Updates on Global Biomarker Standardization Projects

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The Biomarker Team at the UW ADRC

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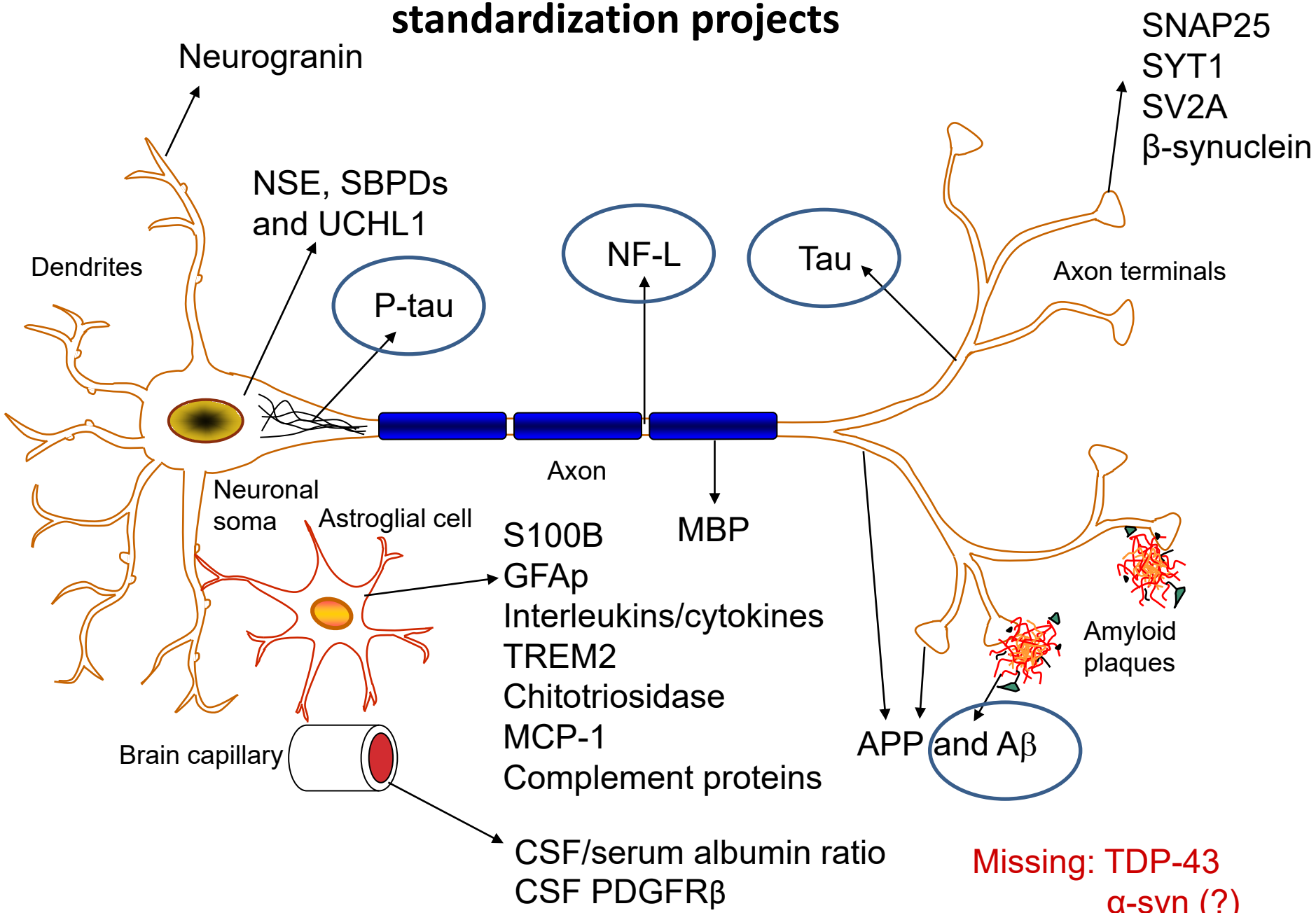
Cindy Jensen
Biospecimen Coordinator

Rachael Wilson
Fluid Biomarker Scientist

Fluid biomarker candidates of potential relevance to standardization projects



Key players in AD biofluid-based biomarker standardization

ifcc International Federation of Clinical Chemistry and Laboratory Medicine

Quick Navigation

Biomarkers of Neurodegenerative Diseases (WG-CSF)

