

# HIGH-THROUGHPUT, FULLY AUTOMATED IMMUNOASSAY **BECKMAN** FOR DETECTING ZYGOSITY OF APOLIPOPROTEIN $\varepsilon 4$ (APOE $\epsilon$ 4) IN PLASMA EDTA

Miklos Szabo, Brian Engel, Katie Hoffmann, Ben Schlichtmann, Kara Curtis, Laura Mediger, Corey Carlson, James Mendoza, Mikaela Nichkova-Doseva Beckman Coulter, Inc., Brea, CA USA

# BACKGROUND

Apolipoprotein E (APOE) and its isoforms (APO  $\varepsilon 2/3/4$ ) shuttle lipids between cellular compartments and organs. The APOE £4 isoform is associated with increased risk of Alzheimer's disease (AD), with higher risk for homozygous APOE  $\varepsilon$ 4+/+ individuals. Similarly, an increased risk of Amyloidrelated imaging abnormalities (ARIA) is observed with lecanemab treatment and APOE  $\epsilon 4+/-$  or  $\epsilon 4+/+$  individuals. Availability of high-throughput assays to determine APOE £4 zygosity would enable reliable use by researchers. We describe the performance of a prototype highthroughput APOE ε4 zygosity assay on the Beckman Coulter DxI 9000 and Access 2 Immunoassay Analyzers.

# **Cross-Reactivity and Interfering Substances**

Studies were performed to assess analytical specificity of the Beckman Coulter APOE £4 RUO assay through response to potential cross-reactants, endogenous interferents, and common drugs.

The study was run on a single Access 2 Immunoassay Analyzer with one reagent lot. Interference and cross-reactivity were

Table 1. Analytical Specificity of the APO ε4 assay

Analyte	Test Concentration	APO ε4 Assay Result (RLU)	APO ε4 Assay Result (% Difference)	Calculated Result
Control	0 µg/mL	8,092	0%	0.002
	10 µg/mL	8,313	3%	0.002
ΑΡΟ ε2	25 µg/mL	8,335	3%	0.002
	50 µg/mL	8,134	1%	0.002
	10 µg/mL	8,260	2%	0.002
ΑΡΟ ε3	25 µg/mL	8,254	2%	0.002
	50 µg/mL	8,148	1%	0.003

assessed on an APO £4 negative and/or | Table 2. Analytical specificity in an APO £4 positive K2 EDTA plasma sample

RESULTS

Table 8. Method correlation between the Beckman Coulter APOE £4 RUO assay on Access 2 or DxI 9000 Immunoassay Analyzers versus the Lumipulse® G Apo ɛ4 RUO assay.

	Dose Ratio	Calculate	d Result
Sample	Lumipulse	Access 2*	DxI 9000*
Sample 1	3.33	7.64	7.69
Sample 2	1.02	4.48	3.73
Sample 3	1.18	4.13	4.10
Sample 4	0.73	3.98	2.81
Sample 5	0.85	3.58	3.02
Sample 6	0.52	3.57	2.01
Sample 7	0.66	3.48	2.81
Sample 8	0.46	3.15	2.73
Sample 9	0.32	2.98	2.59
Sample 10	0.54	2.93	2.67
Sample 11	0.31	2.63	2.13
Sample 12	0.30	2.10	2.05
Sample 13	0.37	1.84	1.47
Sample 14	0.08	0.63	0.44
Sample 15	0.04	0.26	0.14
Sample 16	0.00	0.02	0.00
Sample 17	0.00	0.02	0.00
Sample 18	0.00	0.02	0.00
Sample 19	0.01	0.02	0.00
Sample 20	0.00	0.01	0.00
Correlation with L	umipulse ®	0.89	0.94

### **METHODS**

## **Assay Format**

The research use only (RUO) APOE £4 assay is a multiplex of 2 two-step sandwich assays using paramagnetic particles coated with anti-PanAPOE monoclonal antibody (MAb) and complementary anti-PanAPOE MAb/alkaline phosphatase conjugate or an anti-APO ε4 MAb/alkaline phosphatase conjugate. Samples and reactants are incubated and washed and then a chemiluminescent substrate is added. The light generated is processed through an algorithm that provides a calculated result indicating the APOE £4 zygosity of the sample.

