MEASUREMENT OF BIOMARKERS OF NEUROPATHOLOGY USING DRIED BLOOD SPOTS

Bharat Thyagarajan

Professor

Program Director, Advanced Research and Diagnostics Laboratory

Director, Division of Molecular Pathology and Genomics

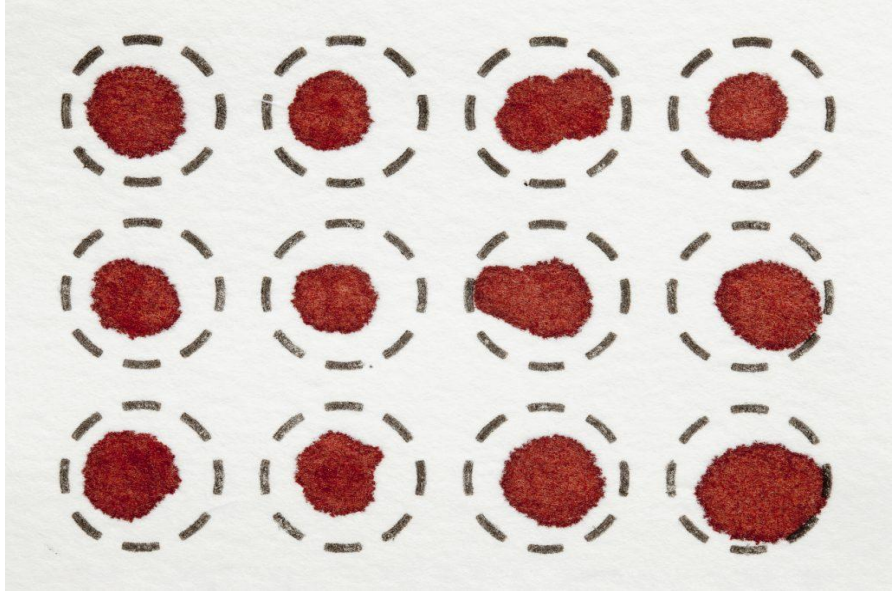
University of Minnesota

BACKGROUND

- Blood based biomarkers of neuropathology are increasingly being used in several research studies
  - Blood based biomarkers have been shown to be associated with dementia
- Blood that has been collected and processed soon after collection for optimal biomarker measurement
  - These methods cannot be practically implemented in resource poor settings
- Alternate methods that do not rely on complex processing or cold storage can improve application of these biomarkers in a more broad research context.

ALTERNATE METHODS: DRIED BLOOD SPOTS (DBS)

- Dried Blood Spots (DBS) is the most commonly used microsampling method
  - newborn screening programs
  - pharmacokinetics
  - toxicology
  - infectious disease
- LIMITATIONS
  - Hematocrit effect
  - Sample heterogeneity
  - Environmental conditions
  - Labor intensive processing in the laboratory