Membership

Name	Position	Country	Term	Time in Office
J. Gobom	Chair	SE	2nd	2021 01 - 2023 12

ALZHEIMER'S ASSOCIATION

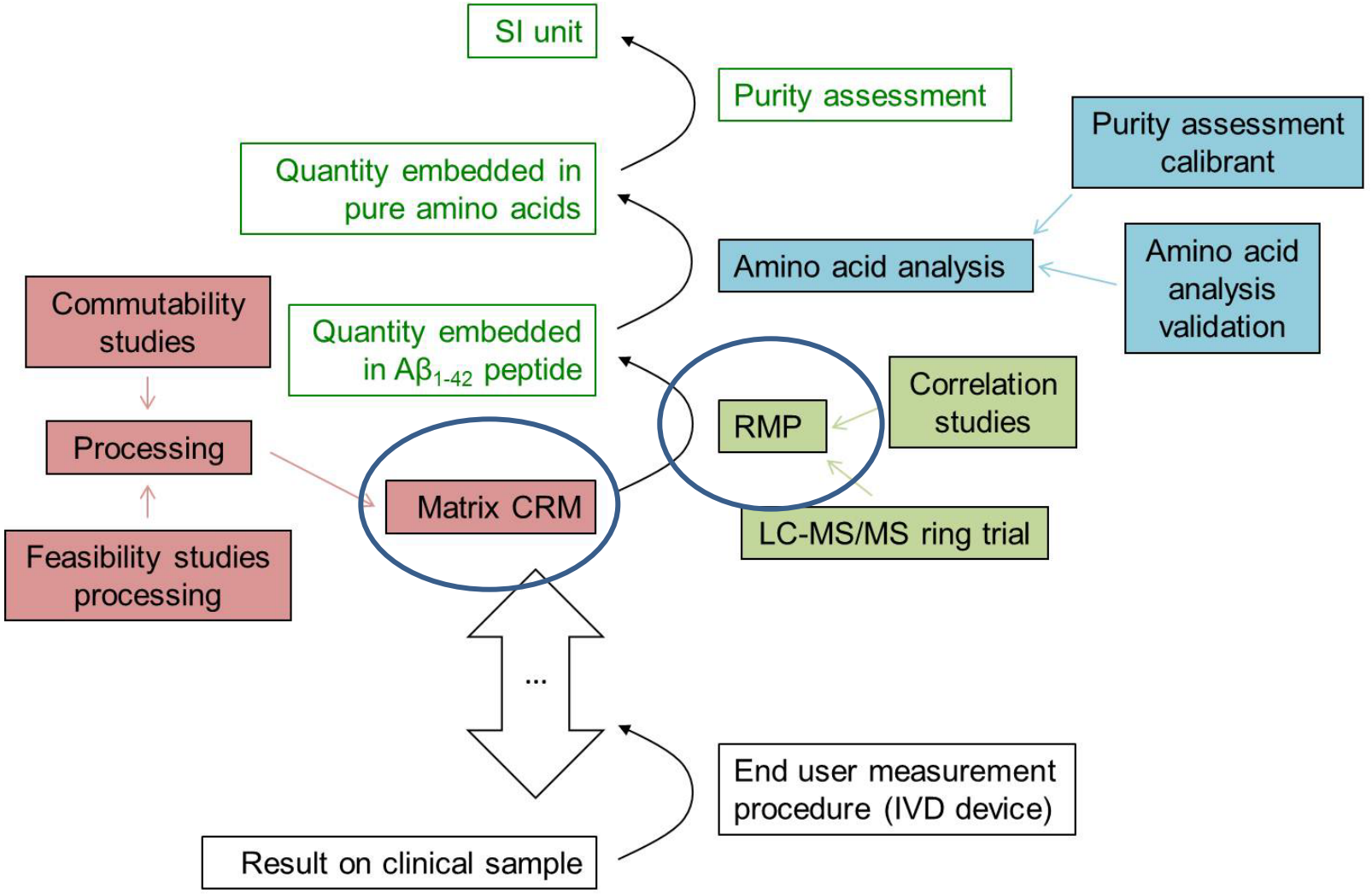
Global Biomarker Standardization Consortium

ISTAART

ALZHEIMER'S ASSOCIATION

Biofluid-Based Biomarker PIA

What is meant by standardization in clinical chemistry?



Why standardization?

