Second-generation fully automated Elecsys cerebrospinal fluid immunoassays demonstrate high precision, reproducibility, and sample stability suitable for clinical use to aid Alzheimer's disease diagnosis

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Sandra Rutz,[1] Ekaterina Manuilova,[1] Laura Mueller-Huebner,[1] Timo Grimmer,[2] Jennifer Powers Carson,[3] Robert H. Christenson,[4] Maria Karpova,[1] Carla Winter,[1] Charlotte E. Teunissen[5]

[1]*Personalized HealthCare Solutions, Roche Diagnostics GmbH, Penzberg, Germany;* [2]*Department of Psychiatry and Psychotherapy, Klinikum rechts der Isar, Technical University of Munich, School of Medicine, Munich, Germany;* [3]*Core Lab for Clinical Studies, Washington University School of Medicine, St Louis, MO, USA;* [4]*Department of Pathology, Department of Medical and Research Technology, University of Maryland School of Medicine, Baltimore, MD, USA;* [5]*Neurochemistry Laboratory, Department of Clinical Chemistry, Vrije Universiteit (VU) University Medical Center, Amsterdam, Netherlands*

Introduction

- Cerebrospinal fluid (CSF) amyloid-beta (1–42) (Aβ42) and phosphorylated Tau 181 (pTau181) biomarker analyses are recommended alongside cognitive evaluation to aid diagnosis of Alzheimer's disease (AD).[1]
  - Aβ42 peptide measured in the CSF is inversely correlated with amyloid positivity detected using positron emission tomography (PET).[2]
  - CSF Aβ42 and pTau181 can discriminate between AD and age-matched controls and predict progression to AD dementia in patients with mild cognitive impairment (MCI).[3]
  - Amyloid positivity as determined by the CSF pTau/Aβ42 ratio strongly correlates with amyloid PET positivity.[4]
- For clinical use, CSF biomarker assays should demonstrate high precision, reproducibility, and sample stability.
- Elecsys® β-Amyloid (1-42) CSF II and Elecsys Phospho-Tau (181P) CSF (Roche Diagnostics International Ltd, Rotkreuz, Switzerland) are electrochemiluminescence immunoassays for the *in vitro* quantitative determination of CSF levels of Aβ42 and pTau181, respectively.

Objectives

- **To evaluate the precision and reproducibility of the second-generation fully automated Elecsys CSF immunoassays on the cobas e 601 analyzer (Roche Diagnostics International Ltd).**
- **To assess the effect of storage conditions on CSF sample stability.**

Methods

- This multicenter study was conducted at four external sites (Amsterdam, Netherlands; Baltimore [MD] and St Louis [MO], USA; and Munich, Germany) and one internal site (Roche Diagnostics GmbH, Penzberg, Germany) between February–December 2021.
- Pooled/frozen spiked/native samples generated from uncharacterized CSF, sourced from third-party vendors and from residual routine clinical samples, and two PreciControl samples were analyzed using the Elecsys β-Amyloid (1-42) CSF II and Elecsys Phospho-Tau (181P) CSF immunoassays on the cobas e 601 analyzer, and pTau181/Aβ42 ratios were calculated from individual Aβ42 and pTau181 measurements.
- Precision was evaluated at the internal site per Clinical and Laboratory Standards Institute (CLSI) EP02-A3: two runs/day in duplicate over 21 days (n=84).
- Reproducibility was determined per CLSI EP05-A3 over 5 days using a single lot across three external sites (site-to-site; Amsterdam, Netherlands; Baltimore and St Louis, USA) and three lots (lot-to-lot) at one internal site.
- Coefficients of variation (CVs) and standard deviations (SDs) for repeatability, intermediate precision, and reproducibility were calculated using variance component analysis.
- Sample stability was determined at one external site (Munich, Germany) from fresh CSF samples, collected per manufacturer's instructions.
- Baseline measurements were taken <6 hours after lumbar puncture and follow-up measurements (from stored aliquots; 1–5 per subject) were taken at defined intervals for ≤8 days at room temperature (25°C; n=13), ≤15 days at 2–8°C (n=14), and for ≤13 weeks at -20°C (n≥25).
- Storage stability was determined using ordinary linear regression; mean percentage recoveries per subject and storage time were used for the analysis.

