Development and Characterization of a Validated Multiplex Panel for Detection of Human Aβ Peptides in Human CSF

Sara Hapip, Aishwarya Ranganathan, Leonid Dzantiev, David Stewart, Jill Dunty, Robert Umek, Pankaj Oberoi, and Jacob N. Wohlstadter

Meso Scale Discovery (MSD), Rockville, MD 20850

1 Abstract

Objectives: To develop and validate a multiplexed assay panel for measurement of candidate biomarkers of Alzheimer's disease (AD) in human cerebrospinal fluid (CSF).

Background: Considered along with neuropsychological and/or neuroimaging data, baseline AD biomarker profiles may be used to stratify and enrich particular patient populations, which may enhance the success of clinical trials and natural history studies. However, challenges with existing AD biomarker assays, including issues with repeatability and reproducibility within and across centers and across manufactured lots, limit their clinical utility. Herein, we report development and analytical validation of a human Aβ peptide multiplex panel for measurement of Aβ38, Aβ40, and Aβ42 in human CSF using fit-for-purpose and Clinical and Laboratory Standards Institute principles.

Methods: The panel was developed using MSD's MULTI-ARRAY® technology and was optimized to minimize CSF matrix effects and interferences. Analytical validation was performed across multiple panel lots to verify consistency in sensitivity, accuracy, and precision.

Results: The human Aβ peptide panel demonstrated excellent sensitivity, performance, and inter-lot reproducibility. Measured levels of the three Aβ peptides in human CSF fell within the quantitative ranges of the assay and were consistent with literature reports. Assay precision, accuracy, and total error were determined from human CSF control samples. Dilution linearity and spike recovery testing demonstrated minimal matrix effects and accurate quantitation of Aβ peptide over the range of the assays. The assays showed no significant cross-reactivity or interference from closely related Aβ peptides.

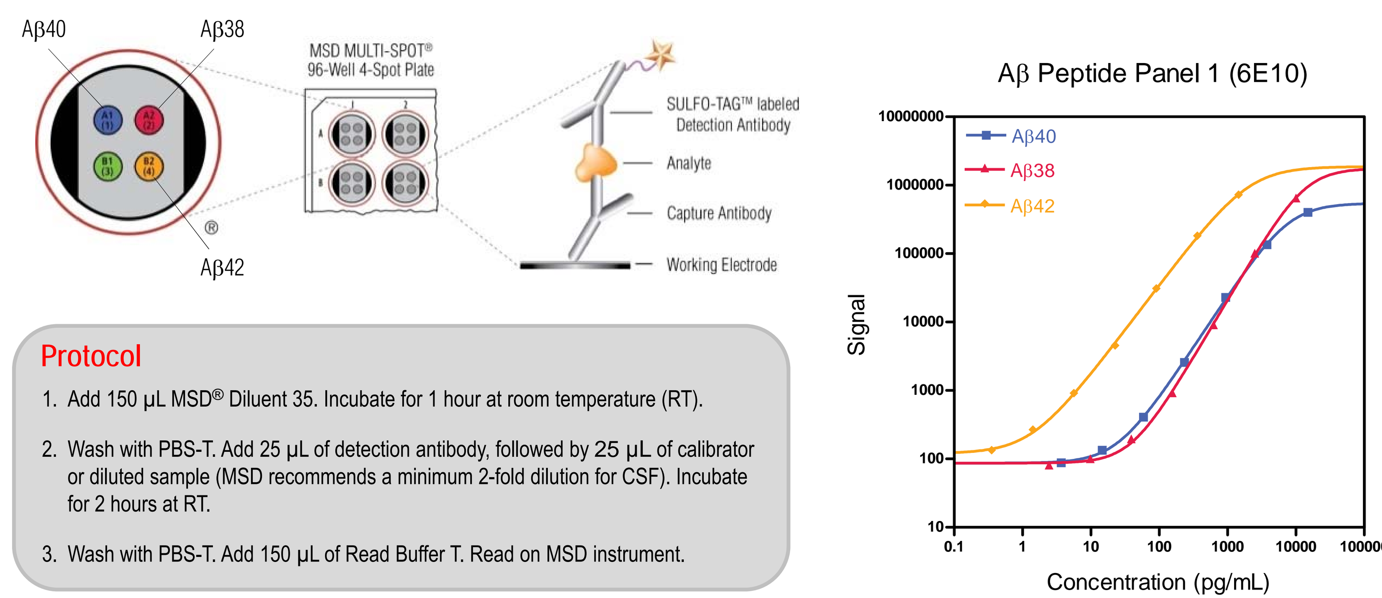
Conclusions: The human Aβ peptide panel was developed and analytically validated to measure Aβ38, Aβ40, and Aβ42 in human CSF. The panel had good analytical performance characteristics, inter-lot consistency, and the ability to measure Aβ peptide levels in human CSF samples. This panel will support ongoing efforts to standardize AD biomarker testing and opens the door to multiplexing with other key biomarker assays such as phosphorylated and total tau.

