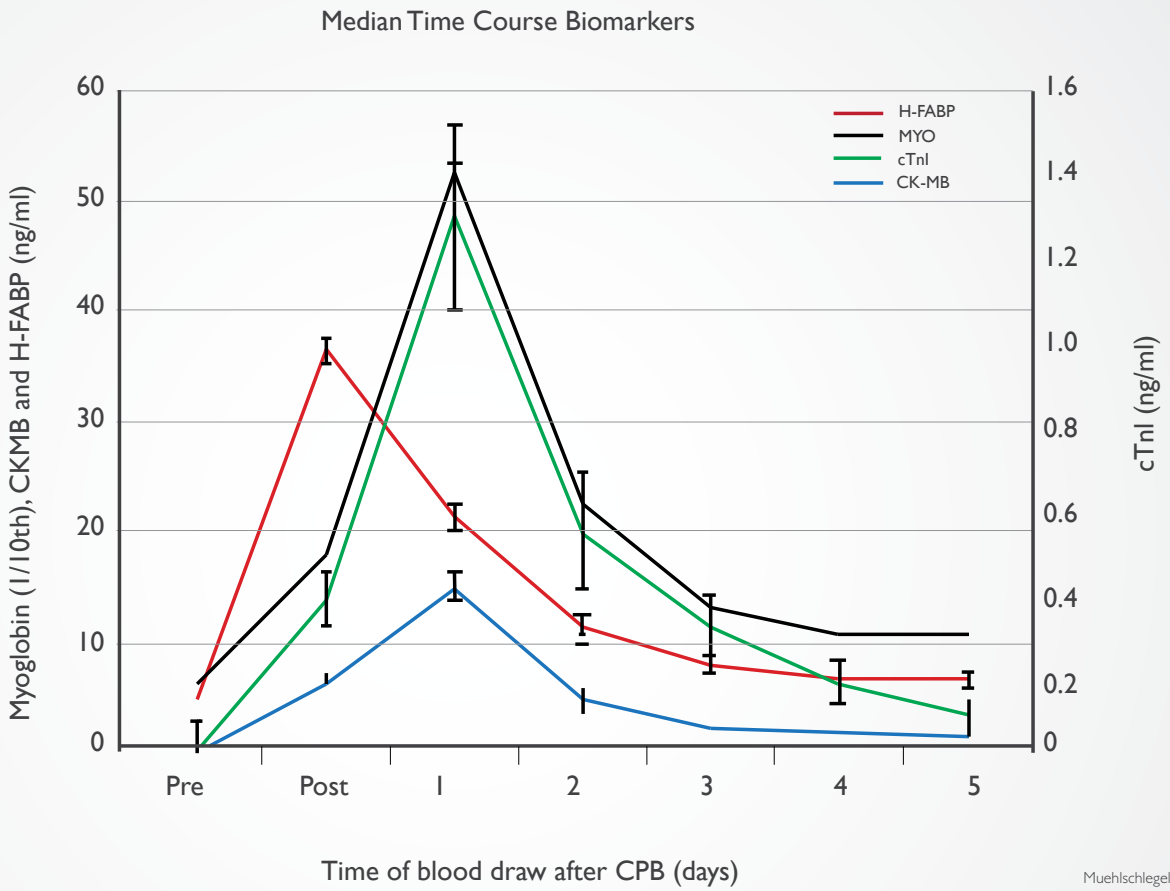
A single measurement of H-FABP on admission, as early as 60-90 minutes from symptom onset might be enough to guide management strategies[20]

- 37 fold increase in risk of 30-day complications[20]
- 99% NPV[20]
- 4.5 fold increase in risk of death[20]

Value in Coronary Artery Bypass Grafting (CABG)

5 year predictive value following CABG

n=1298



Muehlschlegel et al[21]

- The slower release of CK-MB and cTnI generates an inability to discriminate between graft failure with massive tissue necrosis and ischemia reperfusion injury within 24 hours after CABG surgery[21]
- H-FABP is superior to Troponin & CK-MB for the prediction of mortality and ventricular dysfunction[21]
- Enable clinicians to identify patients in need of further diagnostic or therapeutic procedures to reduce loss of myocardial mass or performance[21]

