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Background and Methods

Alzheimer's Disease (AD) is the predominant form of dementia in aging populations, with an estimated 6.7 million people diagnosed in the US and an annual death rate of 122,000¹. One of the diagnostic hallmarks of AD is extracellular deposits of β -amyloid plaques in the cortex and limbic brain region where the major molecular components of β -amyloid plaques is β -amyloid 1-42 (A β 42)²⁻³. Reduced concentrations of A β 42 in cerebrospinal fluid (CSF) and plasma are associated with increased retention of A β tracers in the brain β -amyloid plaques observed with positron emission tomography (PET). Correlation to PET results is further improved by using the ratio of A β 42 to β -amyloid 1-40 (A β 40) to account for variation in total β -amyloid from person-to-person⁴⁻⁶. Although PET imaging and measurements in CSF have been traditionally used to investigate the presence of β -amyloid plaques, these techniques are invasive to the patient⁷.

To provide an alternative, the Sysmex HISCL[®]-5000 Immunoassay System was used to validate chemiluminescence enzyme immunoassays for the measurement of A β 40 and A β 42 in EDTA plasma samples as a laboratory developed test (LDT) to determine the A β 42/40 ratio.

Analytical Validation Results

Analytical performance studies investigated the assay's analytical measurement range, imprecision, potential endogenous interferences, and sample stability.

		Imprecision			
		Low QC	High QC	Plasma Pool 1	Plasma Pool 2*
A β 40	pg/mL	91.31	180.75	116.4	381.72
	Repeatability	1.7%	2.9%	2.1%	2.4%
	Intermediate	4.7%	4.4%	5.7%	4.2%
A β 42	pg/mL	15.01	29.65	11.87	35.35
	Repeatability	1.9%	2.2%	2.7%	3.0%
	Intermediate	4.4%	3.3%	5.3%	5.3%
A β 42/40	Ratio	0.165	0.164	0.102	0.093
	Repeatability	2.9%	3.5%	3.6%	3.9%
	Intermediate	6.0%	4.7%	6.5%	5.5%

*Fortified to create elevated levels of individual measurands

Sample Stability*				
Ambient (20-25 °C)	Refrigerated (2-8 °C)	Frozen (< -10 °C)	Deep Frozen (< -70 °C)	Freeze/Thaw
4 hours	8 hours	8 hours	100 days	1 cycle

*Using Labcorp plasma transfer tubes



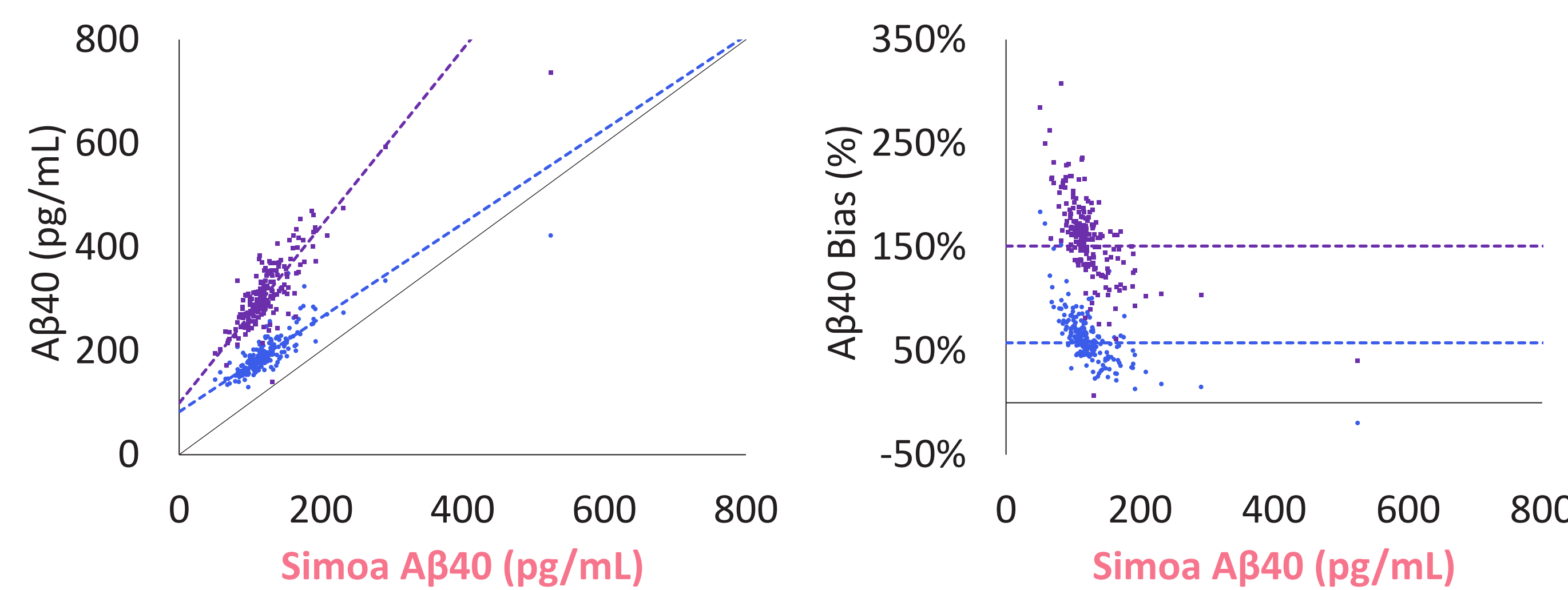
Limits of Quantitation	
A β 40 (pg/mL)	A β 42 (pg/mL)
25 – 2000	3.0 – 2000
Interferent	
Interferent	Acceptable Level
Triglycerides	1500 mg/dL
Hemoglobin	1000 mg/dL
Total Protein	18.5 g/dL
Total IgG	8000 mg/dL
Bilirubin	40 mg/dL
Biotin	3500 ng/mL