2 Development and Validation

The Aβ Peptide Panel 1 (6E10) kit was developed and validated following fit-for-purpose principles and design control procedures. Testing included assessment of accuracy and precision of controls and calibrators, assay specificity, tolerance to matrix and other interferences, and sample testing. The validation was conducted by multiple operators using three independently built kit lots.

Left: Assay format and protocol.

Right: Representative calibrator curves from 18 runs by 2 analysts over 4 days. Signal CVs are typically <8%.



Protocol

1. Add 150 µL MSD® Diluent 35. Incubate for 1 hour at room temperature (RT).
2. Wash with PBS-T. Add 25 µL of detection antibody, followed by 25 µL of calibrator or diluted sample (MSD recommends a minimum 2-fold dilution for CSF). Incubate for 2 hours at RT.
3. Wash with PBS-T. Add 150 µL of Read Buffer T. Read on MSD instrument.

3 Assay Sensitivity and Quantitative Range

Sensitivity: The lower limit of detection (LLOD) is a calculated concentration corresponding to the signal 2.5 standard deviations above the background (zero calibrator). For each kit lot, ≥10 runs were conducted by at least 2 analysts over multiple days.

Range: Upper and lower limits of quantification (ULOQ, LLOQ respectively) were determined by spiking a known amount of calibrator into diluent to assess the accuracy and precision of the samples across multiple lots. Inter-run precision is expressed as the coefficient of variance (CV). The average intra-plate concentration CVs were typically <8%.

	Kit Lot #	LLOD		LLOQ			ULOQ		
		Ave. LLOD (pg/mL)	LLOD Range (pg/mL)	Expected Conc. (pg/mL)	Calc Conc. %CV	% Recovery	Expected Conc. (pg/mL)	Calc Conc. %CV	% Recovery
Aβ40	Across 3 lots	9.98	7.26 - 12.2	25.0			7000		
	Kit Lot 1	10.6	9.12 - 12.2	25.0	14.9	111	7000	7.7	96
	Kit Lot 2	9.86	7.80 - 12.1	20.0	12.2	107	8922	13.2	99
	Kit Lot 3	9.14	7.26 - 10.3	20.0	9.3	103	8922	6.8	89
Aβ38	Across 3 lots	15.6	7.59 - 24.3	60.0			8475		
	Kit Lot 1	18.6	14.6 - 24.3	60.0	10.2	103	8500	8.4	119
	Kit Lot 2	12.7	7.59 - 17.9	45.0	9.4	106	8475	8.1	106
	Kit Lot 3	16.8	14.4 - 20.3	45.0	6.1	111	8475	6.9	99
Aβ42	Across 3 lots	0.372	0.220 - 0.537	3.13			1271		
	Kit Lot 1	0.379	0.288 - 0.518	2.50	14.1	101	1275	6.9	107
	Kit Lot 2	0.356	0.220 - 0.537	3.13	6.3	111	1271	4.8	103
	Kit Lot 3	0.394	0.315 - 0.507	2.00	3.8	110	1271	3.8	99