Certified reference materials, value-assigned to an SI unit concentration (preferably) using a certified reference method will:

-make it mandatory for kit vendors to ”calibrate their calibrators” using the reference material, so that different methods will give the same absolute concentration of the target analyte

-make a diagnostic field less dependent on one or a few companies or labs (in clinical chemistry it is good to have access to several slightly different assays measuring the same analyte in a standardized way)

-make it much easier to get back on track if you run a clinical lab and lose traceability in your assay calibration (it should not happen in the first place but if it happens...)

-facilitate scale-up of assays for de-centralised clinical chemistry laboratory testing (*i.e.*, if we eventually will have to move away from the centralised lab approach for capacity reasons)

A = amyloid pathology

Candidate CRMs

Processing

- Matrix materials: 3 CSF pools (6-7 different patients per pool), not spiked
- Filled 0.5 mL/vial
- 3 levels: low, medium and high $A\beta_{1-42}$ concentration
- Stored at -70 °C

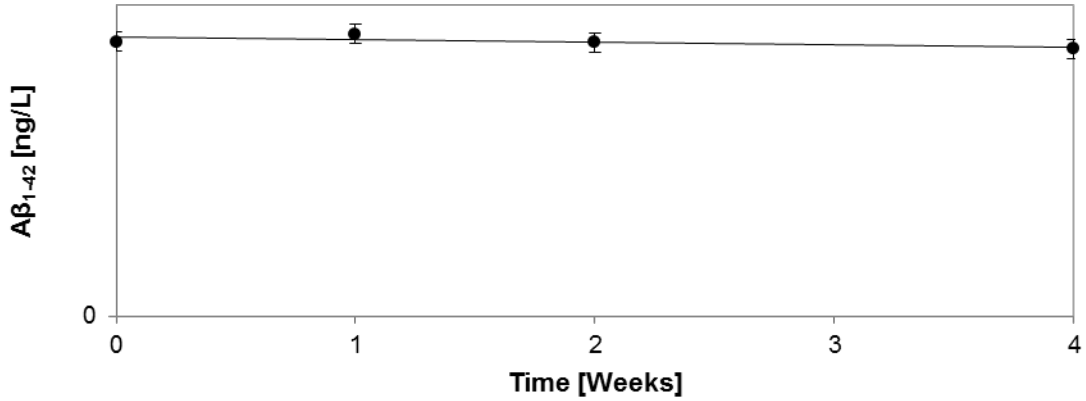
Homogeneity

$A\beta_{1-42}$	Roche			ADx NeuroSciences (Euroimmun)		
	s_{bb} [%]	s_{wb} [%]	u^*_{bb} [%]	s_{bb} [%]	s_{wb} [%]	u^*_{bb} [%]
ERM-DA480/IFCC	2.00	1.72	0.41	1.27	5.05	1.20
ERM-DA481/IFCC	1.12	3.44	0.69	1.26	5.05	1.01
ERM-DA482/IFCC	1.07	1.50	0.36	1.46	5.19	1.23