Samples screened on the DxI 9000 analyzer have a time-to-first result of ~20 minutes, with up to 450 tests/hour. Samples screened on the Access 2 analyzer have a time-to-first result of ~25 minutes, with up to 100 tests/hour.

APO £4 positive K2 EDTA plasma samples.

Stock solutions of potential crossreactants/interferents were spiked into the patient samples to the target concentrations. Control samples were prepared by adding an equal volume of solvent only. Each sample was analyzed in duplicate.

# Imprecision

Studies were performed to assess the imprecision of the Beckman Coulter APOE ε4 RUO assay.

The study was run using two reagent lots on two Access 2 and one DxI 9000 Immunoassay Analyzers. Native K2 EDTA plasma samples with variable APO ε4 status were measured over 5 days, with 5 replicates per run, and 2 runs per day.

Within-run, between-run, and withinlaboratory (total) variances and %CV for the calculated result were then determined for each sample, each reagent lot, and on each Immunoassay Analyzer.

able Z. Analytical specificity	$\mu$ in an AFO 24 positive	

Analyte	Test Concentration	Calculated Result	Calculated Result (% Difference)
Control	0 ng/mL	1.439	0%
Desmin	476 ng/mL	1.530	6%
GFAP	13 ng/mL	1.498	4%
Internexin	238 ng/mL	1.556	8%
Keratin-8	476 ng/mL	1.408	-2%
Nestin	381 ng/mL	1.458	1%
Neurofilament Heavy	952 ng/mL	1.486	3%
Neurofilament Light	5 ng/mL	1.476	3%
Neurofilament Medium	1238 ng/mL	1.584	10%
Peripherin	333 ng/mL	1.666	16%
P-Tau217	952 ng/mL	1.537	7%
Synemin	238 ng/mL	1.701	18%
Vimentin	952 ng/mL	1.601	11%
	Control Desmin GFAP Internexin Keratin-8 Nestin Neurofilament Heavy Neurofilament Light Neurofilament Medium Peripherin P-Tau217 Synemin	Control0 ng/mLDesmin476 ng/mLGFAP13 ng/mLInternexin238 ng/mLKeratin-8476 ng/mLNestin381 ng/mLNeurofilament Heavy952 ng/mLNeurofilament Light5 ng/mLNeurofilament Medium1238 ng/mLPeripherin333 ng/mLP-Tau217952 ng/mLSynemin238 ng/mL	AnalyteTest ConcentrationResultControl0 ng/mL1.439Desmin476 ng/mL1.530GFAP13 ng/mL1.498Internexin238 ng/mL1.556Keratin-8476 ng/mL1.408Nestin381 ng/mL1.458Neurofilament Heavy952 ng/mL1.486Neurofilament Light5 ng/mL1.476Neurofilament Medium1238 ng/mL1.584Peripherin333 ng/mL1.666P-Tau217952 ng/mL1.537Synemin238 ng/mL1.701

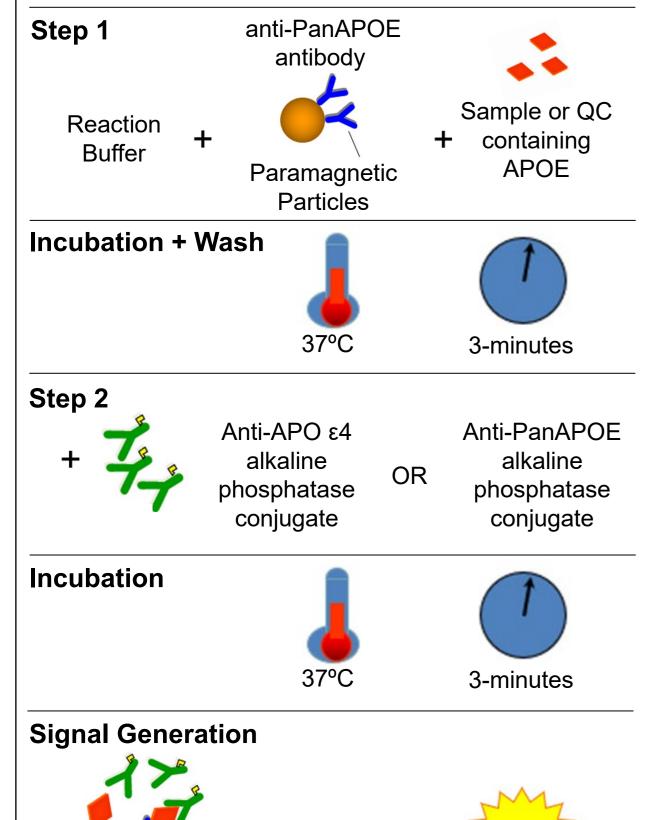
#### Table 3. Analytical specificity (interfering substances)

