

BACKGROUND

The ratio Aβ42/Aβ40 in cerebrospinal fluid (CSF) is considered one of the most reliable biomarkers of Alzheimer's Disease. **ABtest40-IA and ABtest42-IA (IA: immunoassay)** have been widely used for amyloid-β (Aβ) quantification in plasma samples¹⁻². The aim of this study was to validate the application of these tests in CSF samples.

METHODS

Analytical validation of ABtest40-IA and ABtest42-IA was accomplished following FDA and EMA reference guidelines.

Table 1: Methodology followed to analytically validate ABtest-IA with CSF samples.

Calibration curve	Samples	Replicates & runs	Acceptance criteria
Sensitivity	7 different concentration levels of calibrator in dilution buffer	- 3 replicates/level & run	- CV & RE ≤ 20% (25%: ULOQ & LLOQ) - Total error ≤ 30% (40%: ULOQ & LLOQ)
Specificity	Together with "calibration curve" assays	- 6 independent runs	CV ≤ 25%; RE ≤ 25%; Total error ≤ 40%
Precision	3 samples (different Aβ values)	- 2 replicates/level - 1 run	75-125% of nominal values
Accuracy	6 samples with different Aβ levels (LLOQ, Low, Mid, High, ULOQ)	- 6 replicates/run - 6 independent runs - Inter-batch: 3 ABtest-IA lots; 3 runs	- CV ≤ 20% (25%: ULOQ & LLOQ) - RE ≤ 20% (25%: ULOQ & LLOQ)
Recovery	- 5 samples - High, mid & low spiked Aβ conc.	- 2 replicates/level - 1 run	80-120%
Dilution linearity	- 3 samples (dif. Aβ values) - Dil: 1/25, 1/50, 1/75, 1/100	- 2 replicates/dilution - 1 run	- CV between dilutions ≤ 20% - RE vs nominal value ≤ 20%

Correlations between Aβ40, Aβ42 and Aβ42/40 quantified using ABtest-IA and an independent fully automated IVD test, (Lumipulse® G β-amyloid 1-40 and 1-42, Fujirebio), were estimated using 93 CSF samples of Aβ-PET+ (75%) and Aβ-PET- (25%) individuals from AB1601 cohort (aMCI & vMAD). **Differences in CSF Aβ42 and Aβ42/Aβ40 levels** between both Aβ-PET groups were analyzed (Mann Whitney test).

The ability of CSF Aβ42/Aβ40 ratio to discriminate between Aβ-PET positive and Aβ-PET negative subjects was evaluated by means of Receiver Operating Characteristic (ROC) curve analysis, considering Aβ-PET as the gold standard.

CONCLUSION

ABtest40-IA and ABtest42-IA were successfully validated as new **precise and accurate tools for the quantification of Aβ40 and Aβ42** in CSF samples. ABtest-IA measures of Aβ42/Aβ40 ratio in CSF identified individuals with brain amyloid deposition with **equivalent ability to another test commercially available.**

RESULTS

1. Analytical validation

All parameters evaluated in the analytical validation met the acceptance criteria. 1/50 sample dilution was established for Aβ40 and Aβ42 quantification in CSF samples with ABtest-IA.

Table 2: Summary of analytical validation of ABtest-IA for CSF samples.

		ABtest40-IA		ABtest42-IA	
		Mean	Range	Mean	Range
Calibration curve	Dynamic range (pg/mL)	43.9 to 500		5 to 100	
	Accuracy, RE (%)	0.8	-2.3 to 3.5	1.3	-5.5 to 4.9
	Precision, CV (%)	2.6	0.1 to 12.2	3.3	0.8 to 8.7
	Total Error, (%)	4.4	0.5 to 12.4	4.6	0.9 to 11.9
Sensitivity	pg/mL	43.9		5.0	
Specificity	RE (%)	2	0.7 to 2.7	-2.0	-7.4 to 2.3
Precision	Intra-assay, CV (%)	2.4	0.5 to 8.0	3.5	0.7 to 10.6
	Inter-assay, CV (%)	5.2	5.0 to 5.5	7.4	5.1 to 10.9
	Inter-batch, CV (%)	2.5	0.4 to 6.0	5.2	1.8 to 10.9
Accuracy	RE (%)	4.6	-18.8 to 13.2	7.5	-26.3 to 19.2
Recovery	%	93.5	70.4 to 106.7	93.5	78.9 to 108.5
Dilution linearity	CV (%)	4.7	1.3 to 9.8	5.2	2.8 to 8.9
	RE (%)	6.1	-20.7 to 1.9	6.5	-18.5 to 9.8

RE: Relative Error; CV: Coefficient of Variation; Total Error (%): CV (%) + RE (%).