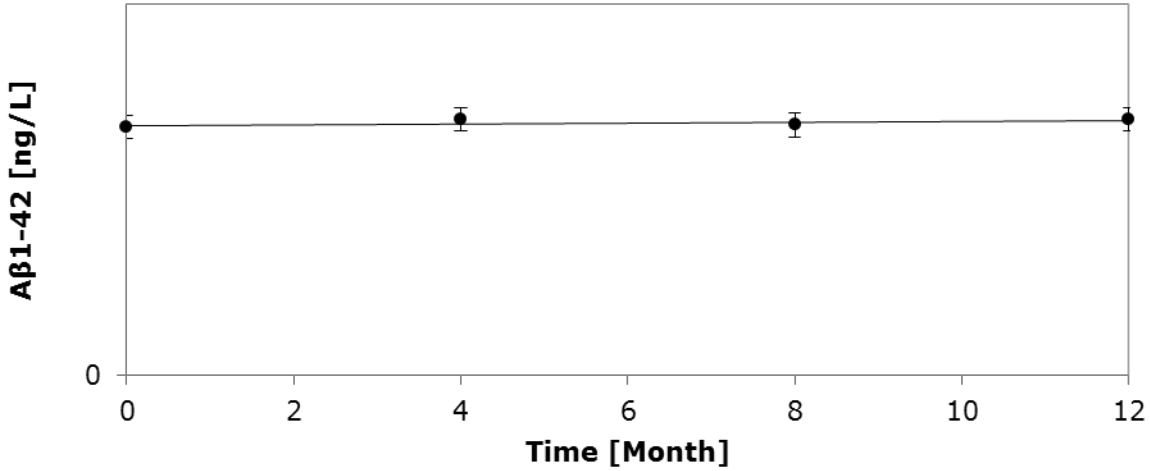
Stability

C
C

Short term stability

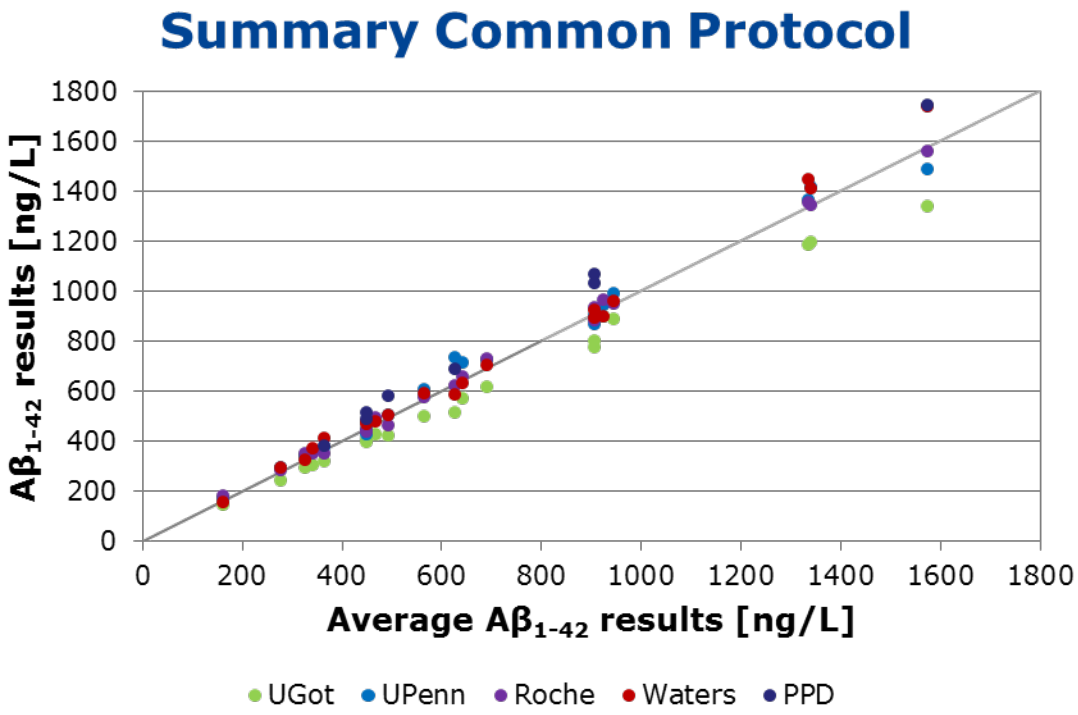


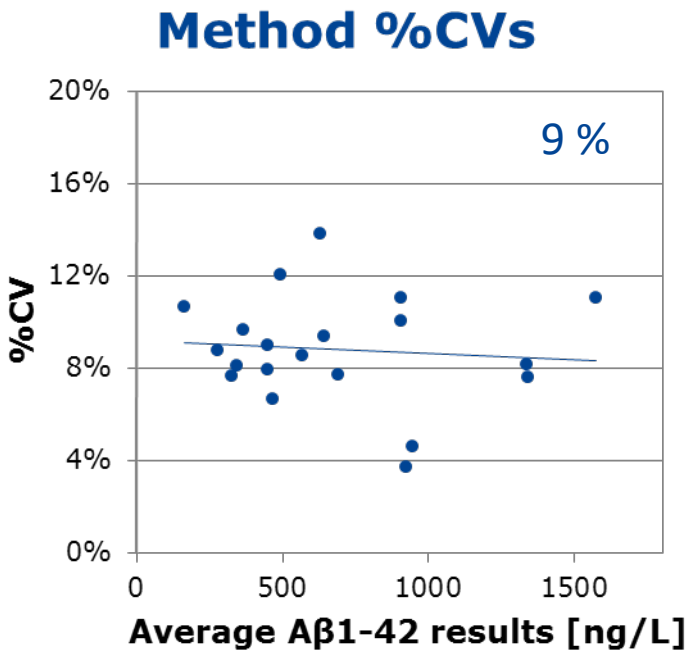
Long term stability



LC-MS Ring Trial II

- 5 labs → common $A\beta_{1\text{-}42}$ calibrant & dilution protocol
- 20 CSF samples





