P1-323

The Impact of Assay Format on Sensitivity and Matrix Tolerance for Plasma Amyloid Beta Peptide (Aβ40 and Aβ42) Measurements

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1 Abstract

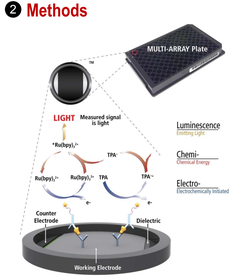
Background: Aβ peptides in blood can be measured with sensitive immunoassays, yet absolute Aβ peptide concentrations differ widely among reports. Assay format, antibody orientation, and matrix interferences contribute to assay variability. Here we describe an evaluation of multiple assay formats with respect to sensitivity and plasma matrix tolerance.

Methods: Immunoassays developed by MSD® include a format that pairs an N-terminal-domain binding antibody (6E10') as capture antibody with peptide-specific (Aβ40 or Aβ42) mouse monoclonal antibody as detector and an alternate format that pairs the peptide-specific antibodies as capture antibody with a 6E10 detector. Assay sensitivity (the lowest calibration point for which recovery was within 20% of the expected concentration and imprecision was less than 20%) was determined for each assay. Studies to evaluate matrix tolerance included dilutional linearity of pooled plasma from 5- to 80-fold dilutions and spike recovery studies with 500 pg/mL Aβ40 and Aβ42 peptides. Experimental samples were measured in duplicate using both assay orientations.

Results: The Aβ42 assay with the peptide-specific capture antibody shows greater sensitivity than the assay using a 6E10 capture antibody (4.12 pg/mL vs. 37 pg/mL). The opposite orientation, using 6E10 capture antibody, is more sensitive for the Aβ40 assay (less than 13.7 pg/mL). Aβ42 concentrations exhibit a proportional bias between the two formats, with absolute measured concentrations 60-90% lower when using the peptide capture antibody format, whereas Aβ40 concentrations exhibit a proportional bias of 20-40% using the peptide-specific capture orientation. Dilutional linearity is better for Aβ42 and Aβ40 assays using the 6E10 capture antibody format, falling between 80–120% for all dilutions. In spike recovery studies, Aβ42 and Aβ40 recover between 80–120% using the 6E10 capture antibody format. In contrast, recovery of Aβ42 in spiked plasma diluted 5-fold is 30% using the peptide capture antibody format. A 40-fold dilution is required to eliminate plasma matrix effects, whereas a 4-fold dilution is sufficient to eliminate matrix effects in cerebrospinal fluid for the Aβ42 assay.

Conclusion: This study suggests that an unidentified component in plasma interferes with binding of peptide-specific antibodies to the Aβ42 C-terminus. An assay format that includes 6E10 as the capture antibody reduces the effect of this interference.

2 Methods



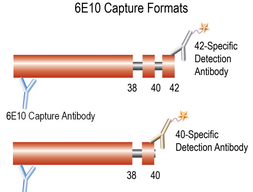
MSD's electrochemiluminescence detection technology uses SULFO-TAG™ labels that emit light upon electrochemical stimulation initiated at the electrode surfaces of MULTI-ARRAY® and MULTI-SPOT® microplates. As part of this study, the same antibody pairs were tested in two different orientations: 6E10 capture antibody or peptide-specific capture antibody. All four assays used the same protocol. We evaluated different diluents to reduce matrix interference. We evaluated matched plasma and serum samples from the same donor.

Protocol

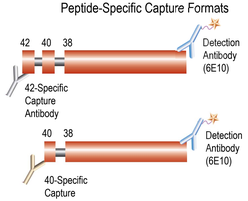
1. Add 150 µL of MSD Diluent 35. Incubate for 1 hour at room temperature.
2. Wash with PBS-T. Add 50 µL of standard or diluted sample. Incubate for 1 hour at room temperature.
3. Wash with PBS-T. Add 25 µL of detection antibody. Incubate for 1 hour at room temperature.
4. Wash with PBS-T. Add 150 µL of MSD Read Buffer T*. Read on MSD SECTOR® Imager.

