Alzheimer's Association Global Biomarkers Standardization Consortium

CSF Round Robin Program

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CSF round robin program

- Initial focus: CSF $A\beta_{1\text{-}42}$
- Analytical methodology: srm/tandem mass spectrometry
- Involve volunteer labs with significant experience in mass spectrometry analyses of biomarkers
- Follow an agreed on protocol
- Assess precision across 4 participating labs using CSF pools
- Share raw data amongst the labs
- Statistical analysis
- Report data to peers
- Publish this pilot study

CSF round robin program

- The 4 participating labs: Waters (Erin Chambers); PPD (Rand Jenkins); UPenn (Les Shaw); UGot (Kaj Blennow)
- Initial reports of methods in literature and in ASMS & AAIC meetings
- Initial pilot study compared performance across 4 participating laboratories; initial report at the AAIC 2013 meeting; manuscript submitted
- N=12 CSF pools (prepared & shipped by UGot to each participant laboratory
- Use of a common sample preparation methodology
- 3 different mass spectrometer systems and 3 different HPLC systems
- 4 different calibration matrices
- Single-plex, triplex or pentaplex methods utilized
- Different batches of high purity rPeptide $A\beta_{1-42}$ standard utilized

Alzheimer's Association Global Biomarker Standardization Consortium

Four Center collaborative study of mrm/tandem mass spectrometry reference methodology for measurement of $A\beta_{1\text{-}42}$ in 12 CSF pool samples

- 3 MS platforms
  - Thermo TSQ Vantage
  - Waters TQ-S
  - ABI Sciex API 5000
- 3 HPLC platforms
  - ACUITY 1D
  - ACUITY 2D
  - Accela 1250
- 4 different surrogate matrices
  - Artificial CSF + 5% rat plasma
  - Artificial CSF + 4 mg/mL BSA
  - Salt and phosphate buffer solution + 4 mg/mL HSA, 0.05 mg/mL IgG, glucose
  - Human CSF using N15 labeled $A\beta_{1\text{-}42}$ as calibrator
- Single-plex, tri-plex or penta-plex methods employed
- **Sample preparation is the same across the 4 centers**

Laboratories participating in the Global Biomarker Standardization Consortium collaborative study

- Erin Chambers & Mary Lame, Waters & Pfizer
- Moucun Yuan, Junlong Shao, William R. Mylott, and Rand Jenkins, PPD
- Magdalena Korecka, John Trojanowski, Leslie Shaw, UPenn
- Henrik Zetterberg, Kaj Blennow, Josef Pannee, Erik Portelius, Johan Gobom, UGot

• mrm LC/MSMS method for Aβ peptides in CSF

- Aβ peptides from rPeptide
- N^{15}-Aβ peptide ISTDs

added to CSF samples

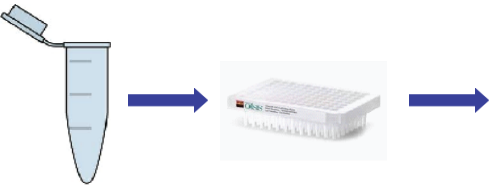
- Guanidine·HCl
- SPE extraction – 96 well