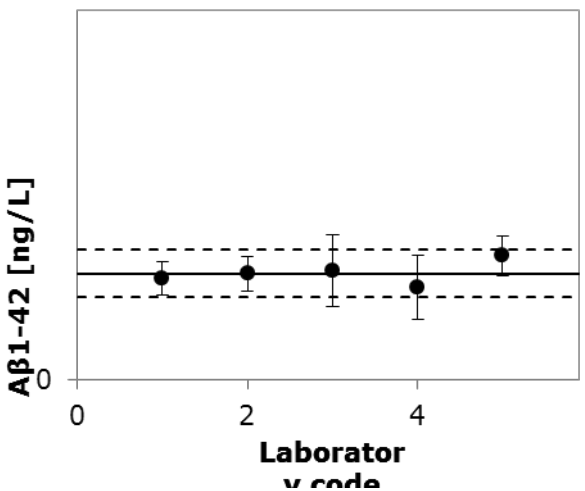
$$u_{char} = \left(\frac{9\%}{\sqrt{5}}\right) = 3.9\%$$

Characterisation

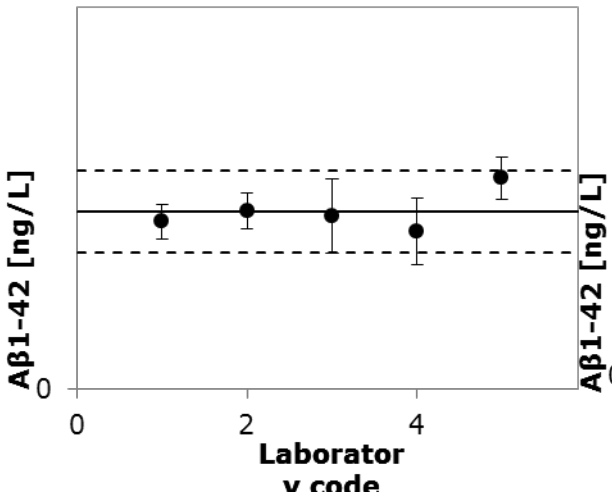
- Certified reference LC-MS methods
- Five laboratories (UGot, Roche, UPenn, PPD, Waters)
- Common Abeta42 calibrant
- 3 days
- 3 samples per day
- Triplicate or duplicate measurements

Results Characterisation

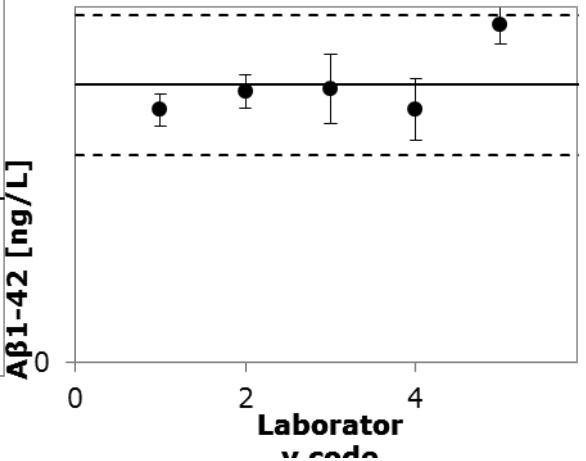
ERM-DA480/IFCC



ERM-DA482/IFCC



ERM-DA482/IFCC



Error bars correspond to 2 * STDEV of all data from the laboratory

Commutability

ERM-DA480/IFCC, ERM-DA481/IFCC and ERM-DA482/IFCC were shown to be commutable for the combination of the following routine measurement procedures:

- EUROIMMUN beta-amyloid (1-42)
- IBL Amyloid-beta (1-42)
- INNOTEST® β-AMYLOID$_{(1-42)}$
- Lumipulse® (Fujirebio Europe N.V., Gent, BE)
- V-PLEX® Aβ Peptide Panel 1 (MSD)
- Roche Elecsys β-amyloid (1-42)

Similar work almost completed for CSF Aβ40 – the CSF Aβ42/Aβ40 ratio will thus be fully standardized soon

Plasma Aβ was depriotized for standardization work by IFCC because of too low correlation between available methods (this may well change with improvement of existing assays)

T = tau pathology

IFCC:

CSF T-tau and P-tau deprioritized in favor of plasma P-tau

The Alzheimer's Association and GBSC Plasma P-tau Round Robin Study

Forty paired large volume plasma and CSF samples representing all clinically relevant P-tau levels (20 CSF AD biomarker-positive, 20 CSF AD biomarker-negative) are being prepared

Candidate reference materials, consisting of plasma spiked at three different concentrations with either GSK3β-phosphorylated recombinant tau or CSF are being prepared

Perform P-tau measurements using all available P-tau methods that are sensitive enough for plasma, including different phospho-forms (P-tau181, P-tau217 and P-tau231)

Examine strengths and slopes of correlations in CSF and plasma – are differences between P-tau forms and assays biological or matrix-dependent?