Assay Comparison

Two hundred clinically defined samples with A β status (determined using PET) were acquired from the Australian Imaging, Biomarkers & Lifestyle Flagship Study of Ageing (AIBL)⁸. These samples consisted of 4 subgroups: cognitively normal (CN)/A β -, CN/A β +, mild cognitive impairment (MCI)/A β +, and diagnosed AD/A β +. These samples were used to perform correlation studies using two existing commercial assay platforms for A β 42/40: Quanterix Simoa[®] and Fujirebio Lumipulse[®] G. In addition, receiver operator characteristic (ROC) analysis was performed to assess diagnostic ability of each assay.

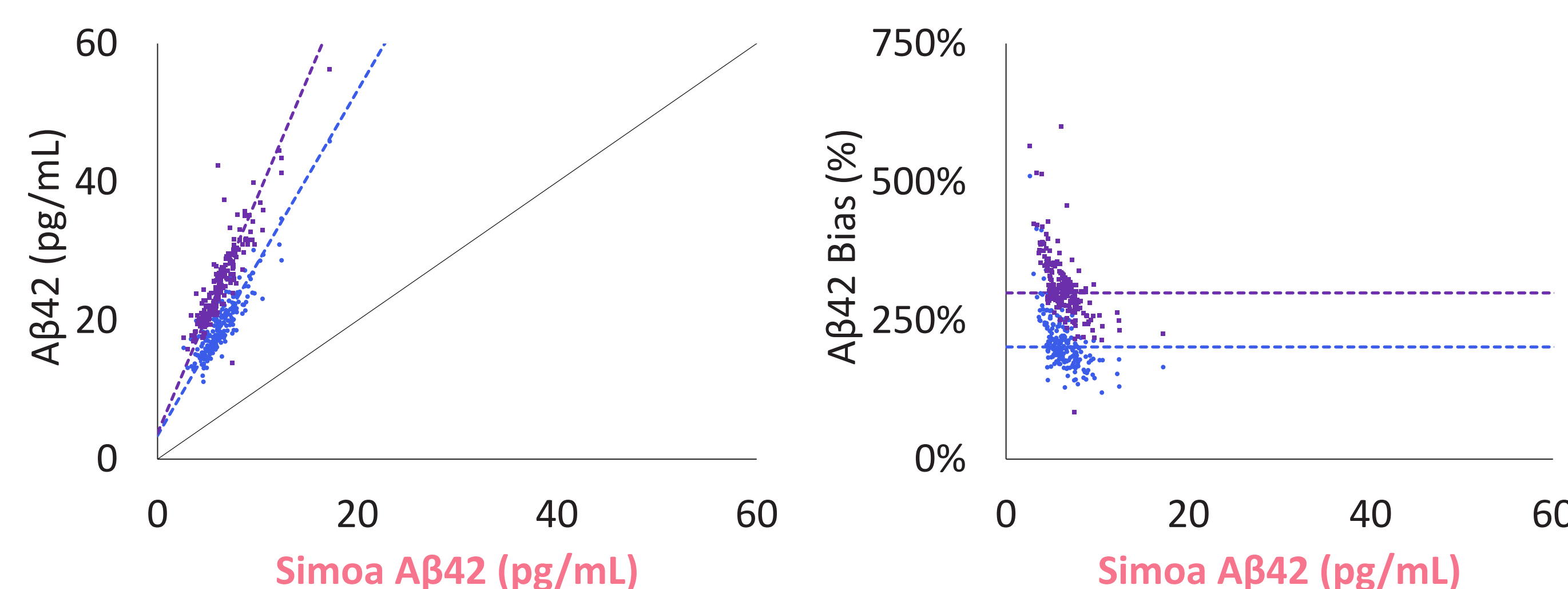
A β 40 Correlative Results as Compared to Simoa[®] Measurements