Results

Precision

- Repeatability CVs were <2.4% for Aβ42, <2.0% for pTau181, and <2.4% for pTau181/Aβ42 ratio (**Table 1**).
- Intermediate precision CVs were <3.4% for Aβ42, <3.8% for pTau181, and <3.9% for pTau181/Aβ42 ratio (**Table 1**).

Reproducibility

- Site-to-site reproducibility CVs were <5.1% for Aβ42, <3.6% for pTau181 and <6.5% for pTau181/Aβ42 ratio (**Table 2**).
- For Aβ42, lot-to-lot reproducibility CVs were <5.5%, except for one sample pool (mean concentration: 322 pg/mL; SD: 34.1 pg/mL [acceptance criterion: ≤48.0 pg/mL]; **Table 3**). Lot-to-lot reproducibility CVs were <3.7% for pTau181 and <6.0% for pTau181/Aβ42 ratio (**Table 3**).

Sample stability

- At maximum sample storage duration of 8 days at room temperature (25°C) (**Figure 1**) and 15 days at 2–8°C (**Figure 2**), mean percent recoveries of 95% and 98%, respectively, were observed for Aβ42, and 97% and 99%, respectively, were observed for pTau181.
- Sample stability assessment at -20°C is ongoing.

Table 1. Repeatability and intermediate precision of the Elecsys β-Amyloid (1-42) CSF II and Elecsys Phospho-Tau (181P) CSF immunoassays.

Biomarker	Sample	Repeatability Mean (pg/mL)	Repeatability SD (pg/mL)	Repeatability CV (%)	Intermediate precision SD (pg/mL)	Intermediate precision CV (%)
Aβ42	PreciControl 1	515	3.88	0.8	12.2	2.4
	PreciControl 2	1767	29.9	1.7	58.3	3.3
	CSF 1	302	3.28	1.1	5.67	1.9
	CSF 2	793	7.88	1.0	17.9	2.3
	CSF 3	1027	11.5	1.1	23.1	2.2
	CSF 4	1305	14.7	1.1	35.7	2.7
	CSF 5	1243	15.3	1.2	28.5	2.3
	CSF 6	2374	40.3	1.7	70.7	3.0
	CSF 7	2317	52.4	2.3	69.0	3.0
pTau181	PreciControl 1	14.2	0.275	1.9	0.526	3.7
	PreciControl 2	47.7	0.788	1.7	0.974	2.0
	CSF 1	15.5	0.263	1.7	0.389	2.5
	CSF 2	22.3	0.268	1.2	0.434	2.0
	CSF 3	26.3	0.398	1.5	0.530	2.0
	CSF 4	33.3	0.579	1.7	0.616	1.9
	CSF 5	58.4	0.764	1.3	0.979	1.7
	CSF 6	108	1.54	1.4	1.79	1.7
	CSF 7	115	1.53	1.3	1.94	1.7

	Sample	Mean ratio	SD	CV (%)	SD	CV (%)
pTau181/ Aβ42 ratio	Ratio sample 1	0.021	0.0005	2.3	0.0008	3.8
	Ratio sample 2	0.028	0.0006	2.0	0.0009	3.1
	Ratio sample 3	0.038	0.0006	1.6	0.001	2.8
	Ratio sample 4	0.041	0.0008	2.0	0.001	2.8
	Ratio sample 5	0.054	0.0009	1.6	0.001	2.5