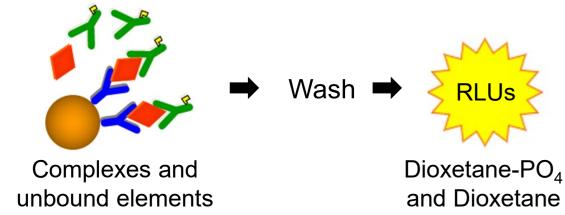
Interferent Concentration		terent			
interierent	Concentration	Calculated Result	% Diff.	Calculated Result	% Diff.
DI Control		1.609		0.002	
Donepezil	300 ng/mL	1.604	0%	0.002	5%
Galantamine	500 ng/mL	1.521	-5%	0.002	7%
Memantine	450 ng/mL	1.556	-3%	0.002	1%
DMSO Control		1.515		0.002	
Aripiprazole	1800 ng/mL	1.497	-1%	0.002	7%
DI Control		1.485		0.002	
Acetaminophen	0.156 mg/mL	1.495	1%	0.002	0%
Ibuprofen	0.219 mg/mL	1.593	7%	0.002	4%
HSA Control		1.463		0.002	
HSA	0.15 g/mL	1.576	8%	0.002	3%
Heparin Control		1.411		0.002	
Heparin	3.3 U/mL	1.649	17%	0.002	1%
Bilirubin Control		1.555		0.002	
Bilirubin - Conj	0.4 mg/mL	1.459	-6%	0.002	-6%
Bilirubin - Unconj.	0.4 mg/mL	1.545	-1%	0.002	1%
Neat Plasma		1.479		0.002	
Cholesterol	2 mg/mL	1.454	-2%	0.002	-2%
Intralipid	15 mg/mL	1.590	7%	0.002	1%

Figure 2. PCR genotype concordance with the APOE ε4 RUO assay. Study demonstrates excellent separation based on APOE £4 zygosity.

	8-	•	Zygosity
	7-		• APOE4-/- • APOE4+/- • APOE4+/+
	6-		A OLAN
t.	F	•	
Calculated Result	<b>J</b> -		
ated F	4-		
alcula		•	
ő	3-		
	2-	<u>.</u>	
	1-		
	•		
	0-		

#### Figure 1. Basic principle of the assay.





# Specificity of the APO ε4 Assay

This study was performed to assess the

# **Method Correlation**

A method correlation study was completed to compare the Beckman Coulter APOE ε4 RUO assay on the Access 2 and DxI 9000 Immunoassay Analyzers with the Lumipulse G Apo ɛ4 (Item #81453) RUO assay on the LUMIPULSE G1200 analyzer. K2 EDTA plasma samples with variable APO ɛ4 status were tested (n=20).

All Samples were tested on one reagent lot in replicates of two for the Access 2 and DxI 9000 Immunoassay Analyzers and in a single replicate for the LUMIPULSE G1200 analyzer.

Correlation between the methods was determined using Passing-Bablok linear regression.

# **PCR Genotype Concordance**

study was performed to assess the concordance of the Beckman Coulter APOE RUO assay with PCR ε4 genotyping.