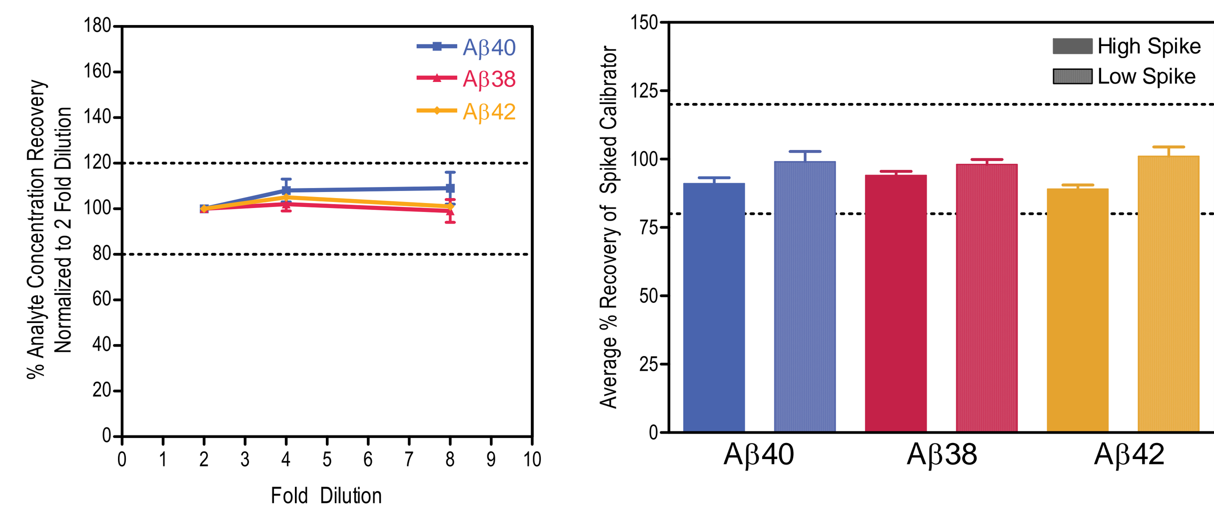
4 Matrix Tolerance

Left: Individual patient CSF samples (n=10) were diluted 2-, 4-, and 8- fold with Diluent 35. Measured concentrations were corrected for dilution factor. Recovery at each dilution was calculated relative to the 2-fold dilution and plotted against dilution factor.

Right: Individual patient CSF samples (n=10) were spiked with two levels of calibrator, diluted 2-fold, and tested for recovery. High and low spikes were as follows: Aβ40, 7000 and 1000 pg/mL; Aβ38, 4000 and 1000 pg/mL; Aβ42 1000 and 100 pg/mL.

% Recovery=measured/expected×100.

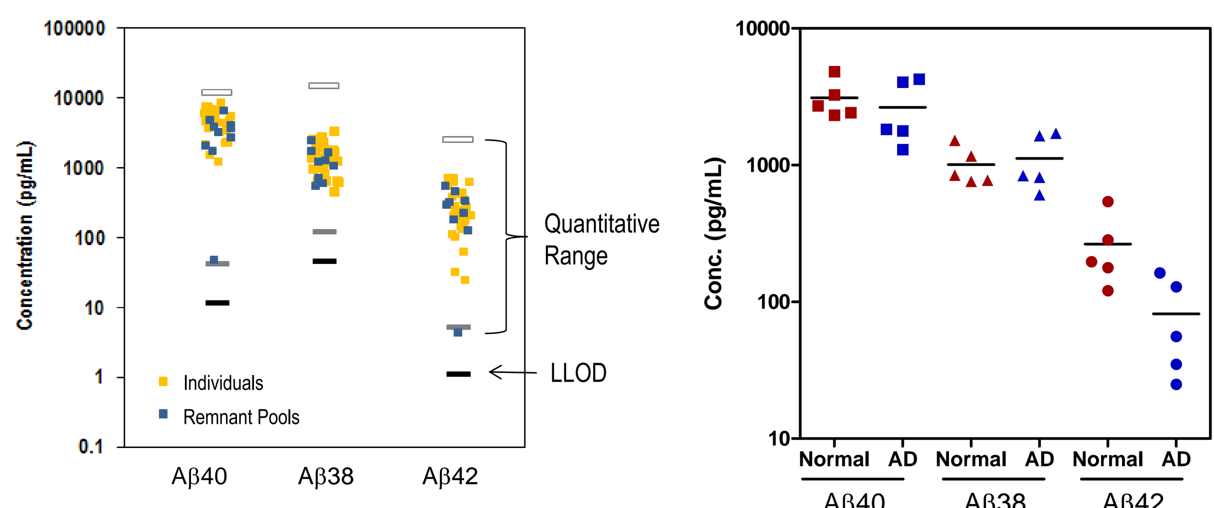
Results are representative of data from two independent MSD kit lots. Error bars: one standard deviation.