	R	Slope	Intercept	Mean Bias
HISCL [®]	0.8289	0.906	82.847	57.7%
Lumipulse [®]	0.8401	1.703	99.751	151.1%



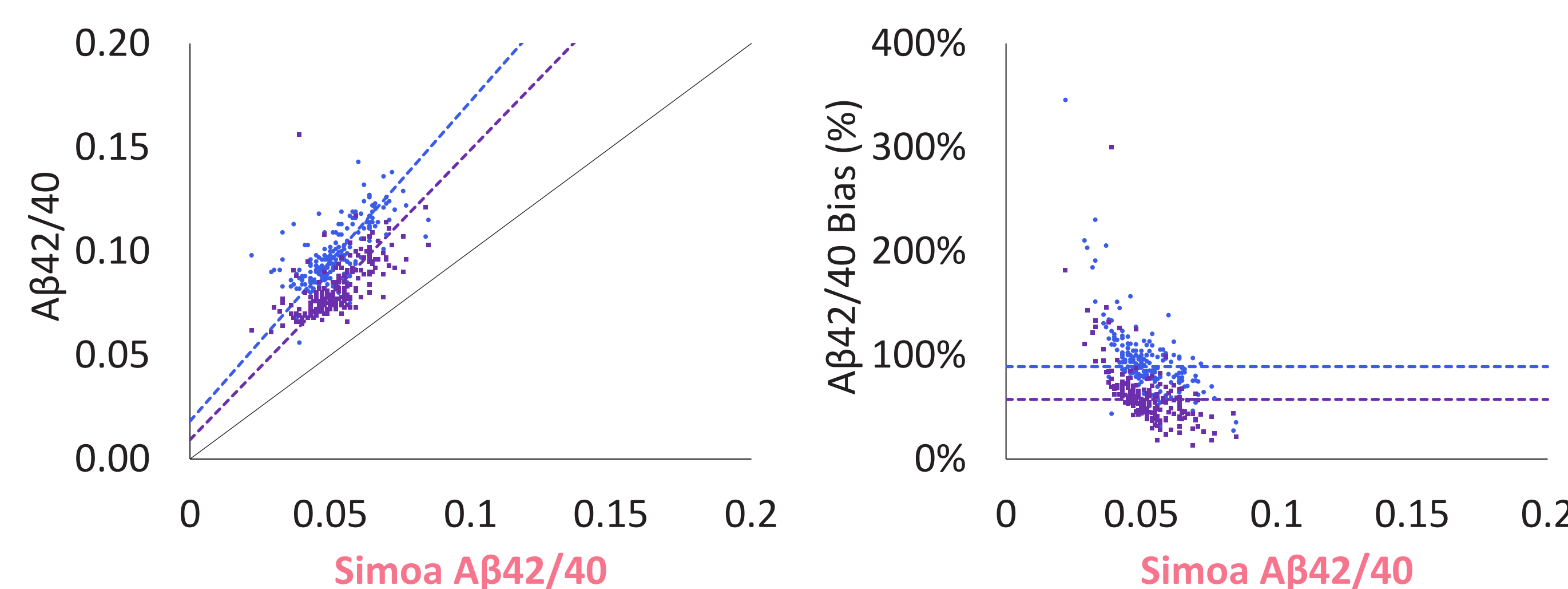
A β 42 Correlative Results as Compared to Simoa[®] Measurements