Format

- 2D HPLC/SRMtandem

mass spectrometry

Korecka M et al, AAIC 2012 Poster #P1-317

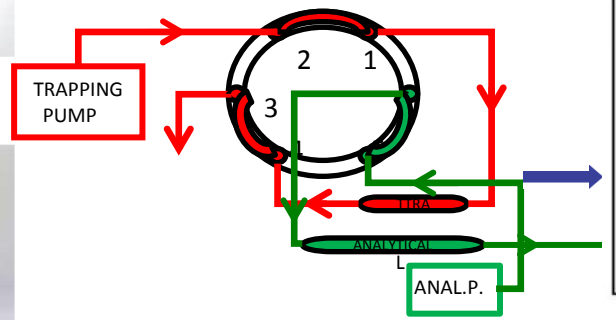


Eppendorf LoBind Tubes

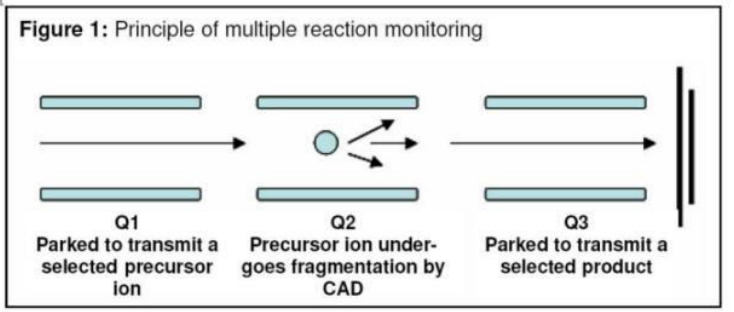
Oasis MCX µElution Plate

ACQUITY UPLC
Trapping column
Analytical column

- UPLC BEH-300
- C18 2.1x150mm, 1.7 µm



Injection position of switching valve - 2D chromatography



m/z 1129.0 → *m/z* 1078.8

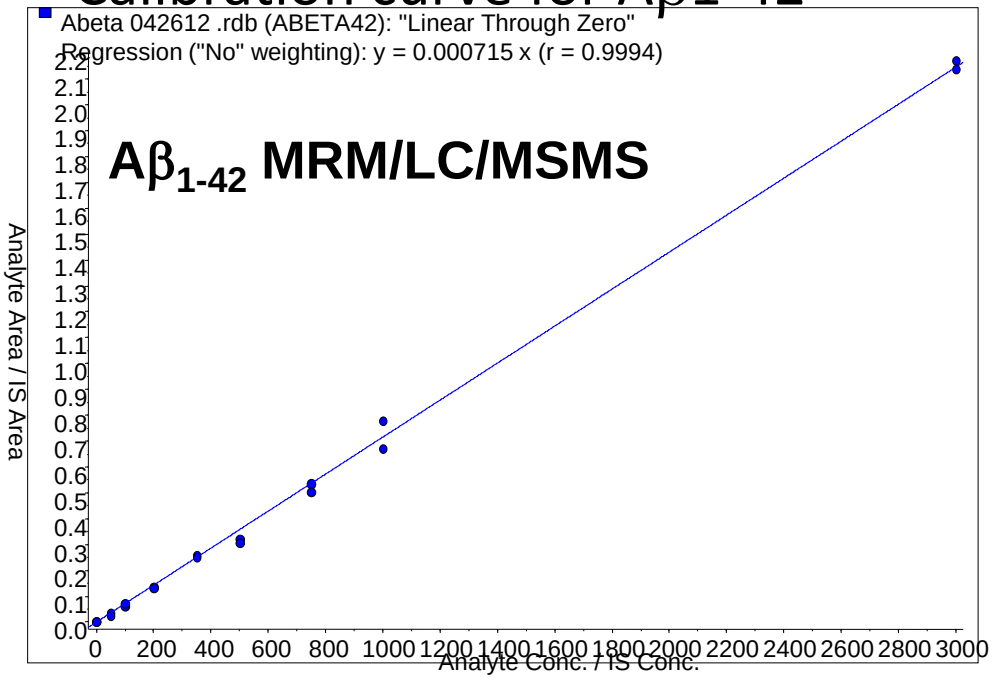
($A\beta_{1-42}$ precursor [4+] ion) (product [4+] ion)