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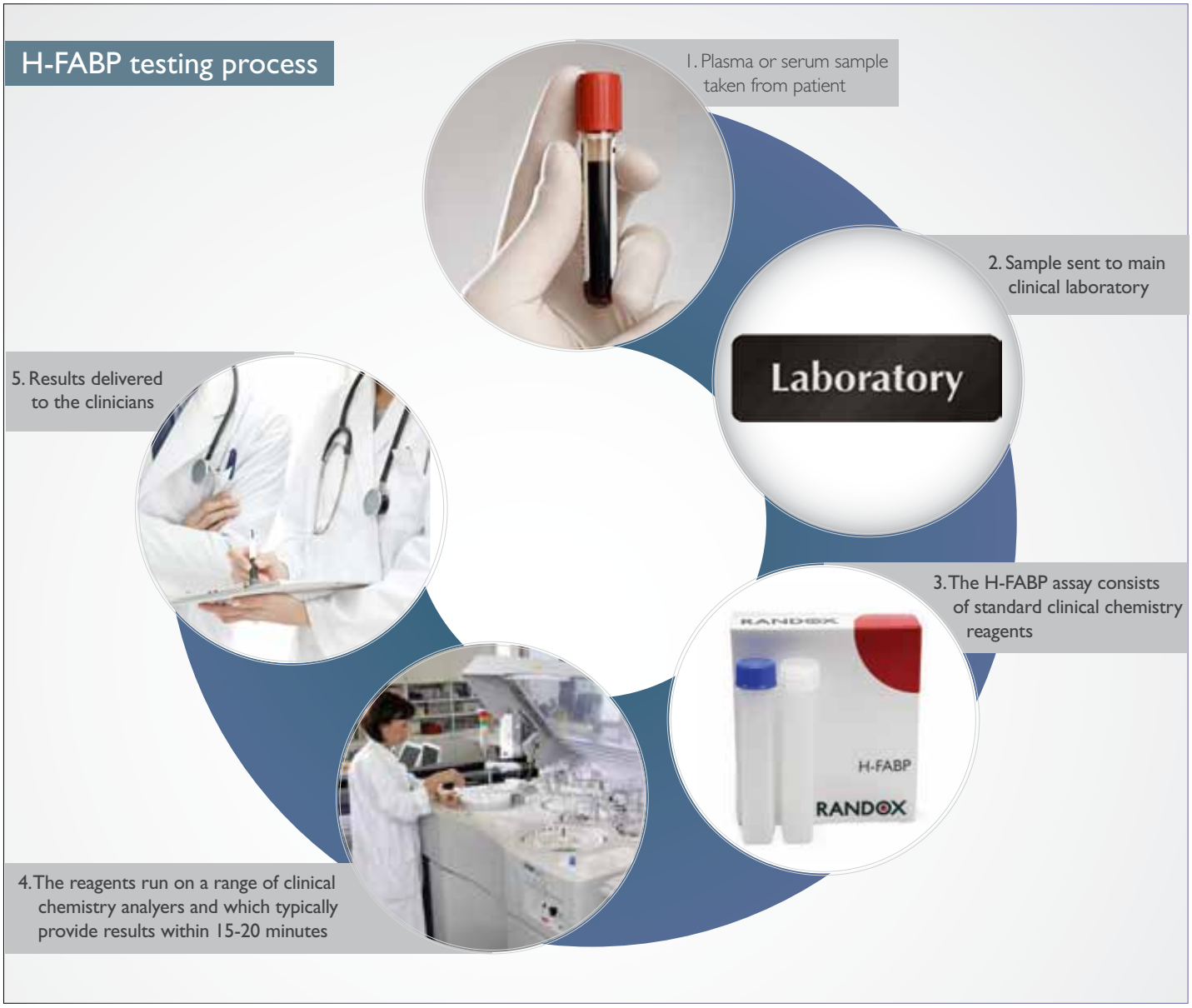
NEW H-FABP Clinical Chemistry Assay

The Randox H-FABP clinical chemistry assay is a latex enhanced immunoturbidemtric assay suitable for fully quantitative measurement of H-FABP in serum and plasma.

The assay can be used on a wide range of manufacturer's clinical chemistry analysers and does not need any dedicated equipment or software.



Assay Specification

Method	Latex-enhanced Immunoturbidmetric assay via a clinical chemistry instrument
Sample	Serum, EDTA & Heparin Plasma
Sample Stability	If not analysed immediately, samples should be stored at -20°C or below
Assay Measuring Range	0.747 – 120 ng/ml
Calibrators	6 x 1ml vials lyophilised
QC	Level 1 (3 x 1ml) & Level 2 (3 x 1ml) lyophilised

Applications Available

Instrument specific applications are available for wide range of clinical chemistry analysers from manufacturers such as Roche, Abbott, Siemens, Beckman Coulter, Olympus & J&J.

Product Details

Catalague Number	Product Description	Kit Contents
FB4025	H-FABP Reagent	R1: Buffer (1 x 19ml) R2: Antibody-latex Reagent (1 x 7ml)
FB4026	H-FABP QC Level 1	3 x 1ml
FB4027	H-FABP QC Level 2	3 x 1ml
FB3134	H-FABP Calibrators	6 x 1ml

Other Products from Randox Cardiology

Biochip Array Technology (BAT)

BAT is a technology developed & pioneered exclusively by Randox, and enables multiple biomarkers to be assessed simultaneously from a single patient sample of plasma, serum or whole blood. The ability to test up to 23 biomarkers from a single sample offers the potential for endless possibilities in both cardiovascular research, and clinical practice.

