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Supplementary Data

Validation of a Multiplex Assay for Simultaneous Quantification of Amyloid-β Peptide Species in Human Plasma with Utility for Measurements in Studies of Alzheimer's Disease Therapeutics

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Supplementary Table 1
Calibration performance summary for validation test batches

Calibrator level	Concentration (ng/L)							
	1[a]	2	3	4	5	6	7	8[b]
$A\beta_{1\text{-}40}$								
Theoretical concentration	1288	515	146	67	31	12	4.0	1.33
Back-calculated mean ($n=43$)	–	456	163	65	30	12	4.1	1.28
CV %	–	4.0	3.9	2.4	2.2	2.3	2.8	1.7
RE %	–	–11.5	11.3	–3.6	–2.6	4.0	3.2	–3.5
$A\beta_{1\text{-}42}$								
Theoretical concentration	495	198	79	32	13	5.0	1.67	0.56
Back-calculated mean ($n=43$)	496	200	77	32	14	4.7	1.87	0.42
CV %	1.3	2.6	2.1	1.8	1.6	1.7	5.4	16
RE %	0.1	1.0	–1.9	0.4	5.3	–5.7	11.9	–25

CV, coefficient of variation; RE, relative error; [a]Level 1 $A\beta_{1\text{-}40}$ calibrator saturated the fluorescence response of the Bio-Plex 200 instrument and was omitted; [b]$A\beta_{1\text{-}42}$ Level 8 calibrator omitted from calibration curve.

[1]Present: Biomarkable, Gent, Belgium.

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Supplementary Table 2
Relative accuracy (as RE) and precision (as CV) of Aβ quantification at ≤ the LLOQ. The V1 plasma pool was serially diluted with kit plasma diluent. Six replicates of each dilution were assayed in a single batch

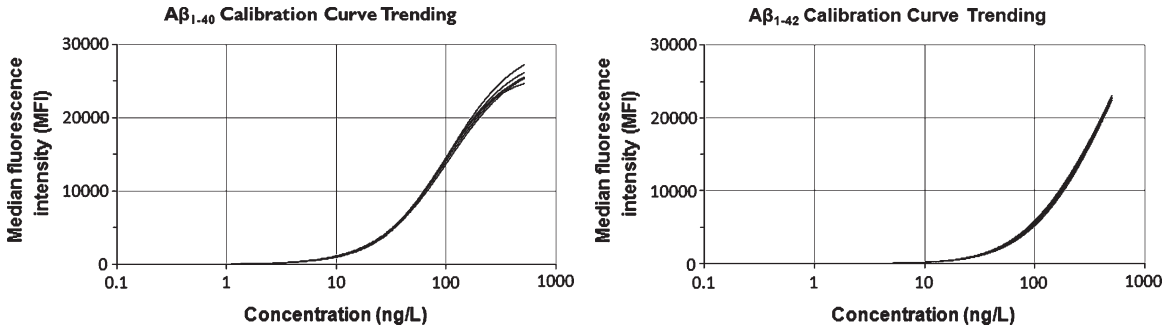
	Concentration (ng/L)	
	$A\beta_{1\text{-}40}$	$A\beta_{1\text{-}42}$
V1 Pool assigned concentration	8.4	7.6
x1 Dilution		
Mean ($n=6$)	7.9	7.6
Intra-assay CV %	2.3	7.9
Intra-assay RE %	–5.9	0
x2 Dilution[a]		
Mean ($n=6$)	3.6	–
Intra-assay CV %	4.5	–
Intra-assay RE %	–14.3	–
x4 Dilution[a]		
Mean ($n=6$)	1.8	–
Intra-assay CV%	3.9	–
Intra-assay RE %	–13.9	–
x8 Dilution[a]		
Mean ($n=6$)	1.1	–
Intra-assay CV%	3.4	–
Intra-assay RE %	0.6	–

CV, coefficient of variation; RE, relative error; [a]Signal out of range for $A\beta_{1\text{-}42}$.