AB SciexTandem mass spectrometer API 5000

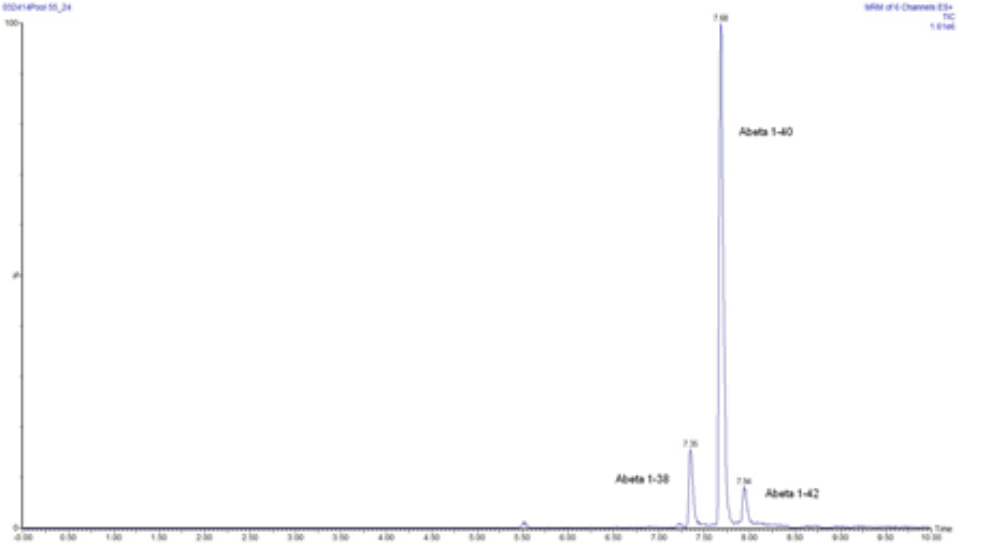
SRM-tandem mass spectrometry

- Established
  - Surrogate matrix
  - LLOQ/UPOQ
  - Linearity
  - Precision performance
  - Recovery from hCSF
  - Freedom from ion suppression
  - Equivalence between surrogate matrix ar

Calibration curve for Aβ1-42



Typical calibration curve for the quantitation of amyloid beta 1-42 (single analyte assay). An artificial CSF with addition of BSA (4mg/mL) is the matrix for calibrators preparation.



Total ion chromatogram of human CSF sample with the following concentrations of three Abeta peptides: 1320pg/mL (Aβ 1-38), 5720pg/mL (Aβ 1-40) and 545pg/mL (Aβ 1-42)

hCSF patient sample

Round robin test on quantification of $A\beta_{42}$ in CSF by mass spectrometry

Josef Pannee[a,*], Johan Gobom[a], Leslie M. Shaw[b], Magdalena Korecka[b], Erin E. Chambers[c],

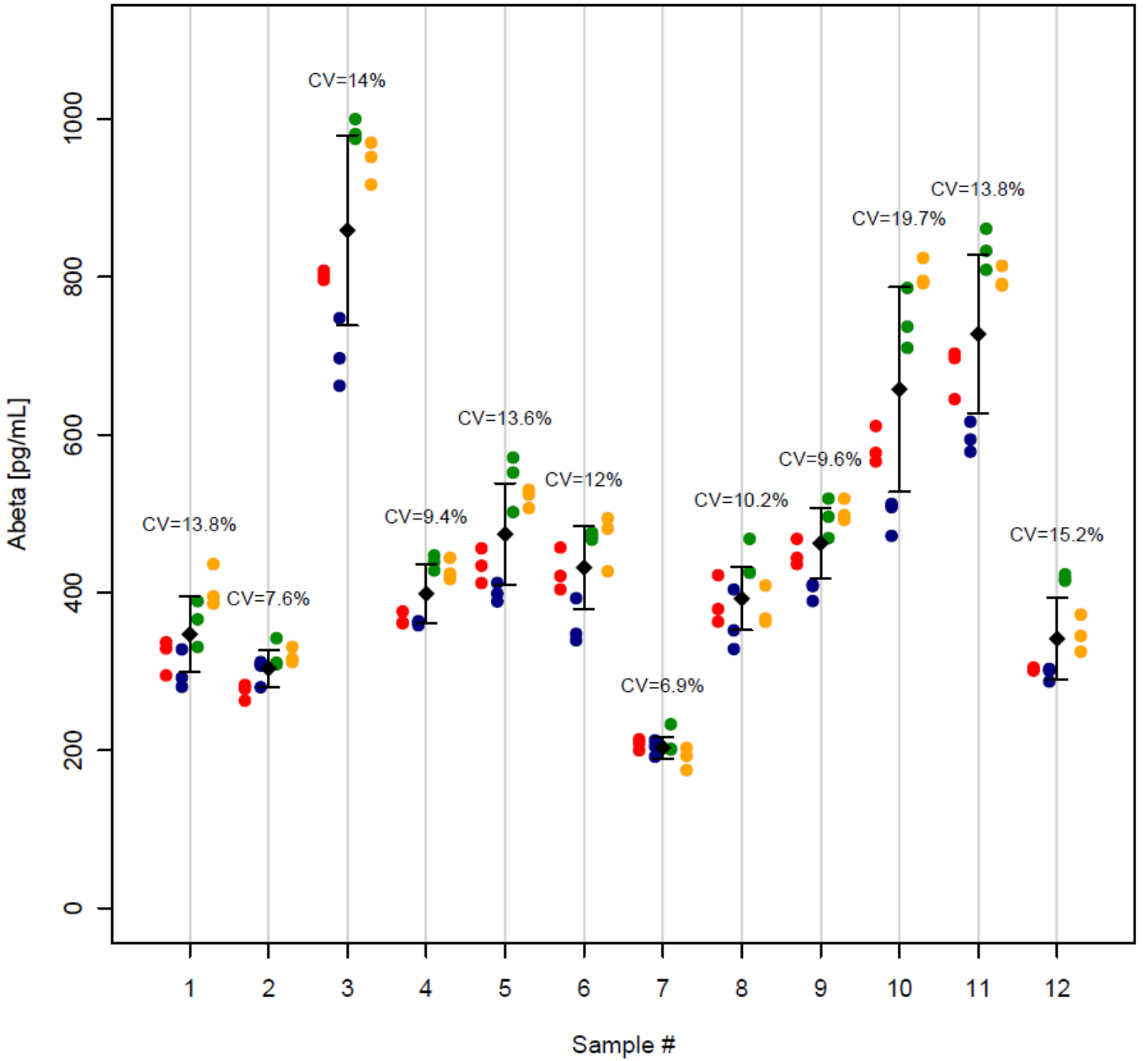
Mary Lame[c], Rand Jenkins[d], William Mylott[d], Maria C. Carrillo[e], Ingrid Zegers[f], Henrik Zetterberg[a,g],

