HIGH-THROUGHPUT, FULLY AUTOMATED IMMUNOASSAY FOR DETECTING ZYGOSITY OF APOLIPOPROTEIN ε4 (APOE ε4) IN PLASMA EDTA

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BACKGROUND

Apolipoprotein E (APOE) and its isoforms (APO ε2/3/4) shuttle lipids between cellular compartments and organs. The APOE ε4 isoform is associated with increased risk of Alzheimer's disease (AD), with higher risk for homozygous APOE ε4+/+ individuals. Similarly, an increased risk of Amyloid-related imaging abnormalities (ARIA) is observed with lecanemab treatment and APOE ε4+/- or ε4+/+ individuals. Availability of high-throughput assays to determine APOE ε4 zygosity would enable reliable use by researchers. We describe the performance of a prototype high-throughput APOE ε4 zygosity assay on the Beckman Coulter DxI 9000 and Access 2 Immunoassay Analyzers.

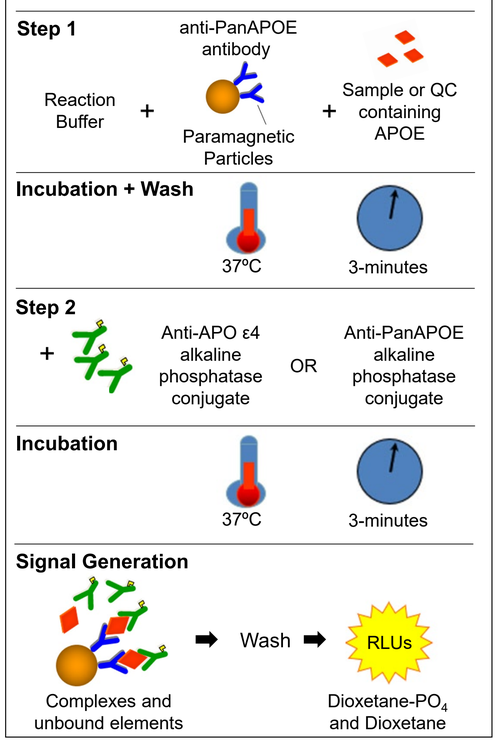
METHODS

Assay Format

The research use only (RUO) APOE ε4 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 ε4 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.

Figure 1. Basic principle of the assay.



Specificity of the APO ε4 Assay

This study was performed to assess the 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 ε2 and APO ε3 protein. The signal from the APO ε4 assay and calculated result were compared with the un-spiked sample. Each sample was analyzed in duplicate.

Cross-Reactivity and Interfering Substances

Studies were performed to assess analytical specificity of the Beckman Coulter APOE ε4 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 assessed on an APO ε4 negative and/or APO ε4 positive K2 EDTA plasma samples.

Stock solutions of potential cross-reactants/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 within-laboratory (total) variances and %CV for the calculated result were then determined for each sample, each reagent lot, and on each Immunoassay Analyzer.

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 ε4 (Item #81453) RUO assay on the LUMIPULSE G1200 analyzer. K2 EDTA plasma samples with variable APO ε4 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

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

The analysis was performed using PCR-genotyped 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.

RESULTS

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
APO ε2	10 µg/mL	8,313	3%	0.002
	25 µg/mL	8,335	3%	0.002
	50 µg/mL	8,134	1%	0.002
APO ε3	10 µg/mL	8,260	2%	0.002
	25 µg/mL	8,254	2%	0.002
	50 µg/mL	8,148	1%	0.003

Table 2. Analytical specificity in an APO ε4 positive K2 EDTA plasma sample

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%

Table 3. Analytical specificity (interfering substances)

Interferent	Test Concentration	APO ε4 Positive K2 EDTA Sample		APO ε4 Negative K2 EDTA Sample	
		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%

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

Sample	N	Calculated Result						
		Mean	Within Run		Between Run		Within Lab	
			SD	%CV	SD	%CV	SD	%CV
Sample 1†	40	0.008	0.001	12.3	0.002	23.8	0.002	26.8
Sample 2†	40	0.014	0.003	19.4	0.006	38.7	0.006	43.3
Sample 3	40	2.08	0.17	7.9	N/A	N/A	0.17	7.9
Sample 4	40	2.76	0.23	8.2	0.17	6.0	0.28	10.1
Sample 5	40	3.73	0.24	6.5	0.23	6.3	0.34	9.0
Sample 6	40	6.38	0.55	8.7	N/A	N/A	0.55	8.7

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

Sample	N	Calculated Result						
		Mean	Within Run		Between Run		Within Lab	
			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

Sample	N	Calculated Result						
		Mean	Within Run		Between Run		Within Lab	
			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

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

Sample	N	Calculated Result						
		Mean	Within Run		Between Run		Within Lab	
			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

Table 8. Method correlation between the Beckman Coulter APOE ε4 RUO assay on Access 2 or DxI 9000 Immunoassay Analyzers versus the Lumipulse® G Apo ε4 RUO assay.

Sample	Dose Ratio	Calculated Result	
	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 Lumipulse ®		0.89	0.94

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

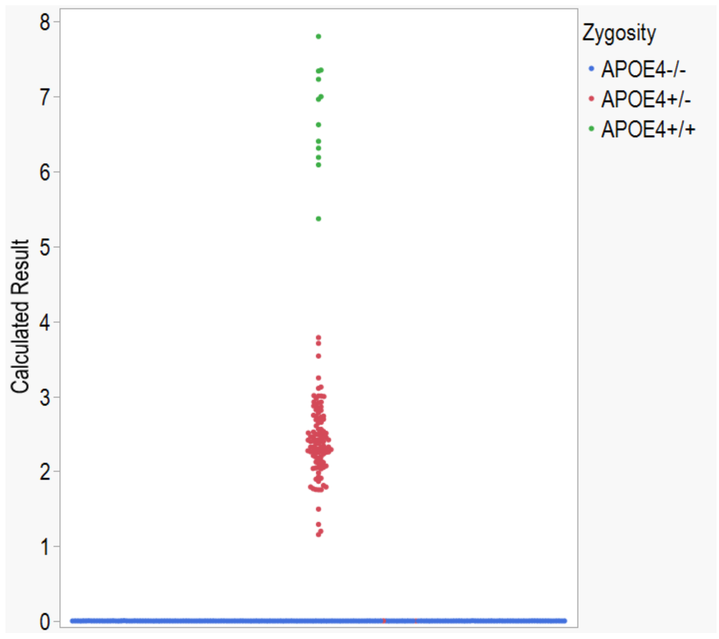


Table 9. Summary of PCR genotype concordance study. The APOE ε4 (RUO) assay shows 99.3% concordance with PCR genotyping (n = 300).

Sample Count			PCR				Concord-ance	Calculated Result Range (DxI 9000)
			APOE ε4 Zygosity			Total		
			-/-	+/-	+/+			
DxI 9000*	APOE ε4 Zygosity	-/-	181	0	0	181	100%	0.003 – 0.007
		+/-	2	105	0	107	98.1%	1.16 – 3.79
		+/+	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 ε4 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 ε4 (RUO) assay may have promise as a foundational blood-based biomarker in research laboratories around the world.

* Full name is DxI 9000 Access Immunoassay Analyzer
† Measured APOE signals are background signals
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