5 Sample Testing: Human CSF

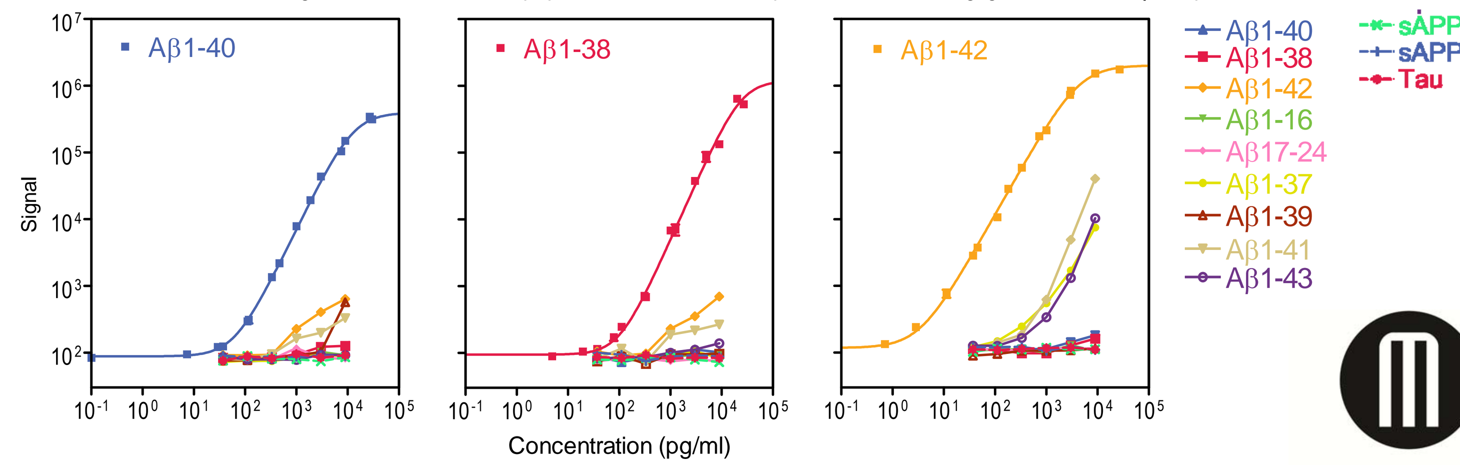
Left: Aβ peptide levels were measured in well-curated individual human CSF samples (n=28) and in pooled remnant samples (n=10). Samples were diluted 2-fold for the assay. The panel measured levels within the assay quantitative ranges for nearly all samples; one pooled remnant sample exhibited very low levels for all three analytes, suggesting the presence of an interferent in the sample. Quantitative range and LLOD are plotted with adjustment for the 2-fold sample dilution.

Right: Aβ peptide levels were measured in individual normal and AD patient samples (n=5 each). The Aβ42 level distinguishes normal and AD samples while smaller differences are observed for Aβ40 and Aβ38. Reported concentrations are adjusted for 2-fold dilution.



6 Specificity

A panel of analytes was spiked into Diluent 35 and tested with the Aβ Peptide Panel 1 (6E10) kit. Concentrations (x-axis) are corrected for sample dilution. The Aβ42 assay exhibits minor cross-reactivity with some related Aβ peptides: Aβ1-41, 2.6%; Aβ1-43 and Aβ1-37, <0.75%. Given the low endogenous levels of these peptides in CSF, this is expected to have a negligible effect on Aβ42 quantification.