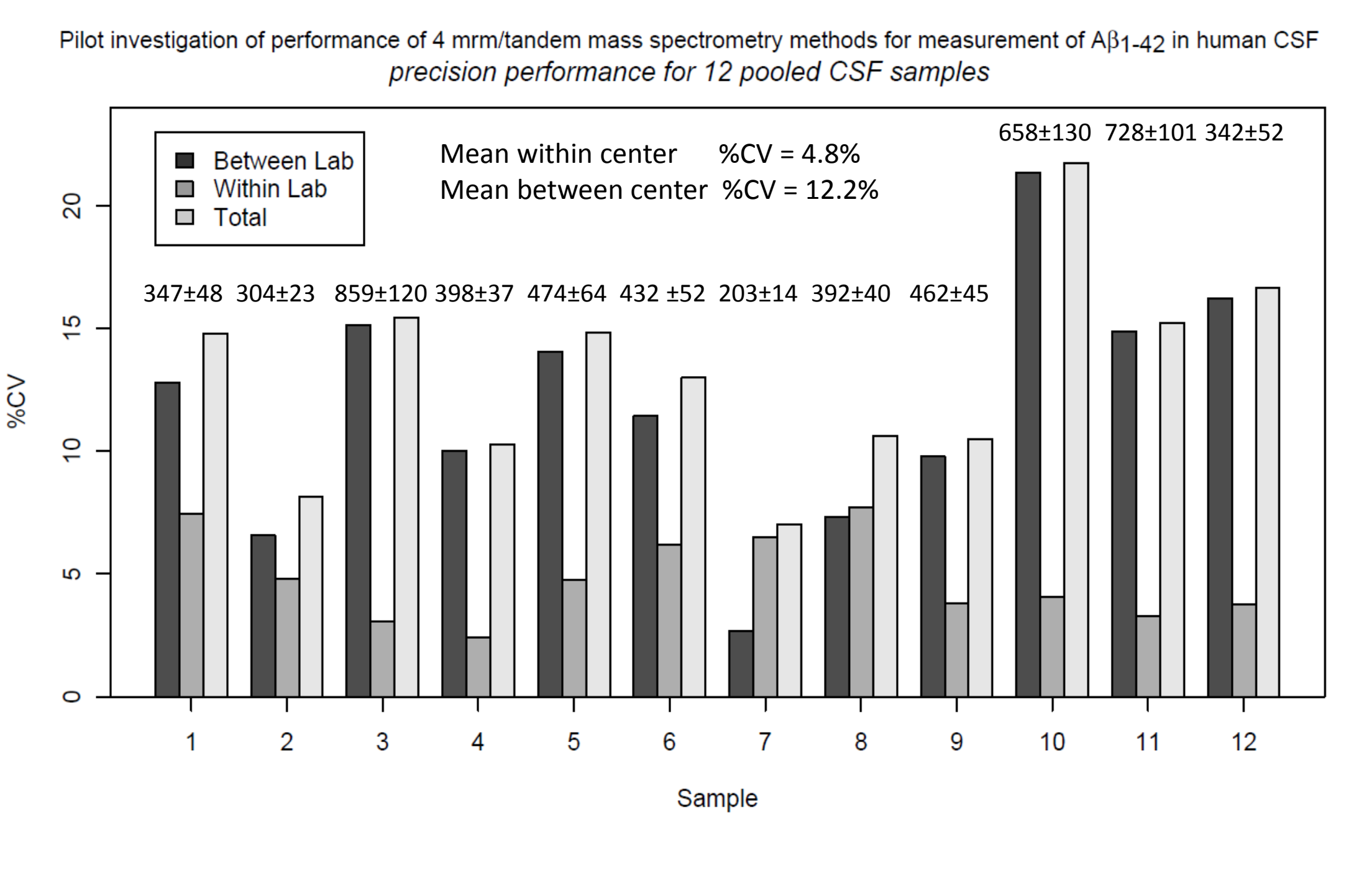
Kaj Blennow[a], Erik Portelius[a;] *manuscript submitted for publication*