Table 2. Site-to-site reproducibility of the Elecsys β-Amyloid (1-42) CSF II and Elecsys Phospho-Tau (181P) CSF immunoassays.
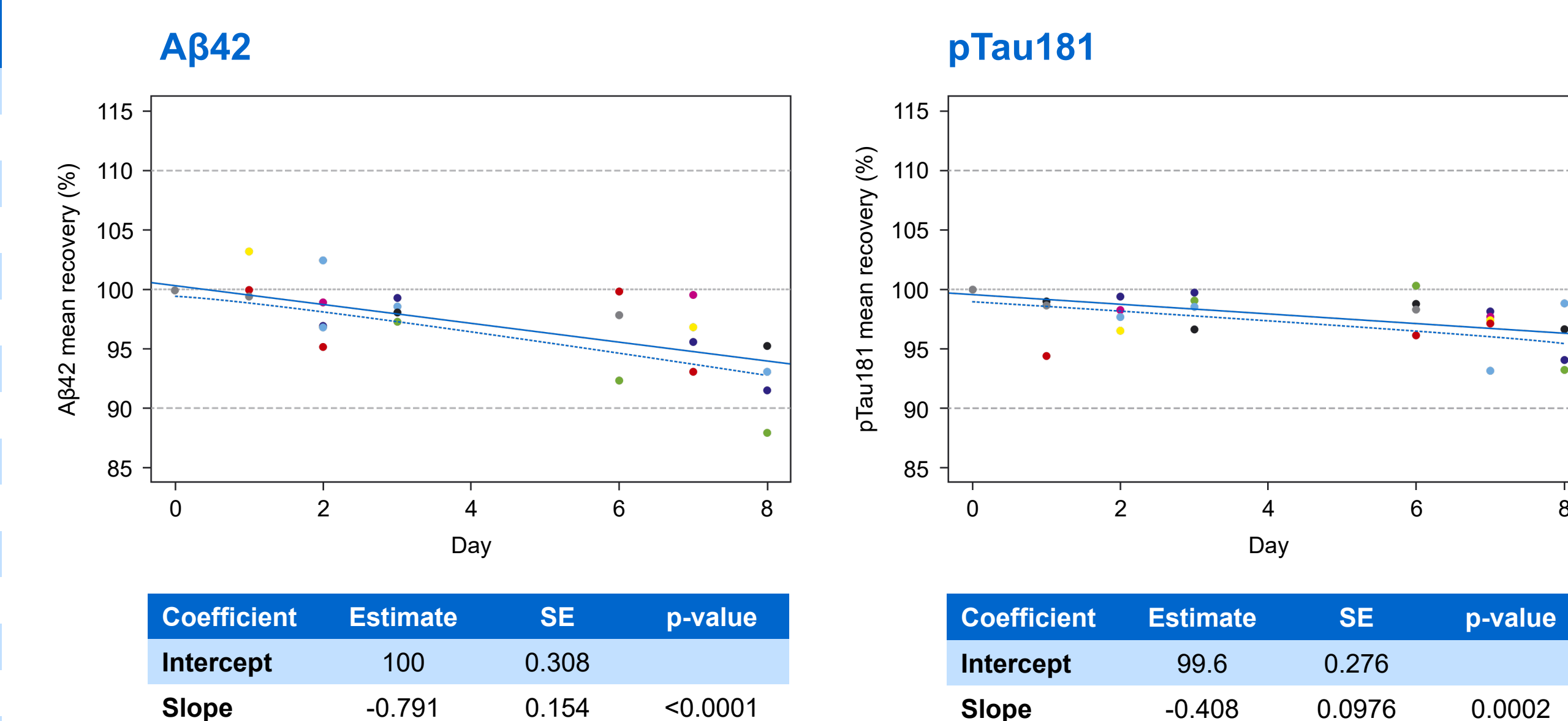
Biomarker	Sample	Mean concentration (pg/mL)	SD (pg/mL)	CV (%)
Aβ42	PreciControl 1	590	18.6	3.2
	PreciControl 2	1751	60.2	3.4
	CSF level 1	397	8.7	2.2
	CSF level 2	856	19.2	2.2
	CSF level 3	1120	21.2	1.9
	CSF level 4	1291	31.4	2.4
	CSF level 5	1358	28.4	2.1
	CSF level 6	2231	111	5.0
	CSF level 7	2448	61.4	2.5
pTau181	PreciControl 1	15.4	0.540	3.5
	PreciControl 2	54.2	1.58	2.9
	CSF level 1	16.5	0.565	3.4
	CSF level 2	22.3	0.682	3.1
	CSF level 3	27.8	0.959	3.4
	CSF level 4	33.6	0.956	2.8
	CSF level 5	61.2	1.71	2.8
	CSF level 6	118	3.12	2.6