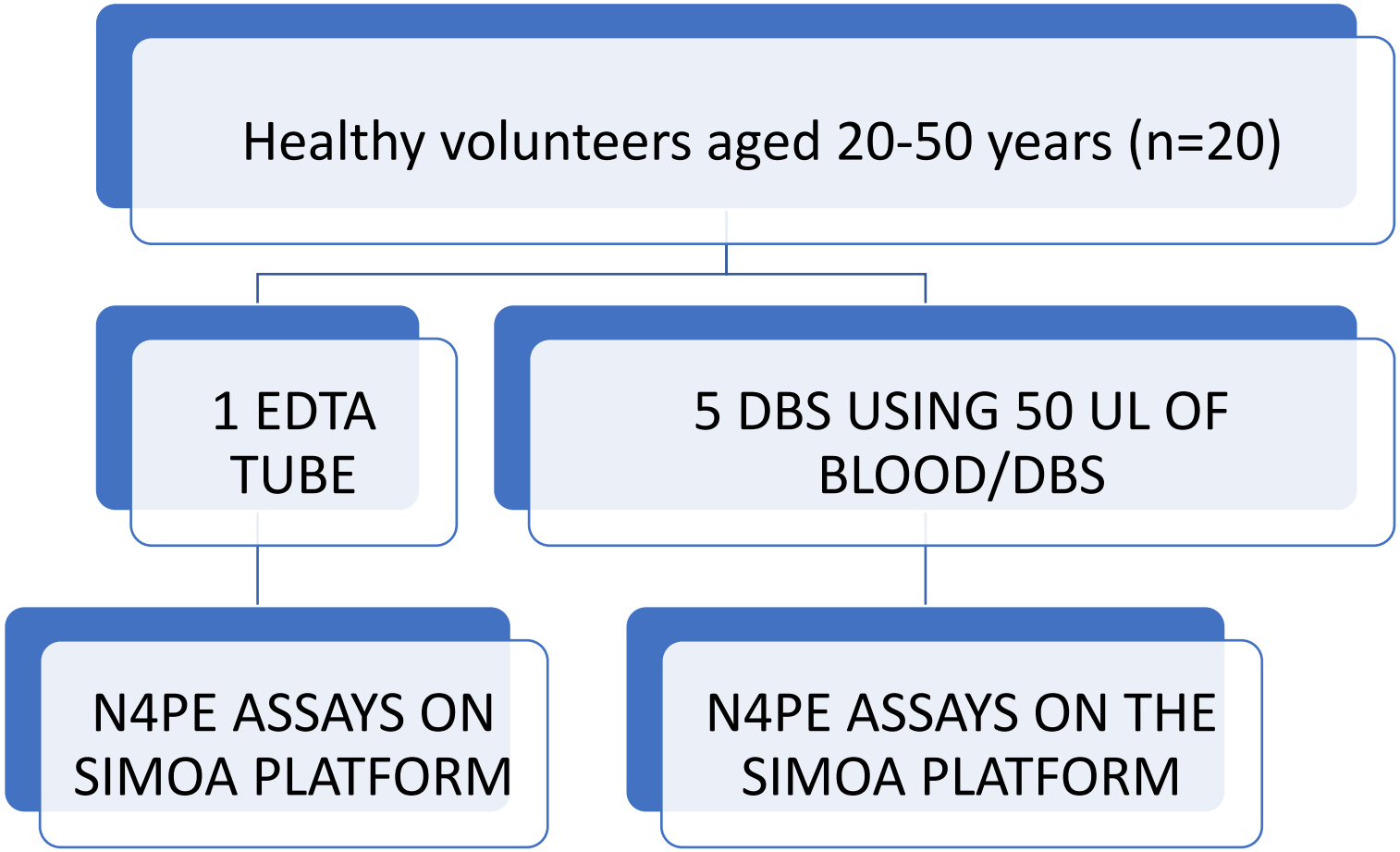
https://www.aacc.org/cln/articles/2022/september/dried-blood-spots-and-beyond
https://autogen.com/wp-content/uploads/2019/04/Dried-Blood-Spots-1024x747.jpeg