Use 1X Read Buffer T for 6E10 capture formats
Use 2X Read Buffer T for peptide-specific capture formats

6E10 Capture Formats



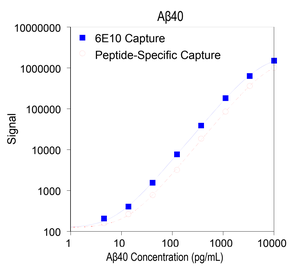
Peptide-Specific Capture Formats

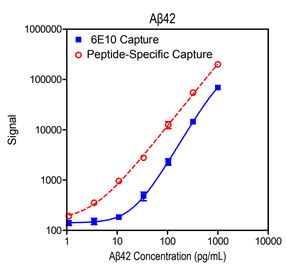


*The 6E10 antibody used in the Aβ plasma assays used in this study is supplied by Covance Research Products, Inc.

3 Standard Curves

The initial experiments were performed with a base diluent containing 1% BSA and 0.1% Tween-20 in PBS. The 6E10 capture format is more sensitive than the peptide-specific format for Aβ40. The opposite format is more sensitive for the Aβ42 assay; the peptide-specific capture is about 10 times more sensitive.





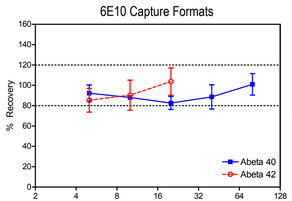
	6E10 Capture		Aβ40 Specific Capture		
Concentration (pg/ml)	Mean Signal	%CV	Mean Signal	%CV	Ratio of Signals
3333	632,699	8.8	362,462	6.1	57%
1111	183,439	3.1	85,719	8.1	47%
370	38,951	12.9	18,655	8.2	48%
123	7,695	2.7	3,189	3.1	41%
41.2	1,546	6.6	772	8.0	50%
13.7	404	5.5	263	6.1	65%
4.57	205	6.3	159	12.8	78%
0	115	5.4	115	11.4	100%

	6E10 Capture		Aβ42 Specific Capture		
Concentration (pg/ml)	Mean Signal	%CV	Mean Signal	%CV	Ratio of Signals
1000	69,365	4.1	200,152	2.2	289%
333	14,507	2.8	54,877	10.6	378%
111	2,313	14.6	12,605	16.3	545%
37.0	484	19.4	2,783	5.4	575%
12.3	183	2.3	961	7.2	524%
4.12	152	14.3	356	0.4	234%
1.37	142	11.5	193	6.1	136%
0	157	17.3	105	8.0	67%

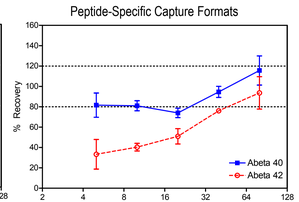
4 Matrix Interference: Dilutional Linearity

To assess matrix tolerance, three human EDTA plasma samples were spiked with 500 pg/mL Aβ40 and Aβ42 peptides, then diluted 5-fold, 10-fold, 20-fold, 40-fold, and 80-fold with the base diluent. The percent recovery at each dilution was calculated relative to the spike concentration and the concentration was adjusted for dilution. The error bars on the graph are the standard deviation between the three samples.
In the 6E10 capture format, the level of plasma Aβ42 was below the approximate LLOQ at dilutions greater than 20-fold. Sample dilutions met standard specifications (100 ± 20 %) and displayed linearity for both peptides when captured with 6E10. The matrix effects were greater when the peptide-specific antibodies were used as the capture.

6E10 Capture Formats



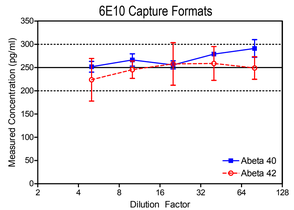
Peptide-Specific Capture Formats



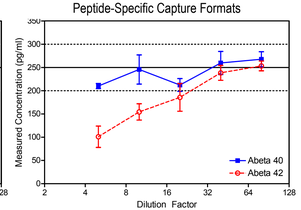
5 Matrix Interference: Spike and Recovery into Diluted Samples

Three human EDTA plasma pooled samples were diluted 5-fold, 10-fold, 20-fold, 40-fold, and 80-fold in base diluent and then spiked with either 250 pg/mL Aβ40 or Aβ42. Peptide concentrations were measured for each assay format. A matrix effect affects Aβ42 peptide-specific capture measurements, therefore plasma must be diluted 40-fold before the peptide spike is accurately recovered (within 20%). In the flipped orientation (6E10 capture), the Aβ42 peptide is recovered accurately at a 5-fold dilution.

