RANDOX

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DEVELOPMENT OF A NEW BIOCHIP ARRAY FOR APOE4 CLASSIFICATION FROM PLASMA SAMPLES USING IMMUNOASSAY BASED METHODS

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INTRODUCTION

Apolipoprotein E (APOE) plays a key role in lipid metabolism and is recognised as one of the most powerful genetic risk factors for dementia and other neurodegenerative diseases. It has become one of the most widely studied gene variants in Alzheimer's disease and constitutes a major consideration for preventive medicine. ApoE exists in three common isoforms (ApoE2, ApoE3 and ApoE4) which are coded by three co-dominant alleles (e2, e3, e4). As such six common ApoE phenotypes exist within the general population E2/E2, E3/E3, E4/E4 (homozygous) and E2/E3, E2/E4, E3/E4 (heterozygous). The presence of the ApoE4 isoform is recognised as a major genetic risk factor for development of Alzheimer's disease. The availability of analytical methods for rapid and reliable ApoE4 classification is therefore advantageous.

Biochip Array Technology (BAT) enables the determination of multiple analytes from a single sample. This technology has been successfully applied to a new biochip array to directly identify from a plasma sample whether patients are ApoE4 heterozygous, homozygous or null through simultaneous detection of both total ApoE levels and specific ApoE4 levels.

METHODOLOGY

Simultaneous chemiluminescent biochip-based sandwich immunoassays for measurement of ApoE4 and total ApoE directly from plasma samples were employed and applied to the Evidence Investigator analyser (EV3602, Randox Laboratories Ltd., Crumlin, UK).

An initial cohort of 272 plasma samples of known genotype were used to establish initial assay parameters. A ratio was calculated using total ApoE ApoE4 protein levels to classify samples as ApoE4 null, homozygous or heterozygous.

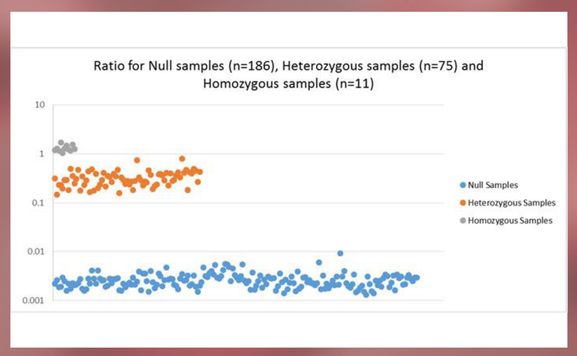
A further cohort of 112 plasma samples of unknown genotype were utilised to verify performance characteristics established employing the initial cohort. Genotype concordance was further investigated by genotyping these same 112 plasma samples from circulating cell free DNA (cfDNA) through the use of another biochip array platform, based on a combination of multiplex PCR and biochip array hybridisation, which allows simultaneous detection of APOE specific single nucleotide polymorphisms (SNPs).

Receiver Operating Characteristics (ROC) curve was used to establish the sensitivity and specificity of the assay using the combined cohort of 384 plasma samples.

Assay time: within 3 hours

RESULTS

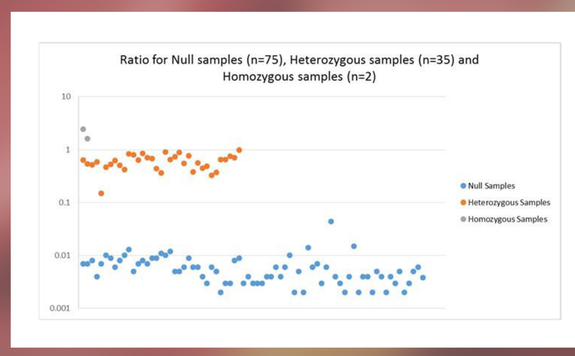
From the initial cohort of 272 samples with known genotypes, 100% were correctly identified as null, homozygous or heterozygous for ApoE4 by the biochip array.



	Null Samples	Heterozygous Samples	Homozygous Samples
Median	0.002	0.294	1.261
Min	0.001	0.147	1.037
Max	0.009	0.793	1.712

15.056-062, 065, 133-137, 139, 142, 143, 149, 150, 154.102RDRT

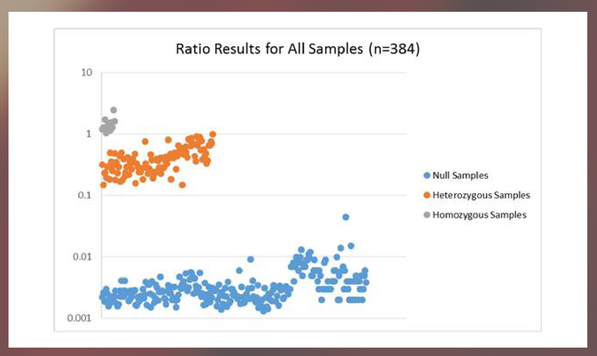
From the additional 112 plasma samples, analysed using BAT for protein and SNPs detection, 100% concordance was found between both approaches.



	Null Samples	Heterozygous Samples	Homozygous Samples
Median	0.005	0.617	2.02
Min	0.002	0.148	1.6
Max	0.044	0.985	2.4

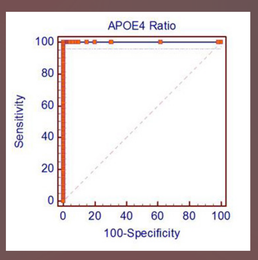
15.225-227.102RDRT

Ratio results for all the 384 plasma samples.



Based on 384 plasma samples, ROC analysis showed that patient samples could be identified as APOE4 positive or negative with 100% sensitivity and 100% specificity.

ROC curve	
Variable	APOE4 Ratio
	APOE4 Ratio
Classification variable	Diagnosis
Sample size	384
Positive group [a]	123 (32.03%)
Negative group [b]	261 (67.97%)
Area under the ROC curve (AUC)	
Area under the ROC curve (AUC)	1
Standard Error [a]	0
95% Confidence interval [b]	0.990 to 1.000
Significance level P (Area=0.5)	<0.0001
Youden index	
Youden index J	1
Associated criterion	>0.044
Sensitivity	100
Specificity	100



CONCLUSION

An individual's APOE status has been shown to affect pre-symptomatic risk, diagnosis, prognosis, and treatment response for a variety of diseases, in particular Alzheimer's disease. The results show that BAT can be successfully applied to provide a platform to rapidly and accurately detect an individual's APOE4 status directly from a plasma sample. In combination with medical and family history, medication and lifestyle, this can deliver valuable information for personalised medicine approaches.