Examine fold change between AD and non-AD samples

Examine if candidate CRMs can harmonize/standardize P-tau measurements in a pilot commutability study

Co-principal investigators:
Henrik Zetterberg, University of Gothenburg (henrik.zetterberg@gu.se)
Les Shaw, UPenn
Kaj Blennow, University of Gothenburg

Confirmed labs:
-**Fujirebio** (Manu Vandijck, a prototype Lumipulse-based assay for P-tau181)
-**Janssen** (Hartmuth Kolb, plasma P-tau217 on Simoa)
-**Lilly** (Jeff Dage, MSD assays for P-tau181 and P-tau217)
-**Quanterix** (Quanterix Simoa assay for P-tau181)
-**UCSD** (Robert Rissmann, Quanterix Simoa assay for P-tau181)
-**UPenn** (Les Shaw, Quanterix Simoa assay for P-tau181)
-**UGOT** (Kaj Blennow and Henrik Zetterberg, in-house Simoa assays for P-tau181 and P-tau231, IP-MS methods for P-tau181 and P-tau217 – candidate reference methods?)
-**ADX** (Erik Stoops)
-**Abbvie** (Anthony Bannon, P-tau231)
-**ALZPath** (Andreas Jeromin, P-tau217)

The Alzheimer's Association and GBSC Plasma P-tau Round Robin Study

Current status:

Samples are being analysed

Results will be presented at AAIC this summer

N = neurodegeneration

Included NfL assays

Table 1. Information about the assays

Manufacturer	Platform	Detection	Analyzed by
Quanterix	HD-X	Simoa	Quanterix
Quanterix	HD-X	Simoa	UHB
In-house	HD-X	Simoa	UHB
Olink	Olink Target 96	PEA	Olink
ProteinSimple	Ella	Fluorescence	ProteinSimple
Siemens Healthineers	Atellica® Solution	CL	Siemens Healthineers

Abbreviations: Simoa, single molecule array; PEA, proximity extension assay, UHB, University Hospital Basel; CL, Chemiluminescence

Pairwise correlations

Pearson's

Method	1	2	3	4	5	6	7
1		1.00	1.00	0.99	0.99	0.99	0.99
2	1.00		1.00	0.98	0.99	0.98	0.99
3	0.99	0.99		0.99	1.00	0.99	0.99
4	0.99	0.99	0.99		0.99	0.99	0.99
5	0.99	0.99	0.99	1.00		0.98	0.99
6	0.99	0.99	0.98	0.99	0.99		0.99
7	0.99	0.99	0.99	0.99	1.00	0.99	