	R	Slope	Intercept	Mean Bias
HISCL [®]	0.8891	2.487	3.409	202.5%
Lumipulse [®]	0.8932	3.408	3.740	299.8%

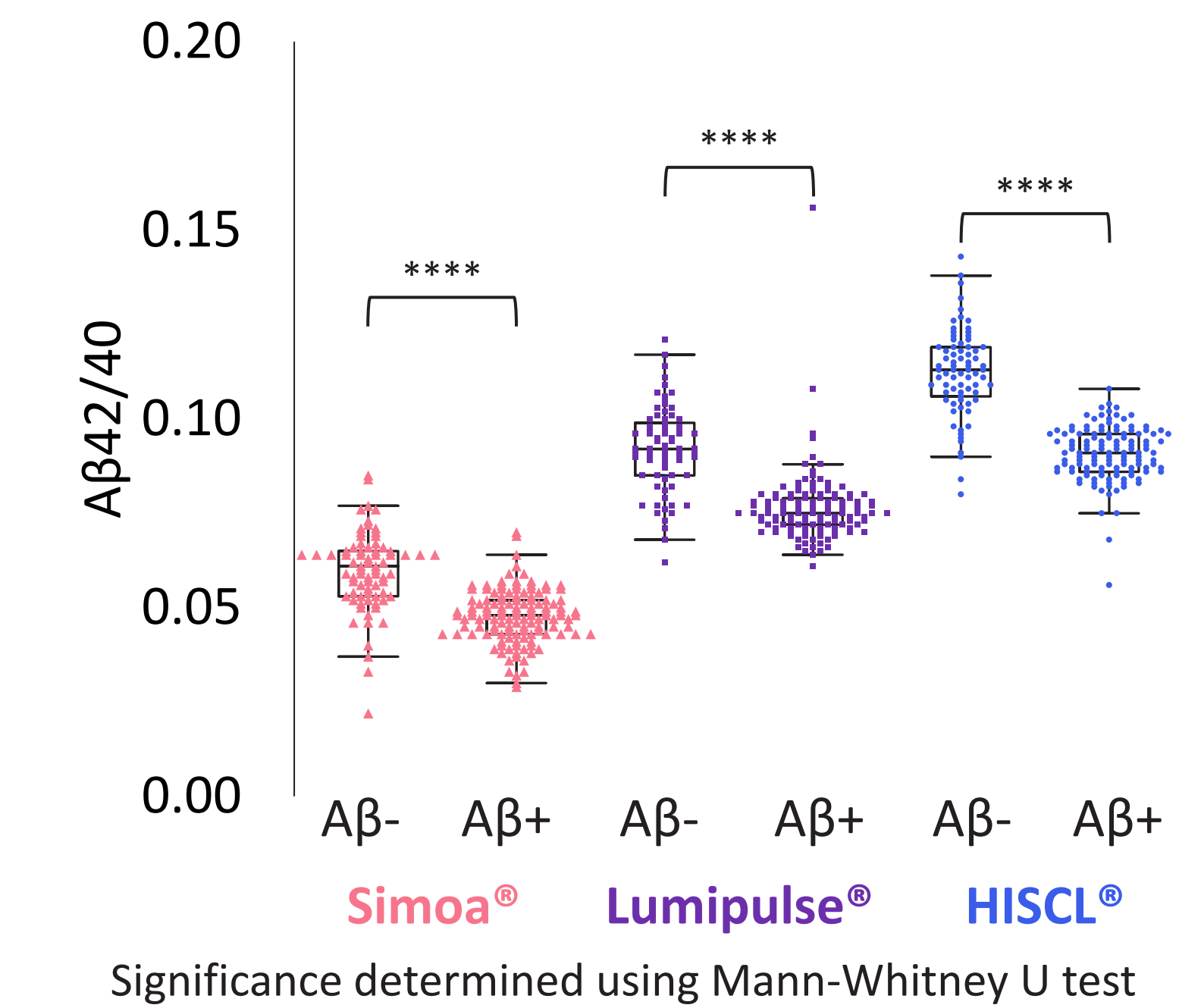


A β 42/40 Correlative Results as Compared to Simoa[®] Measurements

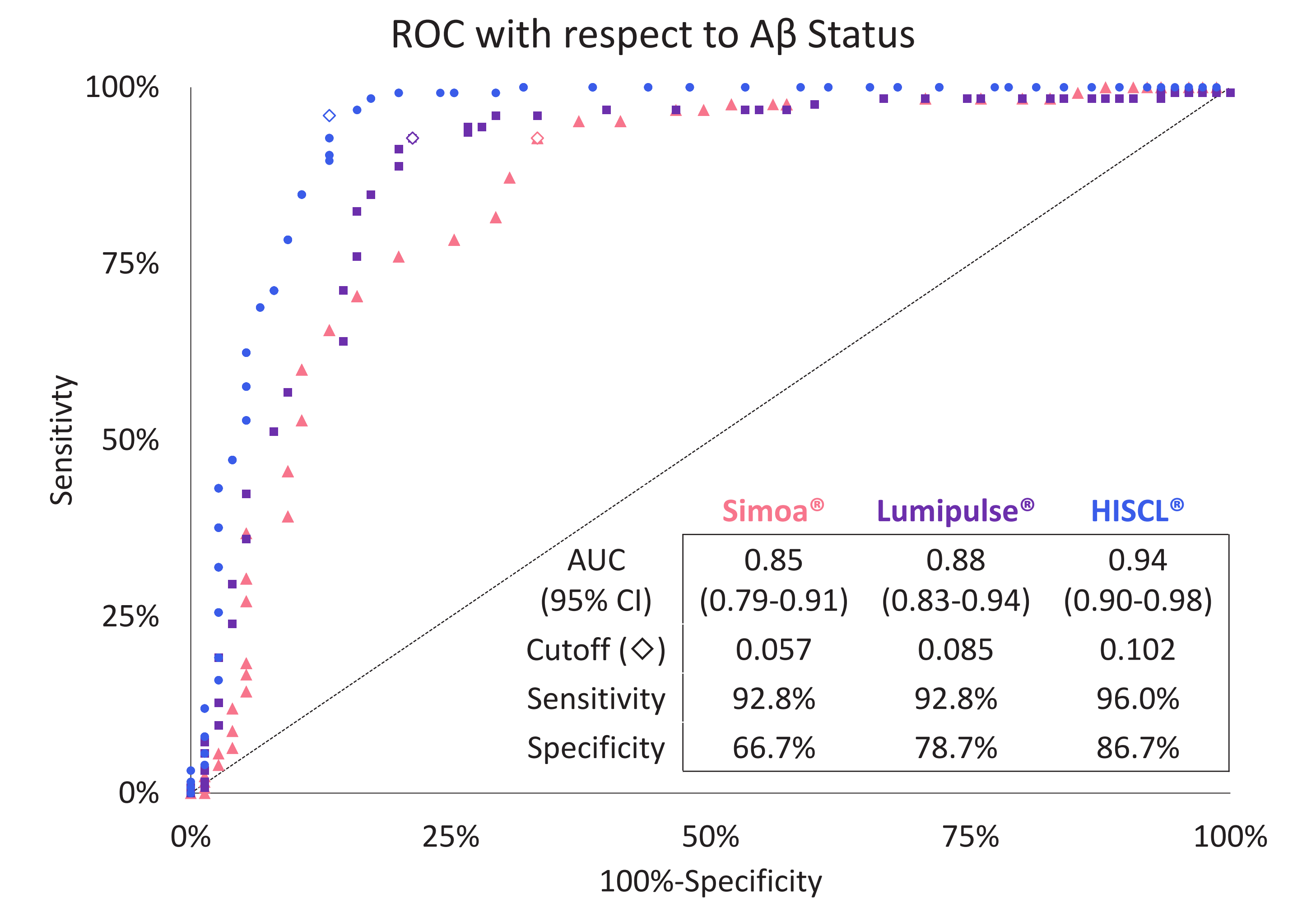
	R	Slope	Intercept	Mean Bias
HISCL [®]	0.6753	1.539	0.018	88.9%
Lumipulse [®]	0.6416	1.396	0.009	57.3%



Clinical Utility Assessment



Due to lack of standardization, discrepant results between assays are not unexpected. However, each assay can distinguish between amyloid beta negative and positive AIBL specimens. ROC results indicated a higher area-under-the-curve (AUC) for the HISCL[®] assay as compared to the other assays. In addition, the cutoff observed for the HISCL[®] assay is consistent with published results⁵.



Conclusions

Measurement of A β 42/40 in plasma samples using a high throughput clinical autoanalyzer is now available as an LDT to assist physicians with identifying patients with β -amyloid plaques and potentially the presence of Alzheimer's disease.

Acknowledgments

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References

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