IMMUNOTURBIDIMETRIC ASSAY KIT FOR THE RAPID MEASUREMENT OF HEART-TYPE FATTY ACID BINDING PROTEIN

Lowry, P.; Szabo, M.; Henry, C.; McConnell, R.I.; Fitzgerald, S.P.

Randox Laboratories Ltd, 55 Diamond Road, Crumlin, Co Antrim, BT29 4QY, UK

Introduction

Heart-type fatty acid-binding protein (H-FABP) is a small cytoplasmic protein, involved in lipid homeostasis. It is abundant in heart muscle and is an early, highly sensitive marker of myocardial injury. As such, its measurement provides early diagnostic information, which is relevant for patient management. In this context, the availability of analytical tools enabling the rapid detection of H-FABP represents an advantage in clinical settings where a short time span for assessment is critical.

We report an immunoturbidimetric (IT) assay kit applicable to automated analysers for the quantitative determination of H-FABP in serum.

Methodology

Principle

H-FABP assay
The principle of the assay is immunoturbidimetric. A latex agglutination complex (read at 694nm) is formed between H-FABP and antibody coated latex particles

Liquid reagents ready for use

The assay was applied to the automated analysers Advia 1650 and RX daytona

Sample

Serum

Reagents and sample volumes

Pipette volumes	RX daytona (µl)	Advia 1650 (µl)
Reagent 1 Volume	160	80
Reagent 2 Volume	40	20
Serum Diluent S. Volume	N/A	30
Serum Diluent Volume	N/A	30
Sample Volume	8	8

Analytical parameters

Sensitivity: sensitivity was assessed by analysis of replicates of serial dilutions of control material and was determined as the lowest concentration with imprecision of <20% and recovery <20% from target for 10 replicates.

Linearity: linearity was determined by assessment of replicates of serial dilutions of control material with an upper limit of 132 ng/ml (these may vary slightly with the lot specific calibrator values of the calibrators in use).

Precision: intra-assay precision performance was established by testing serum samples at defined medical levels, with 20 replicates of each defined level ran concurrently.

Prozone: prozone was assessed by analysis of replicates of serial dilutions of control material with an upper limit of 6400 ng/ml.

Correlation: Correlation studies were conducted by testing serum samples. This assay was applied to Advia 1650 and RX daytona and compared with another commercially available assay. Correlation coefficients were determined by Passing and Bablok regression analysis.

Stability: onboard and calibration stabilities were tested by storing one lot of reagent uncapped on the Advia 1650 analyser for a period of 31 days. The performance was then compared to fresh material.

Calibration frequency: calibration must be performed when this method is implemented on the system and at least at the minimum calibration frequency of seven days. Calibration frequency was derived from quality control performance observed during the onboard stability study.

Results

H-FABP immunoturbidimetric assay:

Performance outline on Advia 1650

Sensitivity, linearity

Sample type	Sensitivity (ng/ml)	Linearity* (ng/ml)
Serum	3.49	132

11/002/FABP, 11/004/FABP

*This may vary with the lot specific calibrator values of the calibrators in use.

Precision

Sample	H-FABP concentration	Intra-assay precision %CV
Control level 1(n = 20)	5.75 ng/ml	**7.94**
Control level 2 (n = 20)	40.3 ng/ml	**1.33**
Control level 3 (n = 20)	91.9 ng/ml	**1.47**

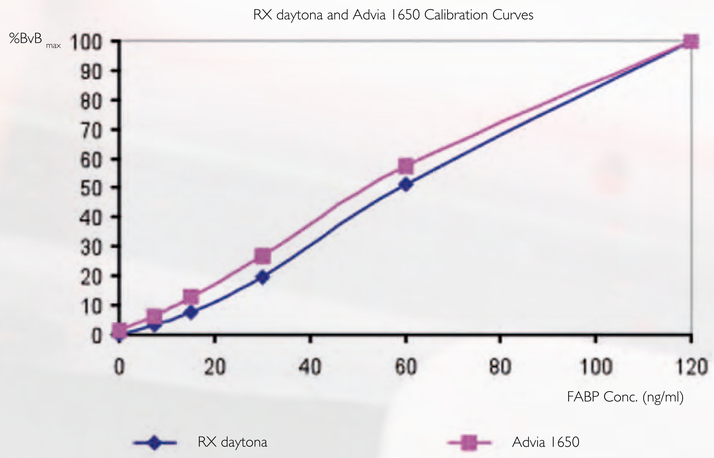
11/001/FABP

Expected values

Human serum from 250 apparently normal donors. 131 males, 119 females.