Table 1. Mass spectrometry methods summary for the 4 centers

	Waters	PPD	U. Penn.	U. Got.
IS concentration	1 ng/mL	2 ng/mL (spiked from DMSO)	2 ng/mL	1.6 ng/mL
CSF Volume	200 µL	100 µL	250 µL	200 µL
Calbrator matrix	aCSF with 5% rat plasma	aCSF with 4 mg/mL HSA + IgG, glucose	aCSF with 4 mg/mL BSA	Human CSF
LC System	ACQUITY, 1D	ACQUITY; 2D Trapping/Eluting	ACQUITY; 2D Trapping/Eluting	Accela 1250
Dilution (injection)	50 uL + 25 µL H2O (10µL)	50 uL + 50 uL H2O (30 µL)	50 µL + 50 µL H2O (50µL)	No dilution. Dried eluate resuspended in 25 µL, 20 µL injected.
LC mobile phases	A- 0.3% NH4OH B- 90:10 ACN/MP A	A- 0.3% NH4OH B- 90:5:5 ACN/TFE/H2O	A- 0.1% NH4OH B- 75:25:5 ACN/MeOH/TFE	A: 0.1% NH_4OH, 5% ACN B: 0.03% NH4OH, 95% ACN
Column	BEH 300 2.1 x 150 mm, 1.7 µm, 50 C	BEH 300 2.1 x 150 mm, 1.7 µm, 50 C	BEH 300 2.1 x 50 mm, 1.7 µm, 60°C	ProSwift RP-4H 1x250 mm
Flow rate	200 µL/min	300 µL/min	200 µL/min	300 µL/min
Mass Spectrometer	Xevo TQ-S	Xevo TQ-S	API 5000	TSQ Vantage
Transitions, m/z	1129.0→1078.5	1129.0→1078.5	1129.0→1078.5	1129.58→1054.03, 1078.79, 1107.06
Run time	8.5 mins	8.5 minutes	12 minutes	14 minutes

Alzheimer's Association Global Biomarker Consortium mrmMSMS Study data for 12 CSF pools