DRIED BLOOD SPOTS: LABORATORY PROCESSING

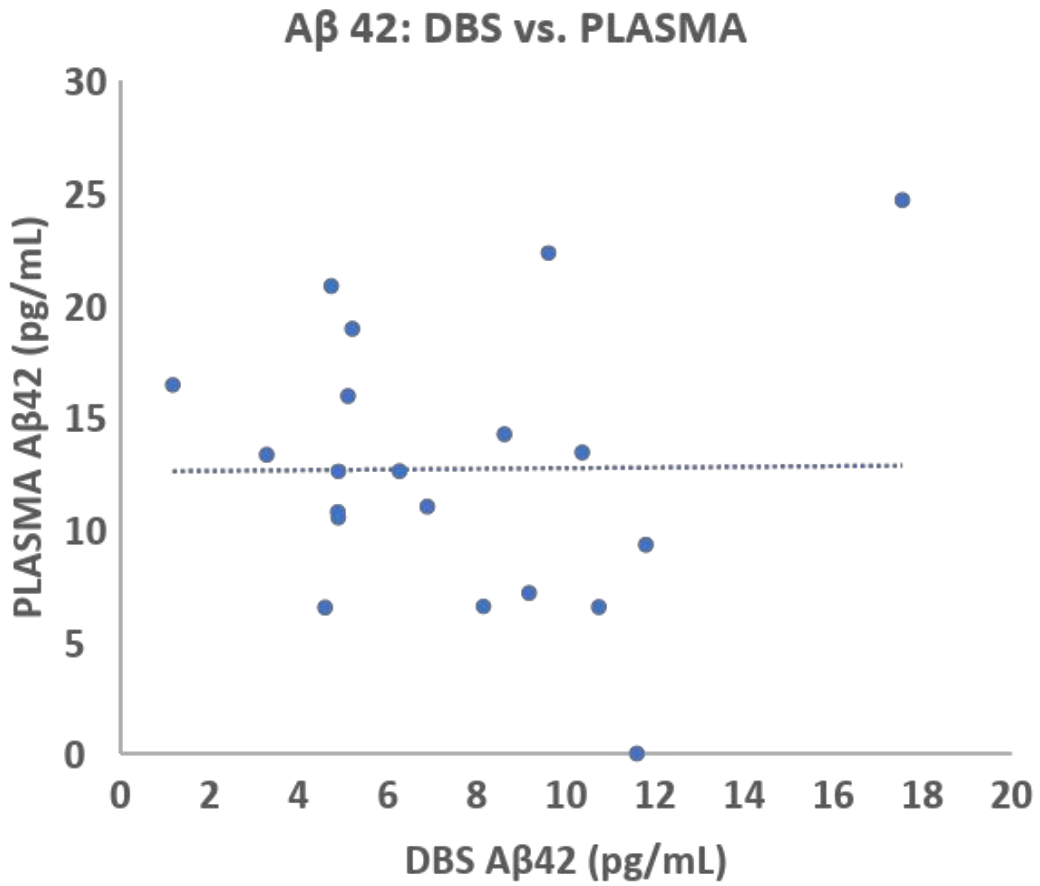
- DBS placed in a shaker (500 rpm) for 4 hours with 200 µl of sample diluent (sample diluent provided by Quanterix Inc.)
- 30 minutes of sonication
- Centrifugation for 10 minutes (9900 rcf)

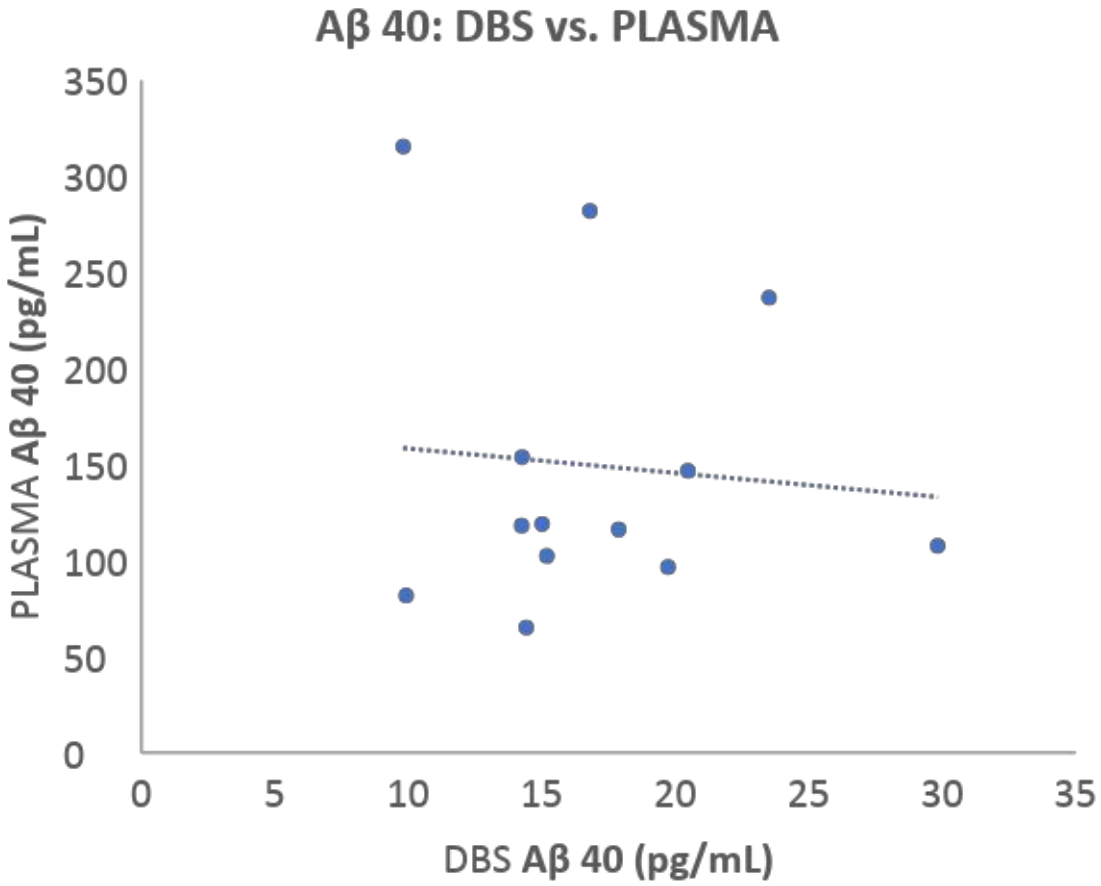
- Control sample: 25 ul of plasma + 200 µl of sample diluent
  - Control used to estimate % recovery of biomarkers with the processing method used in the laboratory