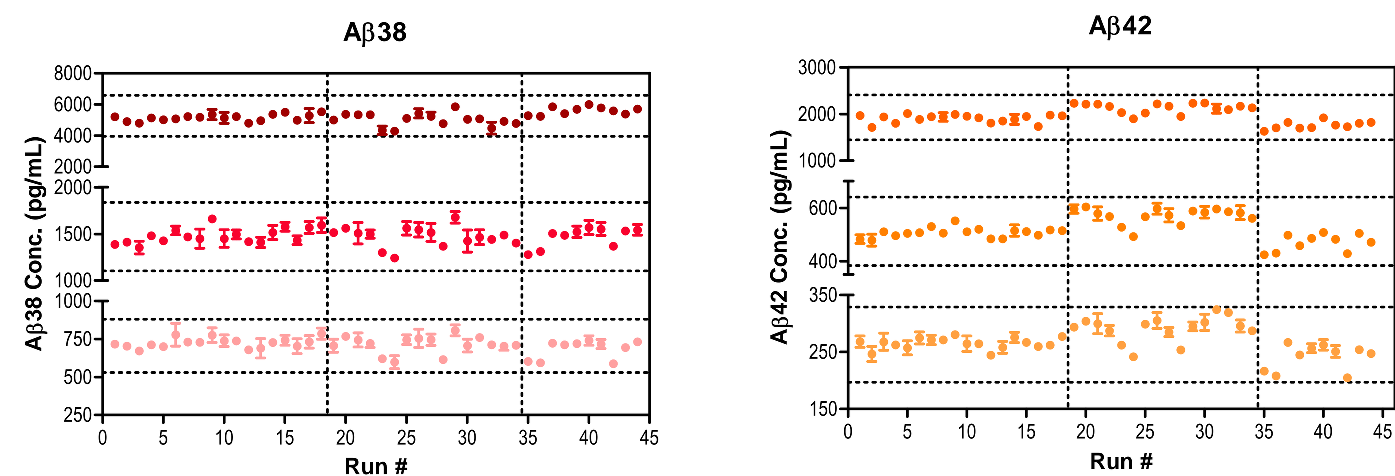
7 Reproducibility of Quality Controls

To evaluate assay reproducibility, 6 quality control samples spanning the expected analyte range were measured over 38 runs across three kit lots by 5 analysts. Results for 3 controls built using pooled spiked human CSF are reported below; comparable results were observed with 3 controls built using diluent that mimics CSF (not shown).

Top Left: Summary of measured concentrations and precision across three kit lots.

Control Charting: All controls tested were within the target range of ±20% of assigned concentration (dashed lines). Vertical dashed lines separate runs from the 3 kit lots. On each plot: top - CSF Control 1; middle - CSF Control 2; bottom - CSF Control 3.

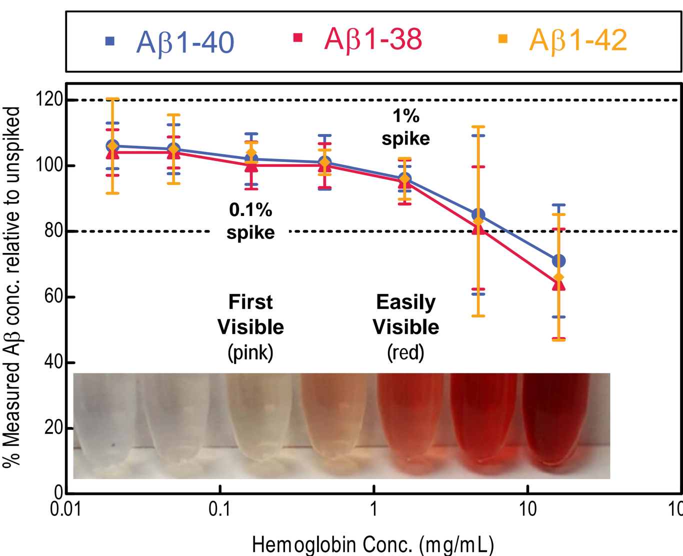
	Control ID	Ave. Conc. (pg/mL)	Ave. Intra-run CV	Ave. Inter-run Conc. CV	Inter-Kit Lot Conc. CV
Aβ40	CSF Ctl 1	9415	4.4%	6.0%	5.5%
	CSF Ctl 2	3111	4.2%	6.9%	6.1%
	CSF Ctl 3	1933	4.9%	8.3%	4.2%
Aβ38	CSF Ctl 1	5276	3.1%	6.1%	5.3%
	CSF Ctl 2	1471	3.6%	7.2%	0.6%
	CSF Ctl 3	705	4.0%	7.8%	3.0%
Aβ42	CSF Ctl 1	1925	3.0%	5.1%	9.2%
	CSF Ctl 2	513	2.4%	5.4%	9.0%
	CSF Ctl 3	263	2.7%	7.0%	8.0%