2. Correlation between methods

Aβ40, Aβ42 and Aβ42/Aβ40 quantified with ABtest-IA strongly correlate with Lumipulse data.

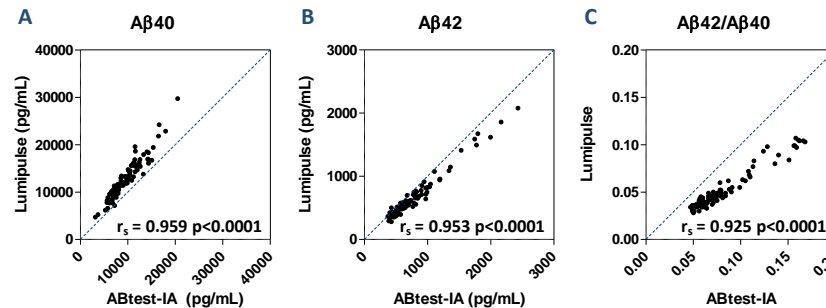


Figure 1: Correlations between ABtest-IA and Lumipulse for Aβ40 (A), Aβ42 (B) and Aβ42/40 (C). r_s: Spearman rank coefficient.

3. Differences in Aβ CSF levels between Aβ-PET+ and Aβ-PET- groups

As expected, the Aβ-PET+ group presents significantly lower Aβ42 (-47.1%) and Aβ42/Aβ40 (-42.7%) levels than the Aβ-PET- group.

Table 3: Levels of Aβ42 and Aβ42/40 in Aβ-PET+ and Aβ-PET- groups.

	Aβ42		Aβ42/Aβ40	
	Aβ-PET+	Aβ-PET-	Aβ-PET+	Aβ-PET-
Mean (pg/mL)	636	1202	0.069	0.121
SD (pg/mL)	171.7	568.8	0.015	0.040
% relat. dif. †	-47.1		-42.7	
p value	< 0.0001		< 0.0001	

†% relative difference: 100*((Aβ-PET+ - Aβ-PET-)/Aβ-PET-).

The magnitude of these differences was very similar for Fujirebio data (data not shown).

4. ROC Analysis and CSF Aβ42/Aβ40 – Aβ-PET agreement

The AUC value in the ROC analysis was slightly better for the ratio Aβ42/Aβ40 than for the Aβ42 biomarker alone.

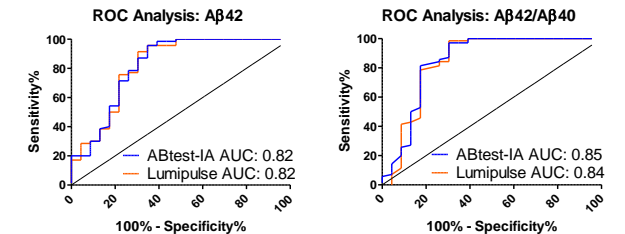


Figure 2: ROC analysis for Aβ42 (left) and Aβ42/Aβ40 (right).

Youden's index maximum was used to establish a cutoff of CSF Aβ42/Aβ40 positivity. Aβ-PET and CSF Aβ42/40 biomarkers classified equally 90% of the subjects (n=84). Among the 9 discordant cases, 78% were CSF Aβ42/40 positive and Aβ-PET negative. This supports that CSF Aβ42/Aβ40 is an earlier biomarker of cerebral amyloid deposition than Aβ-PET. Similar results were found with Fujirebio (data not shown).

1. Doecke JD et al., Total Aβ(42)/Aβ(40) ratio in plasma predicts amyloid-PET status, independent of clinical AD diagnosis. *Neurology*. 2020;94(15):e1580-e91.
2. Perez-Grijalba V et al., Plasma Abeta42/40 ratio alone or combined with FDG-PET can accurately predict amyloid-PET positivity: a cross-sectional analysis from the AB255 Study. *Alzheimers Res Ther*. 2019;11(1):96.