STUDY DESIGN: COMPARISON OF DBS AND PLASMA



RESULTS: DBS VS. PLASMA

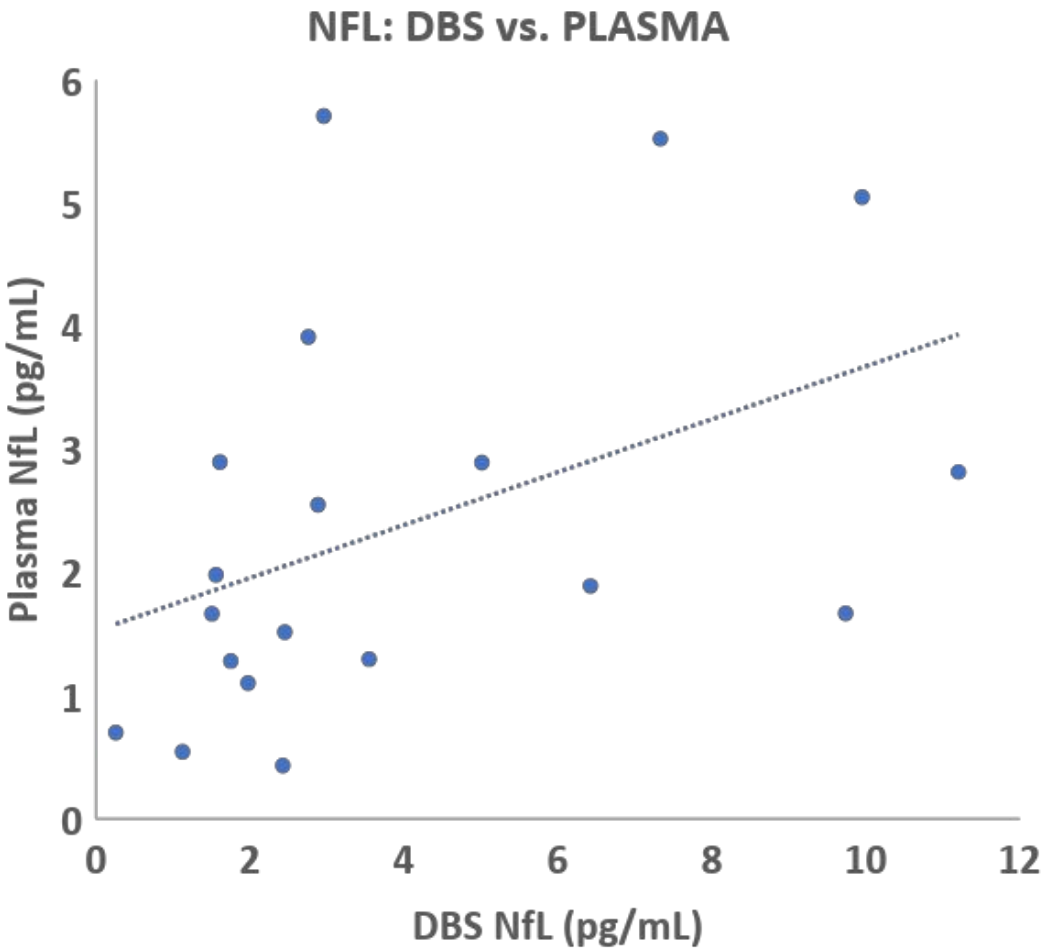