Pilot investigation of performance of 4 mrm/tandem mass spectrometry methods for measurement of Aβ1-42 in human CSF
precision performance for 12 pooled CSF samples
Between Lab
Within Lab
Total
Mean within center %CV = 4.8%
Mean between center %CV = 12.2%
347±48
304±23
859±120
398±37
474±64
432 ±52
203±14
392±40
462±45
658±130
728±101
342±52
%CV
0
5
10
15
20
1
2
3
4
5
6
7
8
9
10
11
12
Sample

Summary

- Initial pilot study comparing performance across 4 participating laboratories completed
- The 4 participating labs: Waters (Erin Chambers); PPD (Rand Jenkins); UPenn (Les Shaw); UGot (Kaj Blennow)
- N=12 CSF pools (prepared & shipped by UGot to each participant laboratory
- Use of a common sample preparation methodology
- 3 different mass spectrometer systems and 3 different HPLC systems
- 4 different calibration matrices
- Single-plex, triplex or pentaplex methods utilized
- Different batches of rPeptide $A\beta_{1\text{-}42}$ standard utilized
- **Very good agreement across the 4 laboratories is consistent with the ruggedness of the methodologic approach and supports their working together on the IFCC ref method assignment of accurate $A\beta_{1\text{-}42}$ concentrations to planned CSF-based standard reference material**
- **The 4 centers have committed to a follow-up interlab study, as part of an IFCC/IRMM guided-study effort, that is planned and there are individual studies completed addressing areas of interest:**
  - **Calibrator matrix comparison studies**
  - **CSF stability**
- The mrm/tandem mass spectrometry-based methodology with high conc GuHCl followed by mixed-bed (ion exchange/RP) cartridge sample preparation is a suitable candidate reference method for assigning accurate and precise $A\beta_{1\text{-}42}$ values on CSF-based reference material.

Next steps

- Use the IRMM preparation of $A\beta_{1\text{-}42}$
  - Pilot: 2 lab (UGot and UPenn) study using 10 CSF pools prepared at UPenn→lab to lab comparison, just completed
  - Prliminary testing of the IRMM dilution protocol
  - conduct the full IRMM – guided “ring” trial
    - Compare across participating centers
    - Use gravimetric protocol for preparation of calibrators and include calibrators prepared by individual lab protocol in the two replicate runs
    - 20 patient CSFs, a set of neat and spiked CSFs
    - Statistical analyses
    - Report results
  - Use these qualified methods for assignment of concentration to the CSF pools for creation of reference materials
- Applications of mass spectrometry-based $A\beta_{1\text{-}42}$ analysis:
  - Comparisons to existing and new immunoassays including analytical performance and clinical performance
  - Provides an accuracy-based “anchor” in methods comparisons
  - Studies in various patient populations of $A\beta_{1\text{-}42}$, and various metabolites for assessment of age and or disease related changes in metabolism

Applications of mass spectrometry-based $A\beta_{1-42}$ analysis- comparison of clinical performance to an existing immunoassay