Biomarker	Sample	Mean ratio	SD	CV (%)
pTau181/ Aβ42 ratio	Ratio sample 1	0.018	0.0008	4.2
	Ratio sample 2	0.031	0.0019	6.1
	Ratio sample 3	0.049	0.0018	3.6
	Ratio sample 4	0.047	0.0014	3.0
	Ratio sample 5	0.047	0.0014	3.1
	Ratio sample 6	0.020	0.0013	6.4
	Ratio sample 7	0.024	0.0014	5.6

Table 3. Lot-to-lot reproducibility of the Elecsys β-Amyloid (1-42) CSF II and Elecsys Phospho-Tau (181P) CSF immunoassays.

Biomarker	Sample	Mean concentration (pg/mL)	SD (pg/mL)	CV (%)
Aβ42	PreciControl 1	546	29.3	5.4
	PreciControl 2	1892	66.5	3.5
	HSP 1	322	34.1	10.6
	HSP 2	886	46.0	5.2
	HSP 3	1042	45.8	4.4
	HSP 4	1235	55.4	4.5
	HSP 5	1447	57.2	4.0
	HSP 6	2391	108	4.5
	HSP 8	2694	126	4.7
pTau181	PreciControl 1	14.1	0.394	2.8
	PreciControl 2	48.1	1.42	3.0
	HSP 1	15.5	0.552	3.6
	HSP 2	20.9	0.608	2.9
	HSP 3	26.2	0.679	2.6
	HSP 4	30.2	0.736	2.4
	HSP 5	59.3	1.28	2.2
	HSP 6	115	2.22	1.9