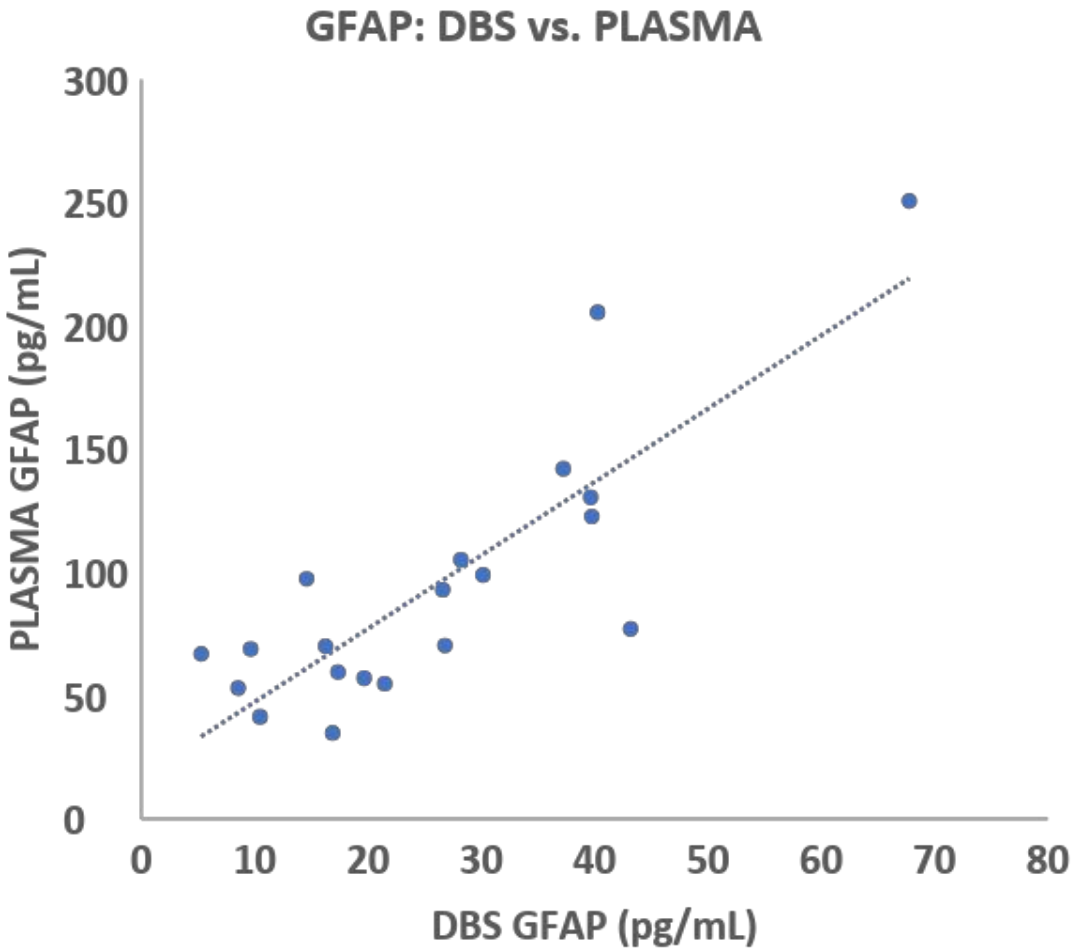
Average CV DBS: 38%
Average CV Plasma: 28%