41 autopsy-proven AD cases and 41 living age- and gender-matched controls*

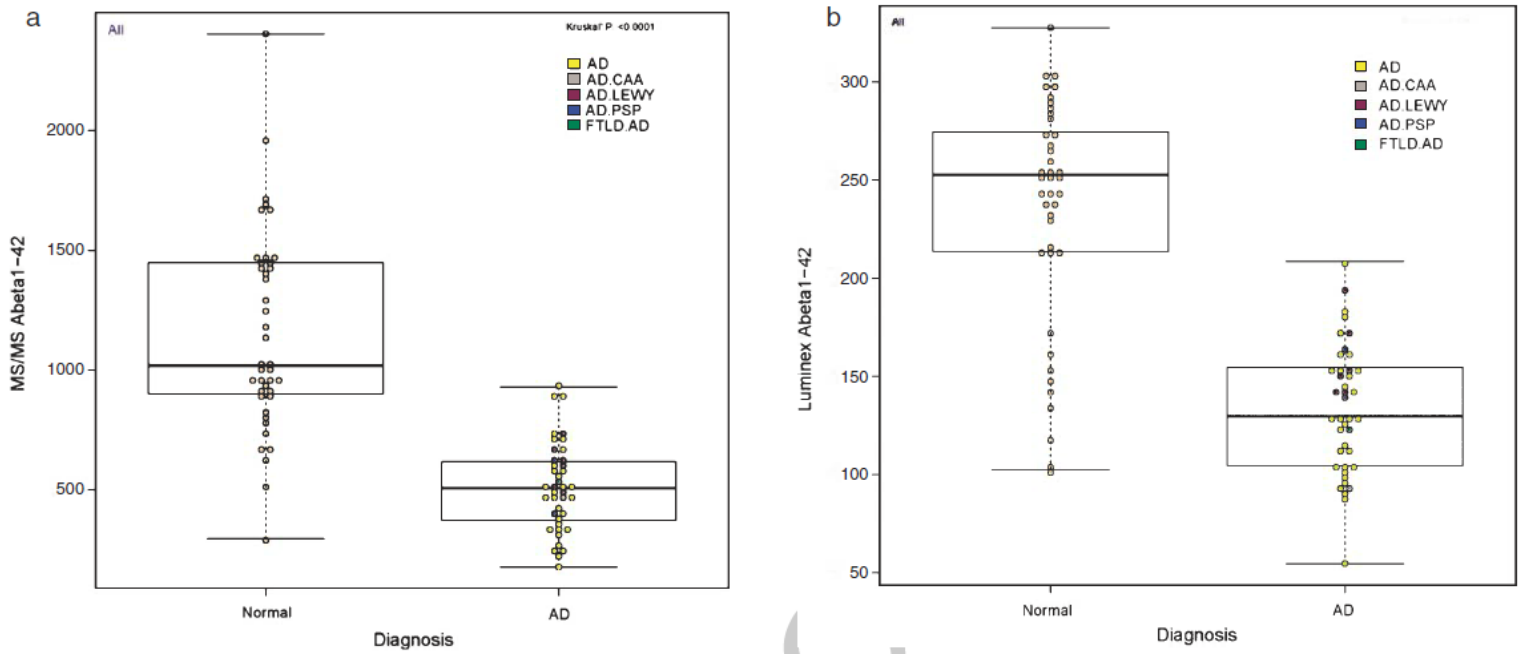
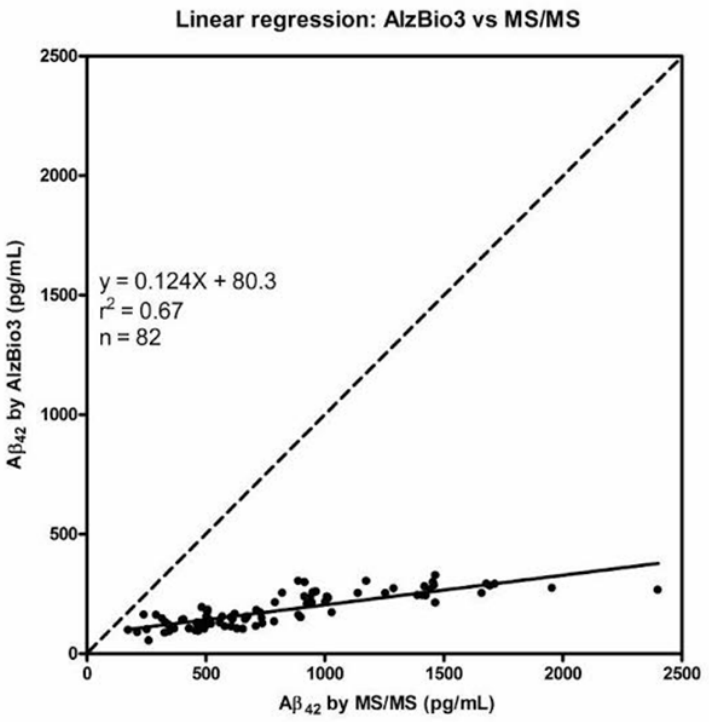


Fig. 3. Distribution of $A\beta_{42}$ results in the group of 41 autopsy proven Alzheimer's disease subjects and 41 age matched control group; A) 2D-UPLC-MS-MS, B) AlzBio3 Luminex.