6E10 Capture Formats



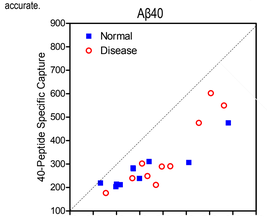
Peptide-Specific Capture Formats



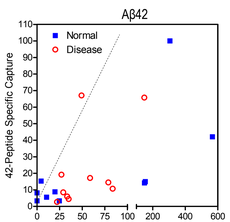
6 EDTA Plasma Samples: 6E10 Capture and Peptide-Specific Formats

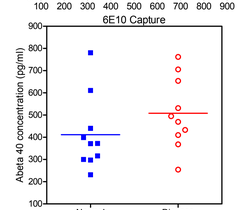
Ten EDTA plasma samples from normal patients and ten EDTA plasma samples from patients with Alzheimer's Disease (AD) were tested with all four assay formats at a 2-fold dilution. Serum from the same patients was also measured. **Top/Left:** The results for Aβ40 are correlated across formats, although the peptide-specific capture reports lower concentrations. **Top/Right:** The Aβ42 results differed between the two formats. **Bottom:** Neither Aβ40 nor Aβ42 levels in the normal samples were detectable using the 6E10 capture format. Only four normal samples and four diseased samples had Aβ42 levels greater than the approximate LLOQ of 50 pg/mL. Median levels for the ten normal and AD plasma samples measured are indicated by the blue and red lines. The approximate LLOQ of the Aβ42 peptide capture assay was 10 pg/mL (data not shown). The matrix interference observed in the dilution linearity and spike recovery experiments make it difficult to identify which format is more accurate.

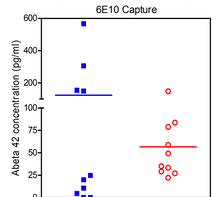
Aβ40



Aβ42

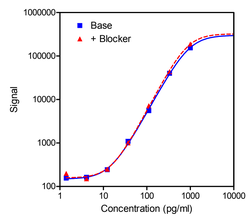




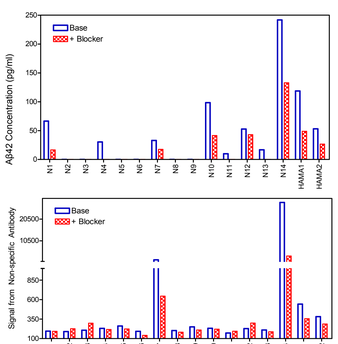


7 Diluents to Reduce False Positive Responses

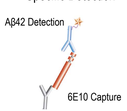
Addition of blocking agents, heterophilic antibodies, and IgGs can be used to reduce the effects of human-anti-mouse antibodies (HAMA) and rheumatoid factors (RF) on immunoassays. We tested the addition of these blockers to our base diluent to determine if high peptide concentrations were an artifact of non-specific antibody interactions. We assayed fourteen normal plasma samples and two HAMA positive samples. **Top:** The standard curves for the two assays (with and without blockers) were nearly identical with less than 20% difference. **Middle:** Sample ECL signals were reduced substantially by the addition of blockers; concentrations of the samples with the highest peptide levels were reduced by more than 2-fold. **Bottom:** A non-specific detection antibody (mouse anti-human IL-6) was paired with the 6E10 capture. High signals observed for several samples were suppressed with the addition of blockers.



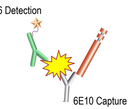
	Base Diluent		+ Blockers	
Conc. of Aβ 42 (pg/mL)	Mean	% Signal CV	Mean	% Signal CV
1000.0	154,419	9.4	188,175	7.6
333.3	40,191	13.1	43,619	1.9
111.1	5,548	16.6	7,186	6.9
37.0	1,093	3.9	1,007	2.2
12.3	243	21.5	249	11.9
4.1	163	19.6	150	10.9
1.4	156	6.3	199	38.4
0.00	171	19.0	175	7.7



Specific Detection



Non-Specific Detection



8 Conclusions

Assay format, antibody orientation, and matrix interferences contribute to variable quantitation of Aβ peptides in plasma. Assay sensitivity is dependent on antibody orientation: the peptide-specific capture for Aβ42 is more sensitive than the 6E10 capture antibody. However, we observed that the peptide-specific capture was more susceptible to matrix interferences. The matrix interferences are likely due to a combination of blocking interferences that reduce signals and bridging interferences (like HAMA or RF) that give false measurements. Aβ40 could be measured using either antibody orientation without matrix interference. Development of a plasma assay for Aβ42 will require considerable diluent optimization and antibody development.

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