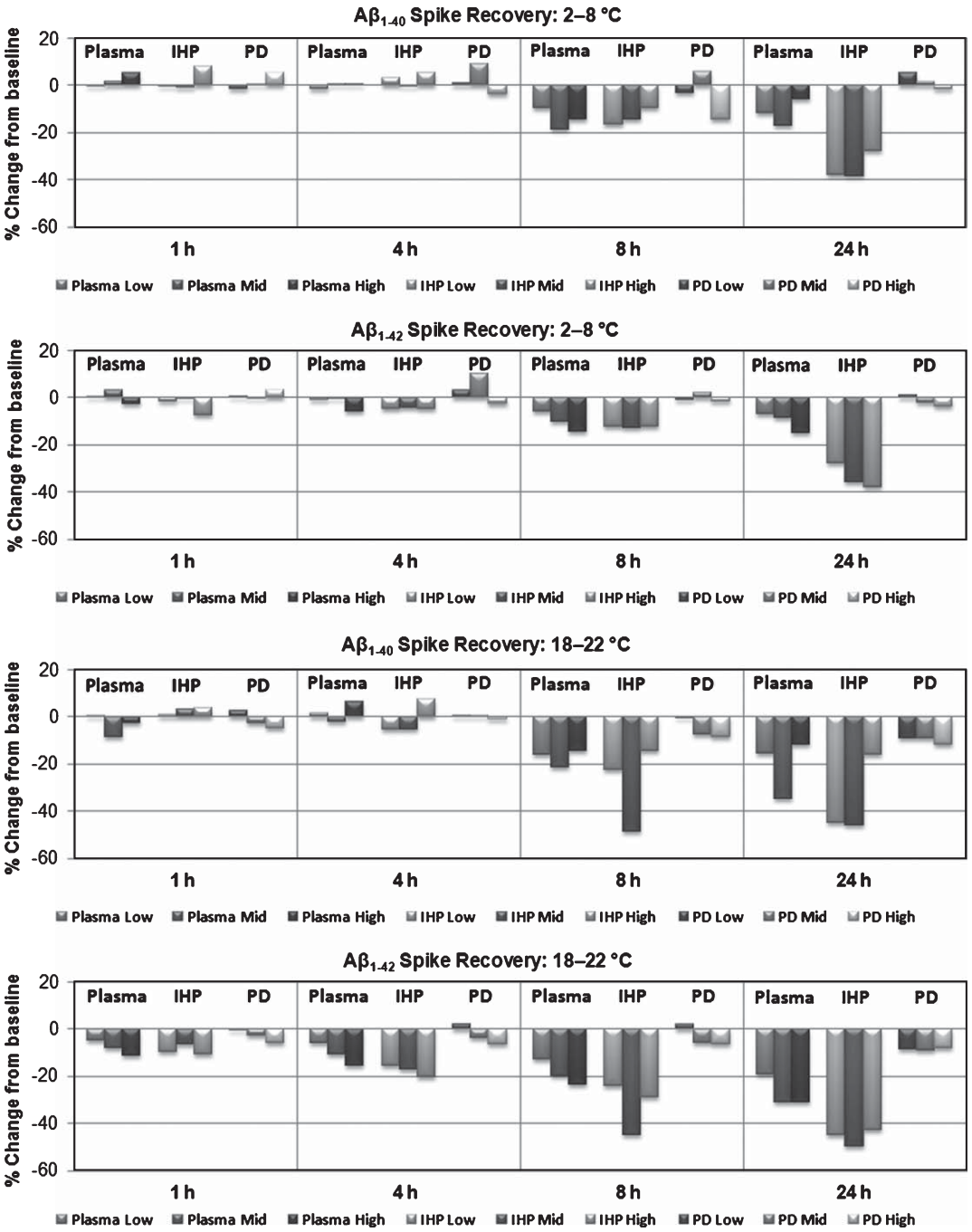
Supplementary Table 3
Quantification of $A\beta_{1\text{-}40}$ and $A\beta_{1\text{-}42}$ in the presence of three investigational drug compounds (up to 2× C_{max} concentration)

	$A\beta_{1\text{-}40}$ (ng/L)					$A\beta_{1\text{-}42}$ (ng/L)				
solanezumab (mg/L)	0	50	250	500[a]	1000	0	50	250	500[a]	1000
Mean ($n=3$)	155	79	85	91	106	36	16	16	17	16
CV %	1.9	2.0	2.8	2.7	2.6	1.3	0.0	1.1	2.4	2.7
% difference from control		–49	–45	–41	–32		–57	–55	–53	–54
semagacestat (mg/L)	0	0.5	2.5	5[a]	10	0	0.5	2.5	5[a]	10
Mean ($n=3$)	173	179	178	177	174	36	37	38	37	37
CV %	1.8	4.5	1.1	1.5	2.1	0.7	1.7	0.1	0.8	1.2
% difference from control		3.9	3.2	2.7	0.9		2.5	3.1	1.8	2.2
LY2811376 (mg/L)	0	0.1	0.5	1[a]	2	0	0.1	0.5	1[a]	2
Mean ($n=3$)	102	98	94	92	91	24	23	23	23	23
CV %	9.4	2.7	3.4	4.3	5.9	1.0	2.3	1.5	2.2	2.1
% difference from control		–4.4	–8.3	–10.1	–10.7		–3.1	–2.5	–4.6	–5.1