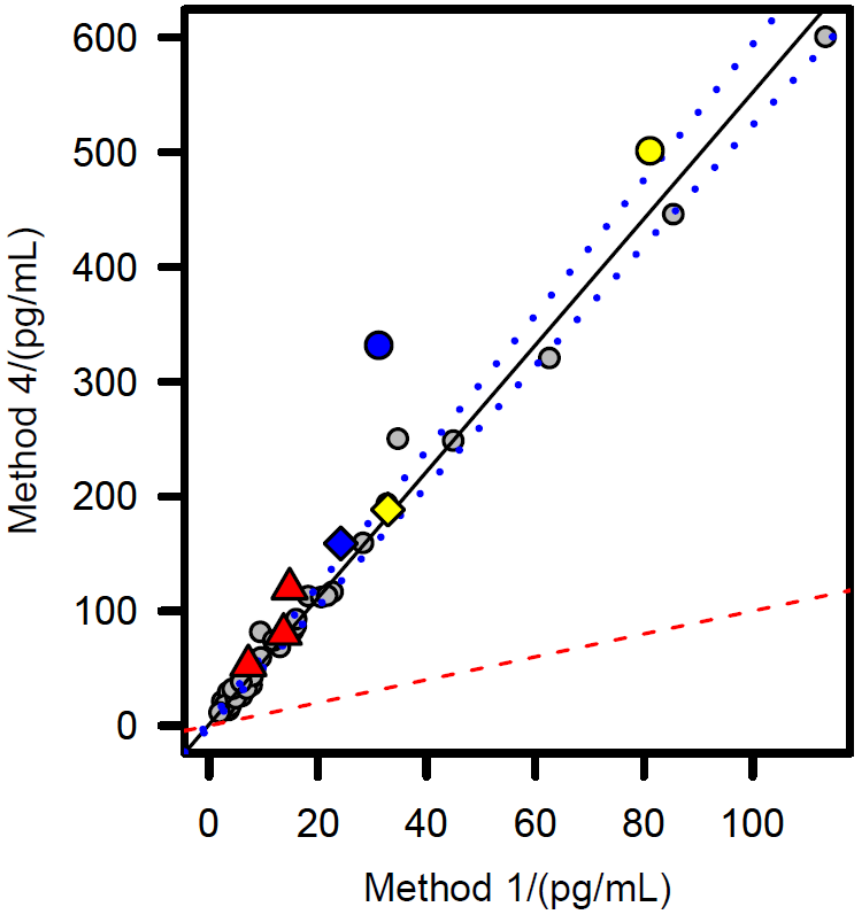
Lower left: Plasma
Upper right: Serum

Spearman's

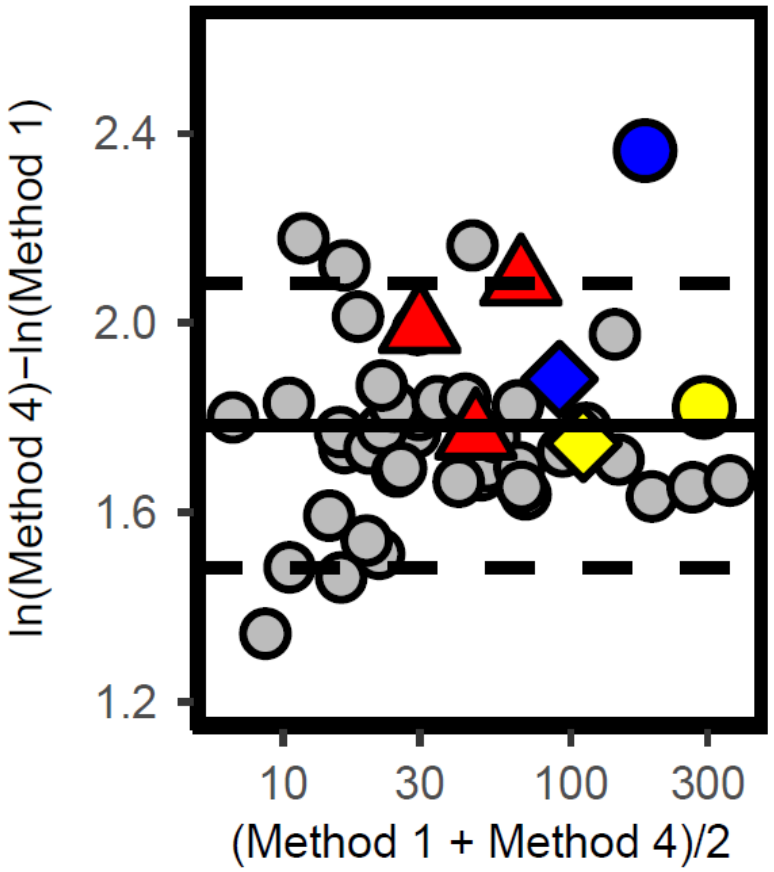
Method	1	2	3	4	5	6	7
1		1.00	0.99	0.97	0.98	0.95	0.97
2	0.99		0.98	0.97	0.98	0.95	0.96
3	0.98	0.98		0.97	0.99	0.96	0.96
4	0.98	0.96	0.96		0.97	0.93	0.94
5	0.98	0.98	0.98	0.98		0.96	0.96
6	0.95	0.94	0.95	0.94	0.94		0.93
7	0.97	0.98	0.96	0.95	0.97	0.94	