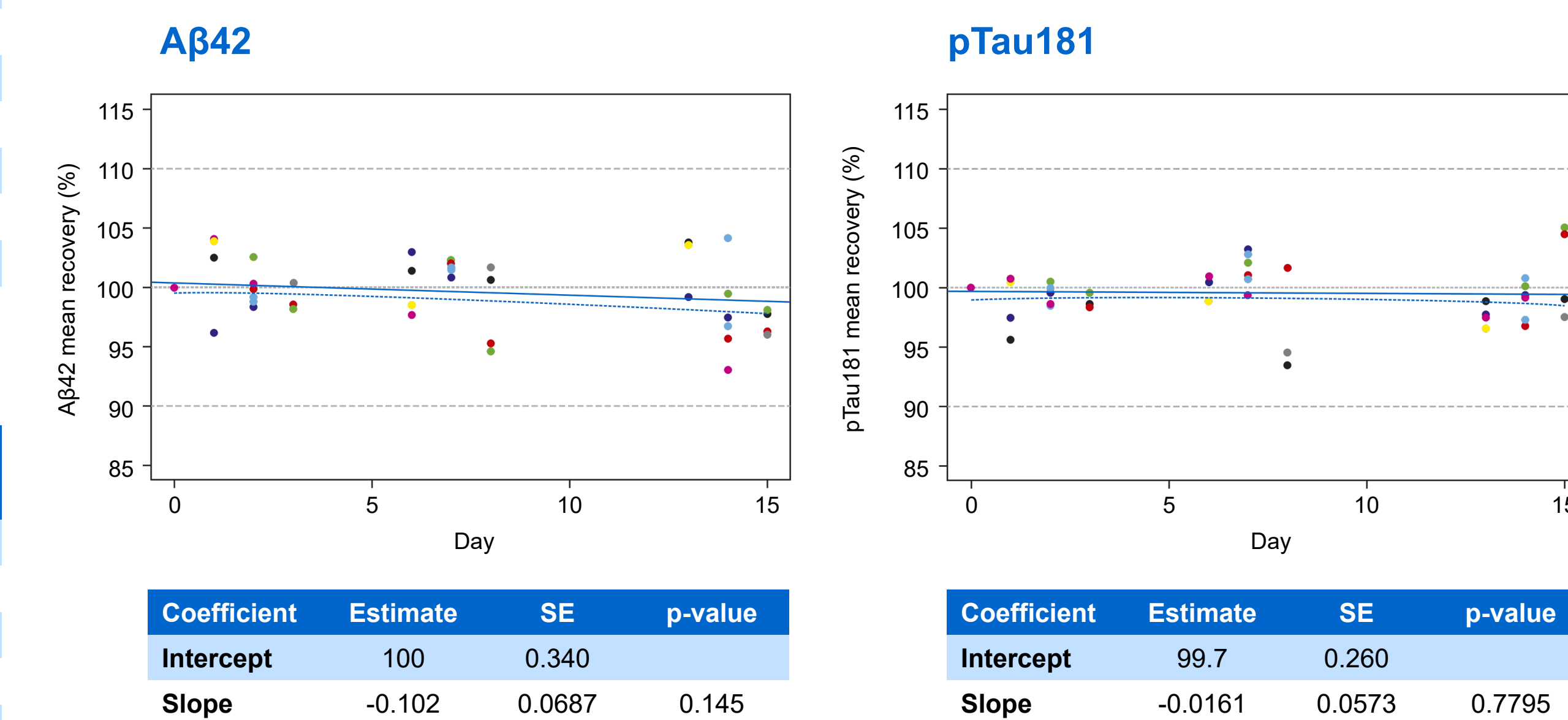|	Sample	Mean ratio	SD	CV (%)
pTau181/ Aβ42 ratio	HSP 6	0.0205	0.00111	5.4
	HSP 7	0.0244	0.00121	5.0
	HSP 8	0.0361	0.00214	5.9
	HSP 9	0.0389	0.00182	4.7
	HSP 10	0.0373	0.00146	3.9

HSP, human sample pool.

Figure 1. Regression analysis of sample stability for 8 days at room temperature (25°C).

Aβ42

Coefficient	Estimate	SE	p-value
Intercept	100	0.308	
Slope	-0.791	0.154	<0.0001

pTau181

Coefficient	Estimate	SE	p-value
Intercept	99.6	0.276	
Slope	-0.408	0.0976	0.0002

Identically colored points denote data from the same subject.
Blue dotted line denotes the limit of the one-sided 95% confidence interval for the regression line, derived using Jackknife approach.
SE, standard error.

Figure 2. Regression analysis of sample stability for 15 days at 2−8°C.

Aβ42

Coefficient	Estimate	SE	p-value
Intercept	100	0.340	
Slope	-0.102	0.0687	0.145

pTau181

Coefficient	Estimate	SE	p-value
Intercept	99.7	0.260	
Slope	-0.0161	0.0573	0.7795

Identically colored points denote data from the same subject.
Blue dotted line denotes the limit of the one-sided 95% confidence interval for the regression line, derived using Jackknife approach.
SE, standard error.

Conclusions

- **The Elecsys β-Amyloid (1-42) CSF II and Elecsys Phospho-Tau (181P) CSF immunoassays demonstrated high precision and reproducibility, supporting their clinical use in AD diagnosis.**
- **Conservative recommendations for CSF sample storage are 5 days at room temperature (25°C) or 14 days refrigerated at 2–8°C.**

References

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