8 Tolerance to Interferences

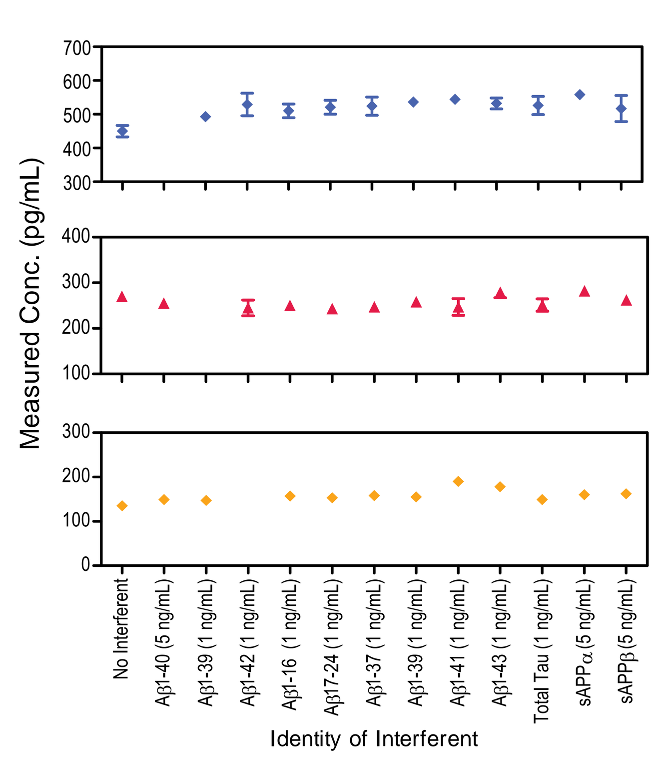
Blood Contamination

Assay tolerance to blood contamination was assessed by measuring Aβ peptide levels in CSF (n=3 pools) spiked with a titration of clarified hemolyzed blood. The measured Aβ peptide concentrations relative to the unspiked sample are plotted. The Aβ Peptide Panel 1 (6E10) assays are tolerant of up to 1.6 mg/mL hemoglobin in CSF (1% sample blood contamination). Error bars: one standard deviation.



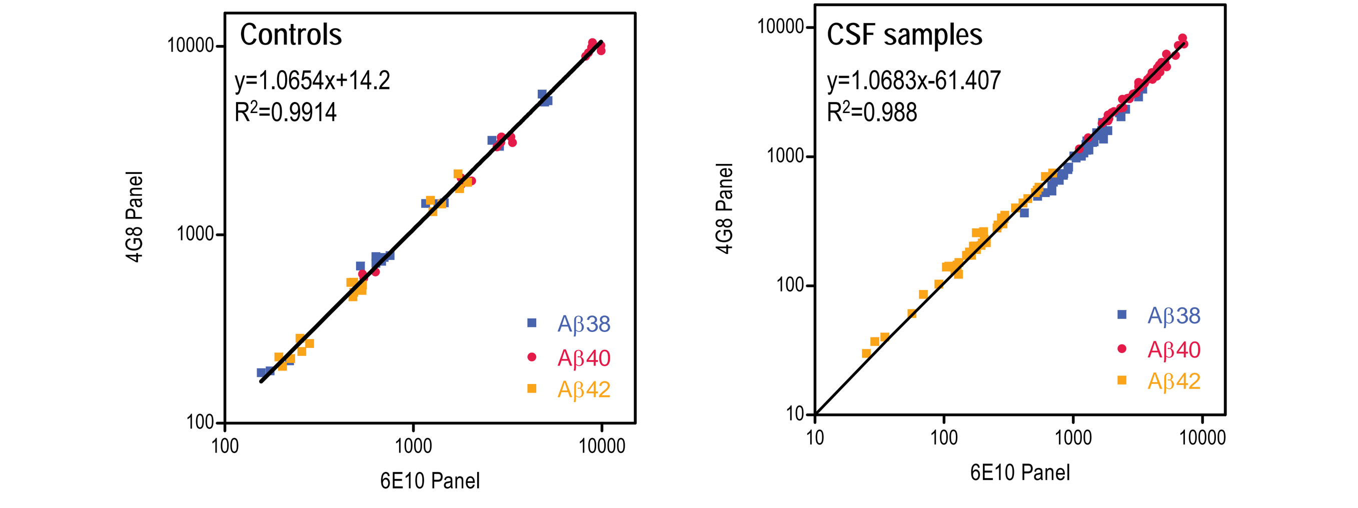
Related Analytes

Aβ40 (top), Aβ38 (middle), or Aβ42 (bottom) peptide calibrators were co-spiked into assay diluent with various Aβ peptides and amyloid precursor proteins at or above expected endogenous levels for these analytes (1 ng/mL except where indicated). Measured Aβ levels were largely within 25% of the sample with no interferent, regardless of the spiked analyte or concentration.