Candidate reference materials

Passing-Bablok

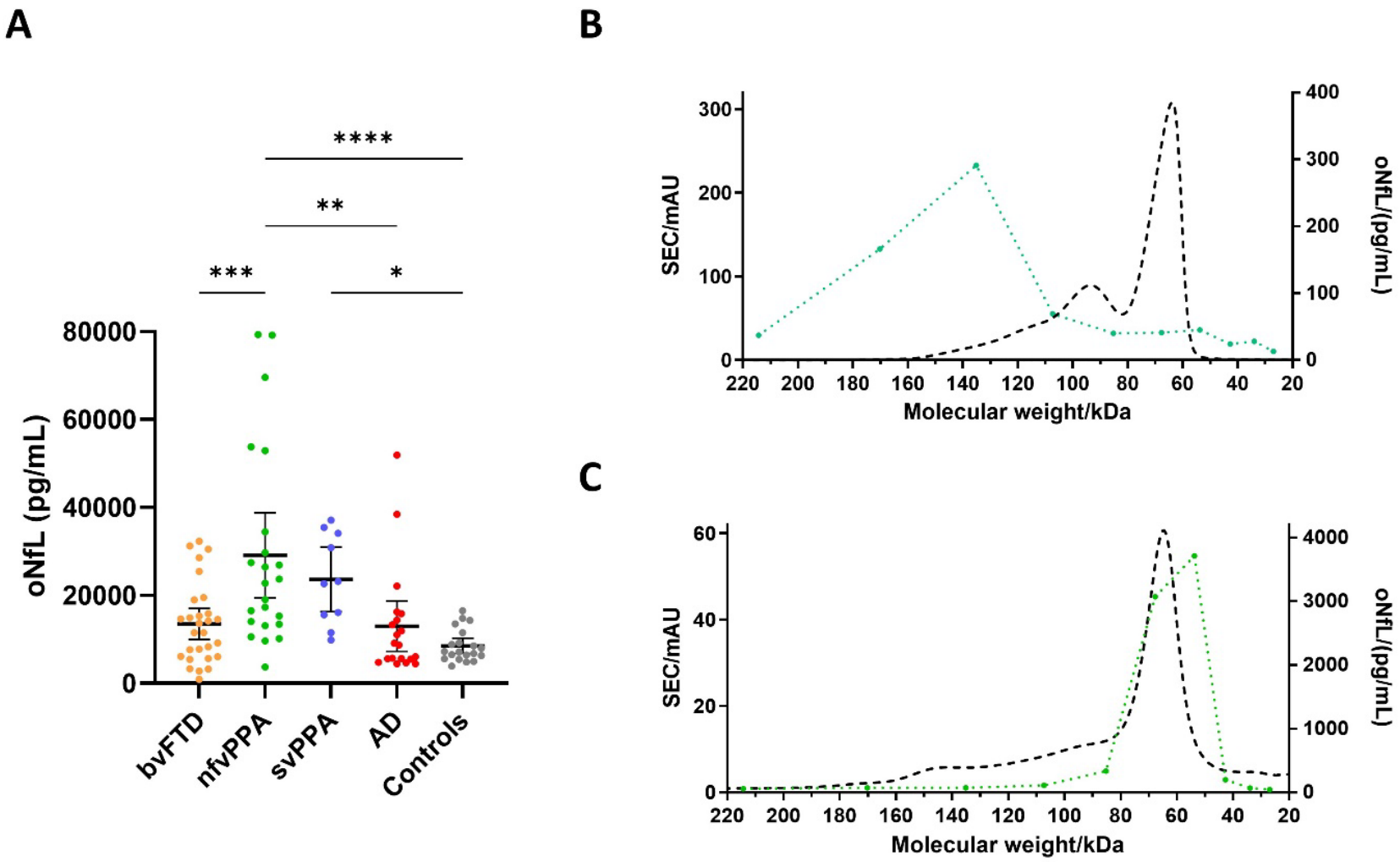


Bland-Altman



Grey= individual samples
Red= Neat candidate RM
Blue= Candidate RM spiked with concentrate
Yellow= Candidate RM spiked with CSF

CSF NfL is a dimer



Meda F *et al*., BMJ Neurol Open, 2023

Conclusions, NfL

The strong pairwise correlations suggest that NfL standardization will be straightforward

CSF seems to be possible to use for spiking a reference material

A new Round Robin and commutability study in which Elecsys, Lumipulse, and MS-based assays are also included is underway

The measurand needs further standardization – CSF NfL and likely plasma NfL exist as a dimer that will likely be measured at a different concentration by MS-based methods compared with immunoassays.

Please get in contact of you want to contribute samples or ideas for standardization projects

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