*same population as described in AoN 2009

Analytical comparison



Clinical utility comparison

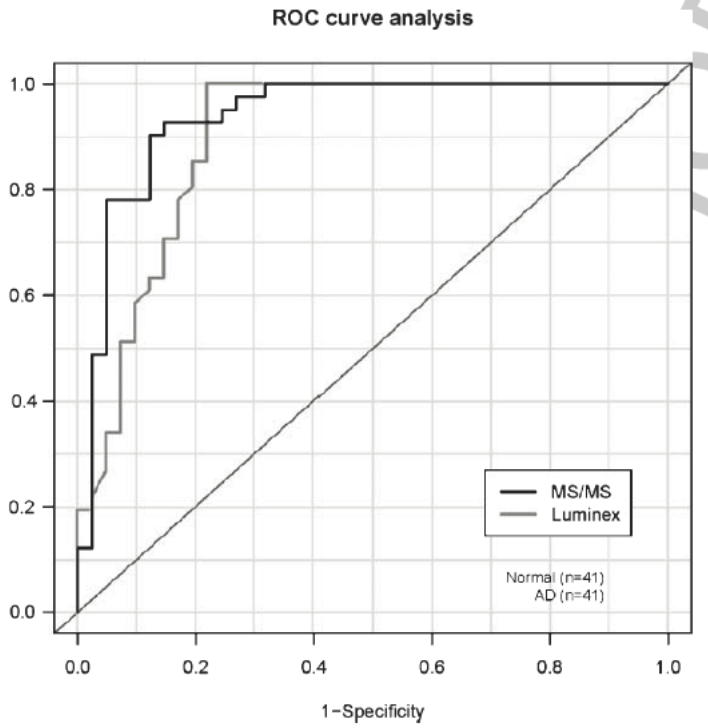


Fig. 4. Comparison of ROC curves for 2D-UPLC-MS-MS and AlzBio3 Luminex. The ROC AUC value for 2D-UPLC-MS-MS was 0.938, and for AlzBio3 Luminex immunoassay the AUC value was 0.900.

ROC analyses

Clinical performance using 41 AD, 41 cog normal controls:

Sensitivity: 92.7%
Specificity: 85.4%
PPV: 86.4%
NPV: 92.1%
Test accuracy: 89%
AUC: 0.94

Clinical performance using the same
41 AD and 41 controls for the AlzBio3 Immunoassay:

	2009 AoN
Sensitivity: 100%	(96.4%)
Specificity: 78%	(76.9%)
PPV:82%	(82%)
NPV:100%	(95.2%)
Test accuracy: 89%	(87%)
AUC: 0.90	(0.91)

Two candidate ref methods (UGot and UPenn)

Clinical Chemistry 60:7
000–000 (2014)

Proteomics and Protein Markers

Mass Spectrometry–Based Candidate Reference Measurement Procedure for Quantification of Amyloid-β in Cerebrospinal Fluid

Andreas Leinenbach,[1†] Josef Pannee,[2†] Thomas Dülffer,[1] Andreas Huber,[1] Tobias Bittner,[1] Ulf Andreasson,[2] Johan Gobom,[2] Henrik Zetterberg,[2,3] Uwe Kobold,[1] Erik Portelius,[2] and Kaj Blennow[2*] on behalf of the IFCC Scientific Division Working Group on CSF proteins

Journal of Alzheimer's Disease 41 (2014) 441–451
DOI 10.3233/JAD-132489
IOS Press

Qualification of a Surrogate Matrix-Based Absolute Quantification Method for Amyloid-β_{42} in Human Cerebrospinal Fluid Using 2D UPLC-Tandem Mass Spectrometry

Magdalena Korecka[a], Teresa Waligorska[a], Michal Figurski[a], Jon B. Toledo[a,d], Steven E. Arnold[b,c], Murray Grossman[c], John Q. Trojanowski[a,d] and Leslie M. Shaw[a,d,*]

- Two candidate ref methods
- 10 CSF pools provided by UPENN
- 3 replicate runs
- Used $A\beta_{1\text{-}42}$ prep

provided by IRMM for the Ring trial & each lab used their calibration dilution protocol.