Table 4. Imprecision on Access 2 Immunoassay Analyzer with reagent lot 1

						0			
	Calculated Result								
Ν	Maan	Withi	n Run	Betwe	en Run	Withi	n Lab		
	mean	SD	%CV	SD	%CV	SD	%CV		
40	0.008	0.001	12.3	0.002	23.8	0.002	26.8		
40	0.014	0.003	19.4	0.006	38.7	0.006	43.3		
40	2.08	0.17	7.9	N/A	N/A	0.17	7.9		
40	2.76	0.23	8.2	0.17	6.0	0.28	10.1		
40	3.73	0.24	6.5	0.23	6.3	0.34	9.0		
40	6.38	0.55	8.7	N/A	N/A	0.55	8.7		
	40 40 40 40 40	Mean400.008400.014402.08402.76403.73	MeanSD400.0080.001400.0140.003402.080.17402.760.23403.730.24	NMeanWithin Run SD $40$ $0.008$ $0.001$ $12.3$ $40$ $0.014$ $0.003$ $19.4$ $40$ $2.08$ $0.17$ $7.9$ $40$ $2.76$ $0.23$ $8.2$ $40$ $3.73$ $0.24$ $6.5$	NMeanWithin RunBetwee40 $0.008$ $0.001$ $12.3$ $0.002$ 40 $0.014$ $0.003$ $19.4$ $0.006$ 40 $2.08$ $0.17$ $7.9$ N/A40 $2.76$ $0.23$ $8.2$ $0.17$ 40 $3.73$ $0.24$ $6.5$ $0.23$	NMeanWithin RunBetween Run400.0080.00112.30.00223.8400.0140.00319.40.00638.7402.080.177.9N/AN/A402.760.238.20.176.0403.730.246.50.236.3	NMeanWithin RunBetween RunWithin40 $0.008$ $0.001$ $12.3$ $0.002$ $23.8$ $0.002$ 40 $0.014$ $0.003$ $19.4$ $0.006$ $38.7$ $0.006$ 40 $2.08$ $0.17$ $7.9$ N/AN/A $0.17$ 40 $2.76$ $0.23$ $8.2$ $0.17$ $6.0$ $0.28$ 40 $3.73$ $0.24$ $6.5$ $0.23$ $6.3$ $0.34$		

Table 5. Imprecision on Access 2 Immunoassay Analyzer with reagent lot 2

				Calculated Result						
S	Sample	Ν	Maan	Withi	n Run	Betwee	en Run	Withi	n Lab	
r			Mean	SD	%CV	SD	%CV	SD	%CV	
•	Sample 1†	40	0.008	0.001	13.1	0.002	22.8	0.002	26.3	
	Sample 2†	40	0.013	0.001	8.1	0.005	35.1	0.005	36.0	
	Sample 3	40	1.89	0.20	10.6	0.15	8.1	0.25	13.4	
	Sample 4	40	2.44	0.27	11.1	0.11	4.5	0.29	12.0	
	Sample 5	40	3.26	0.28	8.7	0.11	3.4	0.30	9.3	
	Sample 6	40	5.77	0.56	8.8	0.35	6.0	0.61	10.7	
		•		•			•	-	•	

#### Table 6. Imprecision on DxI 9000 Immunoassay Analyzer with reagent lot 1

		Calculated Result							
Sample	Ν	Moon	Withi	n Run	Betwee	en Run	Withi	n Lab	
		Mean	SD	%CV	SD	%CV	SD	%CV	
Sample 1	40	0.003	0.000	9.8	0.000	3.7	0.000	10.4	
Sample 2	40	0.003	0.000	7.9	0.000	2.1	0.000	8.2	
Sample 3	40	1.64	0.13	7.7	0.05	3.1	0.14	8.3	
Sample 4	40	2.37	0.22	9.2	N/A	N/A	0.22	9.2	
Sample 5	40	3.61	0.27	7.6	0.26	7.2	0.38	10.4	
Sample 6	40	6.66	0.63	9.4	0.24	3.7	0.67	10.1	
	Sample 1 Sample 2 Sample 3 Sample 4 Sample 5	Sample 140Sample 240Sample 340Sample 440Sample 540	Sample 1         40         0.003           Sample 2         40         0.003           Sample 3         40         1.64           Sample 4         40         2.37           Sample 5         40         3.61	MeanSDSample 1400.0030.000Sample 2400.0030.000Sample 3401.640.13Sample 4402.370.22Sample 5403.610.27	Sample         N         Mean         Within Run           Sample 1         40         0.003         0.000         9.8           Sample 2         40         0.003         0.000         7.9           Sample 3         40         1.64         0.13         7.7           Sample 4         40         2.37         0.22         9.2           Sample 5         40         3.61         0.27         7.6	Sample         N         Mean         Within Run         Between           Sample 1         40         0.003         0.000         9.8         0.000           Sample 2         40         0.003         0.000         7.9         0.000           Sample 3         40         1.64         0.13         7.7         0.05           Sample 4         40         2.37         0.22         9.2         N/A           Sample 5         40         3.61         0.27         7.6         0.26	Sample         N         Mean         Within Run         Between Run           Sample 1         40         0.003         0.000         9.8         0.000         3.7           Sample 2         40         0.003         0.000         7.9         0.000         2.1           Sample 3         40         1.64         0.13         7.7         0.05         3.1           Sample 4         40         2.37         0.22         9.2         N/A         N/A           Sample 5         40         3.61         0.27         7.6         0.26         7.2	$\begin{array}{c c c c c c c c c c c c c c c c c c c $	