Cytokine Array I
Epidermal Growth Factor (EGF)
Interferon-γ (IFN-γ)
Interleukin-1α (IL-1α)
Interleukin-1β (IL-1β)
Interleukin-2 (IL-2)
Interleukin-4 (IL-4)
Interleukin-6 (IL-6)
Interleukin-8 (IL-8)
Interleukin-10 (IL-10)
Monocyte Chemotactic Protein-1 (MCP-1)
Tumour Necrosis Factor-α (TNF-α)
Vascular Endothelial Growth Factor (VEGF)

Cytokine Array II
Eotaxin
Insulin like Growth Factor 1, Free (IGF-1(free)
Interleukin-1 Receptor Antagonist (IL-1Ra)
Interleukin-12p40 subunit (IL-12p40)
Interferon-γ -Inducible Protein 10 (IP-10)
Platelet Derived Growth Factor BB (PDGF-BB)
Regulated on Activation, Normal T Expressed and Secreted (RANTES)

Cytokine Array III
Granulocyte Macrophage Colony Stimulating Factor (GM-CSF)
Interleukin-5 (IL-5)
Interleukin-15 (IL-15)
Macrophage Inflammatory Protein - 1α (MIP-1α)
Tumour Necrosis Factor β (TNFβ)

Cytokine Array IV
Matrix Metalloproteinase-9 (MMP-9)
Soluble IL-2 Receptor α (sIL-2Rα)
Soluble IL-6 Receptor (sIL-6R)
Soluble Tumour Necrosis Factor Receptor I (sTNFRI)
Soluble Tumour Necrosis Factor Receptor II (sTNFRII)

Cytokine Array V (On Evidence Investigator only)
Interleukin-3 (IL-3)
Interleukin-7 (IL-7)
Interleukin-13 (IL-13)
Interleukin-12p70 (IL-12p70)
Interleukin-23 (IL-23)

Cardiac Array
Creatine-Kinase Muscle Brain (CK-MB)
Heart Type Fatty Acid Binding Protein (H-FABP)
Myoglobin(Myo)
Troponin I (cTnI)

Adhesion Molecules Array
E-Selectin
L-Selectin
P-Selectin
Intercellular Adhesion Molecule-1 (ICAM-1)
Vascular Cell Adhesion Molecule-1 (VCAM-1)

Cerebral Array I
Brain-Derived Neurotrophic Factor (BDNF)
Glial Fibrillary Acidic Protein (GFAP)
Heart Type Fatty Acid Binding Protein (H-FABP)
Interleukin-6 (IL-6)

Cerebral Array II
C-Reactive Protein (CRP)
D-dimer
Neuron Specific Enolase (NSE)
Neutrophil Gelatinase-Associated Lipocalin (NGAL)
Soluble Tumour Necrosis Factor Receptor I (sTNFRI)

Metabolic Syndrome Array I
C-peptide
Ferritin
Insulin
Interleukin-1α (IL-1α)
Interleukin-6 (IL-6)
Leptin
Plasminogen Activator Inhibitor-1 (PAI-1)
Resistin
Tumour Necrosis Factor-α (TNFα)

Metabolic Syndrome Array II
Adiponectin
C-Reactive Protein (CRP)
Cystatin C

Endocrine Array
Cortisol
Dehydroepiandrosterone Sulphate (DHEAs)
Leptin
17α Hydroxyprogesterone

Biochip Array Technology - Genetic Arrays

Cardiac Risk Prediction Array

- For assessment of genetic risk for development of cardiovascular disease
- Examines specific genetic elements that will not change over an individual's lifespan

Familial Hypercholesterolemia Array

- FH is a genetic disorder characterised by high levels of LDL
- Most common defects are LDLR, ApoB and PCSK9 gene mutations
- Array assesses 20 SNPs known to influence the function of these three genes

Hypertension Array

- Estimated one billion people worldwide affected by hypertension
- Leading risk factor for stroke, AMI, heart failure and chronic renal failure
- Array contains a number of SNPs indicating genetic predisposition to hypertension

Lipoprotein (a)

Automated chemistry assay for the casual genetic biomarker of CVD

Elevated Lp(a) concentration in plasma is an independent genetic marker correlating with increased risk of atherosclerotic disorders including myocardial and cerebral infarction. Levels are also elevated in nephritic syndrome, patients undergoing renal dialysis, patients with uncontrolled diabetes mellitus and hypothyroidism.

Our highly successful Lipoprotein (a) test is the only method in the world to accurately and reliably measure Lp(a), it is not affected by Apo (a) size related bias like most other methods.

In June 2010, the European Atherosclerosis Society (EAS) published a consensus paper on Lp(a), recommending its widespread use as a screening tool in those at intermediate or high risk of cardiovascular disease

"The evidence clearly supports Lp(a) as a priority for reducing cardiovascular risk, beyond that associated with LDL cholesterol. Clinicians should consider screening statin-treated patients with recurrent heart disease, in addition to those considered at moderate to high risk of heart disease - EAS Consensus Panel[22]

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