Mean age 38 years (from 18 to 85 years)

Patient category	99th percentile
Normal	6.32 ng/ml



Performance outline on RX daytona

Sensitivity, linearity

Sample type	Sensitivity* (ng/ml)	Linearity (ng/ml)
Serum	<2.5	>120

2011/01/11.xls

*Theoretical Sensitivity

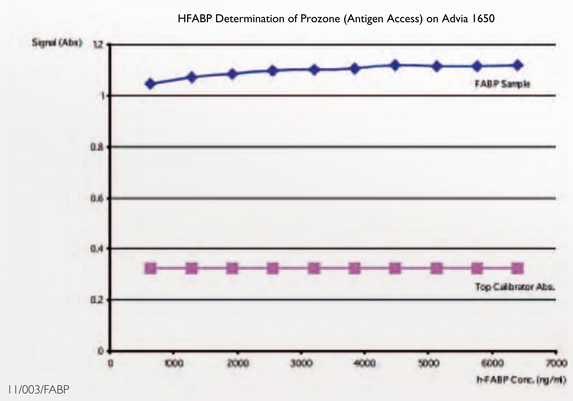
Precision

Sample	H-FABP concentration	Intra-assay precision* %CV
Control level 1(n = 6)	6.84 ng/ml	7.95
Control level 2 (n = 6)	59.72 ng/ml	1.16
Control level 3 (n = 6)	120.48 ng/ml	1.77

2011/01/11.xls

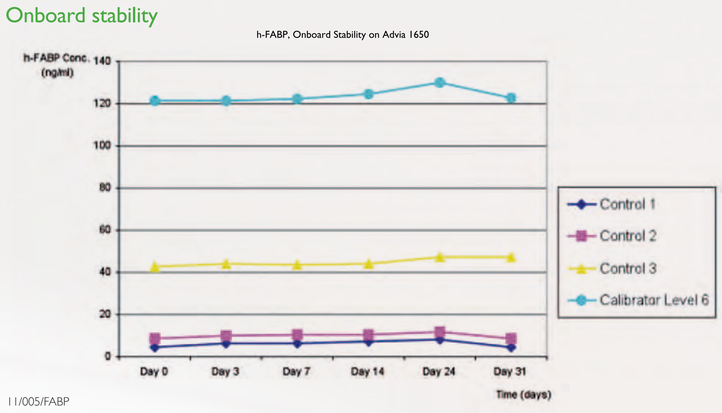
*Determined from the results of 6 replicates of each control level within the same run.

Prozone



11/003/FABP

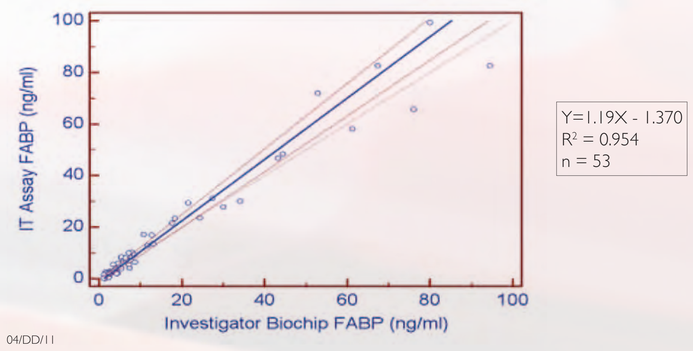
Onboard stability



11/005/FABP

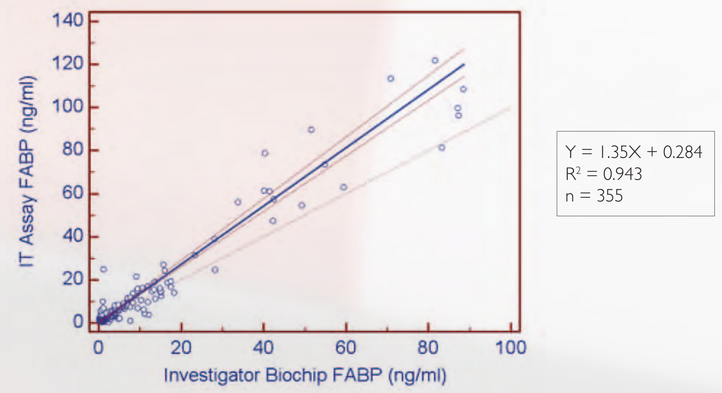
Comparison with commercially available H-FABP assay on biochip array platform

H-FABP: RX daytona/Biochip array platform (on Evidence Investigator analyser)



04/DD/11

H-FABP: Advia 1650/Biochip array platform (on Evidence Investigator analyser)



04/DD/11

Conclusion

The data generated indicates that this immunoturbidimetric assay kit for the measurement of H-FABP is applicable to automated analyzers and facilitates the rapid assessment of early myocardial ischemia in clinical settings.

Randox Laboratories Limited, 55 Diamond Road, Crumlin, County Antrim, BT29 4QY, United Kingdom
T +44 (0) 28 9442 2413 **F** +44 (0) 28 9445 2912 **E** marketing@randox.com **I** www.randox.com