Table 9. Summary of PCR genotype concordance study. The APOE ε4 (RUO) assay shows 99.3% concordance with PCR genotyping (n = 300).

			PCR				Concord-	Calculated	
Sample Count		APOE ε4 Zygosity			Total	ance	Result Range		
			-/-	+/-	+/+	ισται		(Dxl 9000)	
	-/-	181	0	0	181	100%	0.003 - 0.007		
Dxl	APOE ε4 Zygosity	+/-	2	105	0	107	98.1%	1.16 – 3.79	
9000*	Lygosity	+/+	0	0	12	12	100%	5.37 – 7.81	
	Total		183	105	12	300	99.3%	0.003 – 7.81	

# CONCLUSION

The Beckman Coulter APOE £4 RUO assay provides fast and precise results in an automated immunoassay platform on the Beckman Coulter DxI 9000 and Access 2 Immunoassay Analyzers. The assay showed 99.3% concordance with PCR and had high correlation with commercially available APOE ε4 RUO assays. The concordance data show that the Beckman Coulter APOE £4 (RUO) assay may have promise as a foundational blood-based biomarker in research laboratories around the world.

APOE specificity of the APO ε4 assay. The study was run on a single Access 2 Immunoassay Analyzer with one reagent lot. A K2 EDTA plasma APO ε4 negative sample was spiked with stock solutions of APO  $\epsilon^2$  and APO  $\epsilon^3$  protein. The signal from the APO ɛ4 assay and calculated result were compared with the un-spiked sample. Each sample was analyzed in duplicate.

2024-13544

The analysis was performed using PCRgenotyped K2 EDTA plasma samples tested in singlicate on a single DxI 9000 Immunoassay Analyzer, with one reagent lot. (n=300). Each sample was tested in a single replicate.

APOE zygosity from PCR genotyping was compared with the Calculated Result of the APOE ε4 RUO assay.

 Table 7. Imprecision on DxI 9000 Immunoassay Analyzer with reagent lot 2

	Calculated Result								
Sample	Ν	Maan	Withi	n Run	Betwee	en Run	Withi	n Lab	
		Mean	SD	%CV	SD	%CV	SD	%CV	
Sample 1	40	0.004	0.000	8.5	0.000	2.1	0.000	8.8	
Sample 2	40	0.004	0.000	6.7	N/A	N/A	0.000	6.7	
Sample 3	40	1.45	0.11	7.4	0.07	4.5	0.13	8.7	
Sample 4	40	2.04	0.13	6.3	0.02	1.1	0.13	6.4	
Sample 5	40	3.26	0.22	6.8	0.21	6.4	0.31	9.4	
Sample 6	40	6.32	0.45	7.1	0.17	2.7	0.48	7.6	

danaher.

© 2024 Beckman Coulter. All rights reserved. Beckman Coulter, the stylized logo, and the Beckman Coulter product and service marks mentioned herein are trademarks or registered trademarks of Beckman Coulter, Inc. in the United States and other countries. The Danaher trademark is a proprietary mark of Danaher Corporation. All other trademarks are the property of their respective owners. \* Full name is DxI 9000 Access Immunoassay Analyzer † Measured APOE signals are background signals For research use only. Not for use in diagnostic procedures.