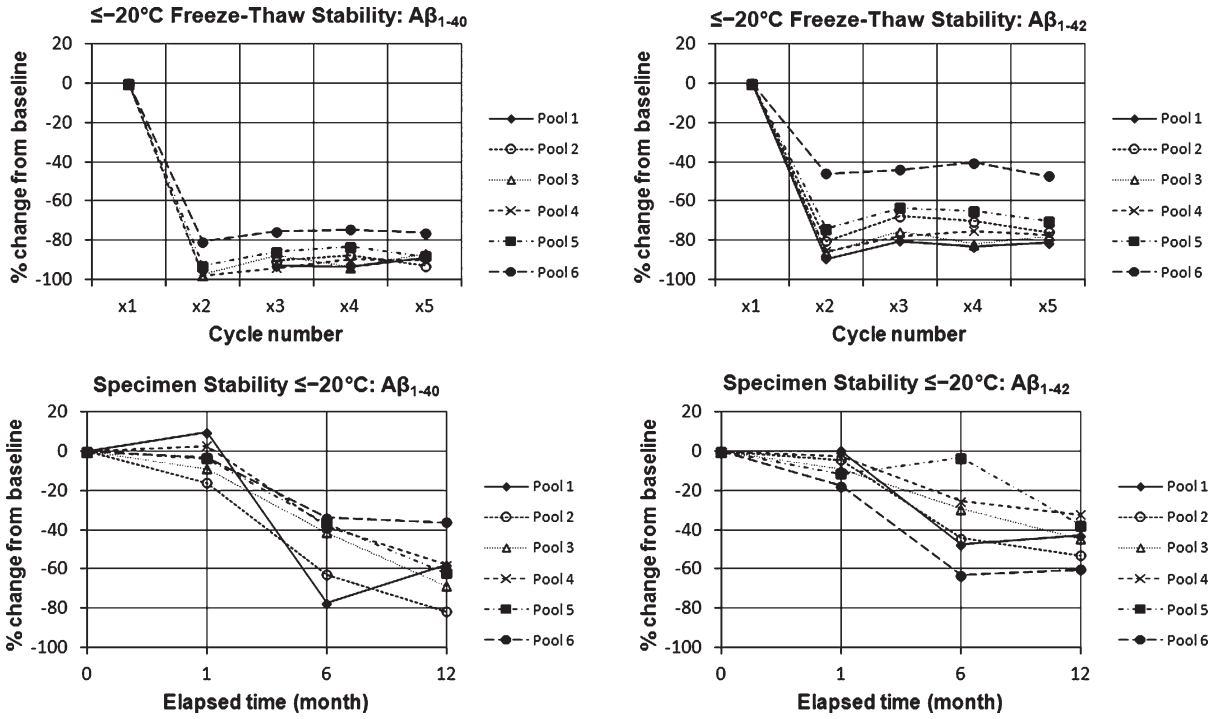
CV, coefficient of variation; RE, relative error; [a]Projected therapeutic C_{max} concentration.



Supplementary Figure 1. Representative overlaid calibration plots (runs 1–6: relative accuracy and precision determinations) for $A\beta_{1\text{-}40}$ and $A\beta_{1\text{-}42}$. Calibration ranges for $A\beta_{1\text{-}40}$ and $A\beta_{1\text{-}42}$ were 1.33–515 ng/L and 1.67–495 ng/L respectively.



Supplementary Figure 2. Analyte spike recovery. The effects of incubation time and temperature on the recovery of Aβ peptides spiked at 3 concentrations into plasma, immuno-depleted human plasma (IHP) and kit plasma diluent (PD). Analyte recovery in each treatment was calculated as percentage of baseline (Time = 0 h) value. Each matrix type was spiked with synthetic $A\beta_{1\text{-}40}$ and $A\beta_{1\text{-}42}$ peptides, respectively (ng/L): Low (50, 25); Mid (200, 100); High (1000, 500).



Supplementary Figure 3. Stability of Aβ peptides in 6 individual plasma pools stored at ≤–20°C up to 12 months and during five freeze-thaw cycles from ≤–20°C.

Supplementary Table 4

Analytical specificity of the INNO-BIA plasma Aβ forms. Purified Aβ peptide species of known concentration in buffer were tested in the INNO-BIA plasma assay using the standard test procedure. Cross reactivity was calculated using measured median fluorescence intensity values, adjusted for analyte concentration

Peptide identity		$A\beta_{1-40}$		$A\beta_{1-42}$	
	Concentration (ng/L)	MFI[a] ($n = 2$ runs)	Reactivity (%)	MFI[a] ($n = 2$ runs)	Reactivity (%)
$A\beta_{1-40}$	75	6602		13	0.2
	2000	27026		134	0.5
$A\beta_{1-42}$	100	46	0.5	7305	
	2000	639	2.4	26295	
$A\beta_{8-42}$	2000	5	0	3	0
	10000	6	0	4	0
$A\beta_{1-40}/A\beta_{1-42}$	75/100	6595	100	6836	94
$A\beta_{1-40}/A\beta_{8-42}$	75/100	6620	100	13	0.2
$A\beta_{1-38}$	2000	5	0	2	0
	10000	6	0	2	0
$A\beta_{1-39}$	2000	7342	27	6	0
	10000	17013	13	17	0
Rat $A\beta_{1-42}$	2000	58	0.2	141	0.5

[a]Median fluorescence intensity.