9 Aβ Peptide Panel 1 with Clone 4G8 Detection

Developed and validated for human CSF in parallel, the Aβ Peptide Panel (6E10) and (4G8) kits exhibit comparable performance in terms of precision and accuracy of calibrator and control measurements, sensitivity, specificity, and tolerance to interferences. Whereas the clone 6E10 detection antibody recognizes only human peptides (epitope: amino acids 18–22), the clone 4G8 detection recognizes both human and mouse peptides (epitope: amino acids 3–8) and is therefore useful in measurement of rodent samples. Below: good correlation in measured concentrations of 6 controls across three kit lots (left) and measured concentrations of 35 CSF samples (right).



10 4G8 Panel: Validated for measurement of Mouse Plasma

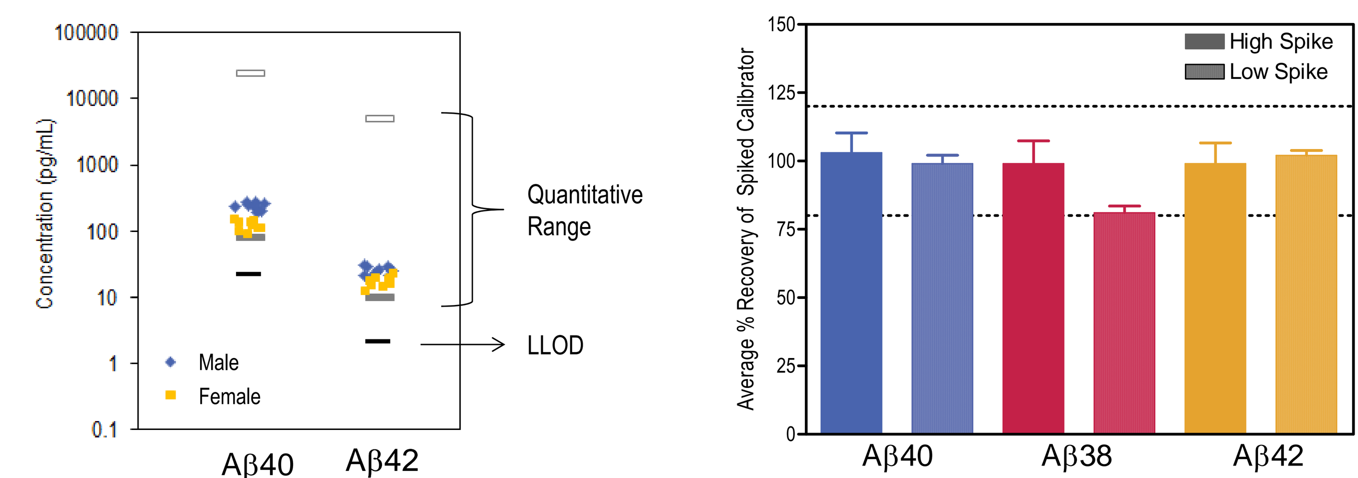
The 4G8 Panel was additionally validated for use with mouse plasma and tested with cell supernatants and mouse brain homogenates.

Right: Individual mouse EDTA plasma samples (n=20) were diluted 2-, 4-, 8-, and 16-fold with Diluent 35. Measured concentrations were corrected for dilution factor. Recovery at each dilution was normalized to 4-fold dilution.

Below: Aβ peptide levels were measured in individual male and female mouse EDTA plasma (n=10 per group). Samples were diluted 4-fold. Aβ40 and Aβ42 levels were within the assay quantitative ranges. Aβ38 levels were below LLOD (not shown).

Below Right: Mouse plasma samples (n=10) were spiked with two levels of calibrator, diluted 4-fold, and tested for recovery. High and low spikes were: Aβ40, 7000 and 100 pg/mL; Aβ38, 4000 and 1000 pg/mL; Aβ42, 1000 and 100 pg/mL.

Results are representative of data from two independent MSD kit lots. Error bars: one standard deviation.



11 Conclusions

The MSD Aβ Peptide Panel 1 (6E10) and Aβ Peptide Panel 1 (4G8) kits have been analytically validated for Aβ40, Aβ38, and Aβ42 measurement in human CSF. The kits were built using highly characterized, critical reagents and improved handling methods. The Aβ Peptide Panel 1 kits exhibit improved robustness and reliability, tolerance to matrix effects and interferences, good analytical performance, inter-lot consistency, and the ability to distinguish between normal and AD samples. The 4G8 kit has also been validated for mouse EDTA plasma and has been used to measure Aβ peptides in cell supernatants and mouse brain homogenates.

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