Average CV DBS: 53%
Average CV Plasma: 8%

RESULTS: DBS VS. PLASMA



Average CV DBS: 23%
Average CV Plasma: 17%



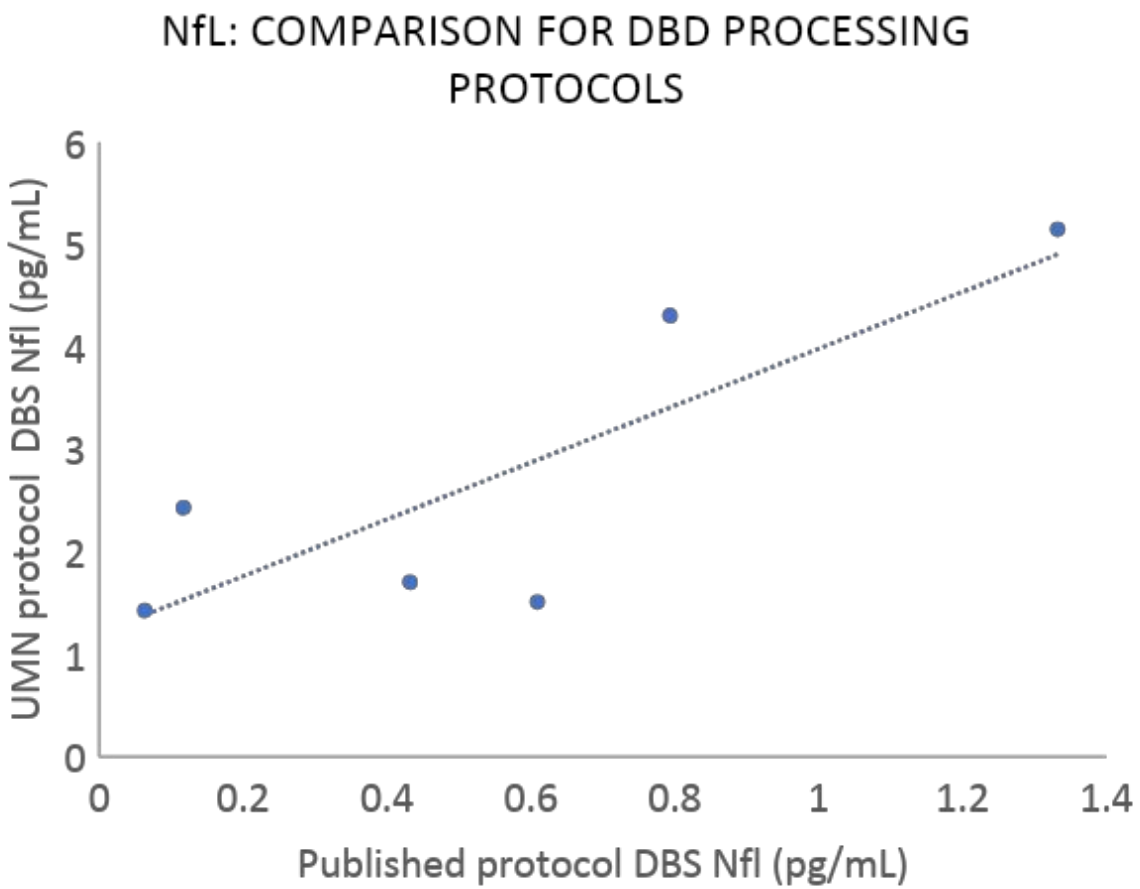
Average CV DBS: 33%
Average CV Plasma: 6%

COMPARISON OF BIOMARKER RECOVERY USING DIFFERENT DBS PROCESSING METHODS

- We compared NfL values (n=6) obtained by our lab protocol to published protocols where NfL was measured from DBS
  - 120 ul of PBS in a shaker (400 rpm) at 37^{0}C for 1 hour.
  - Centrifugation for 10 minutes (2000 rpm)

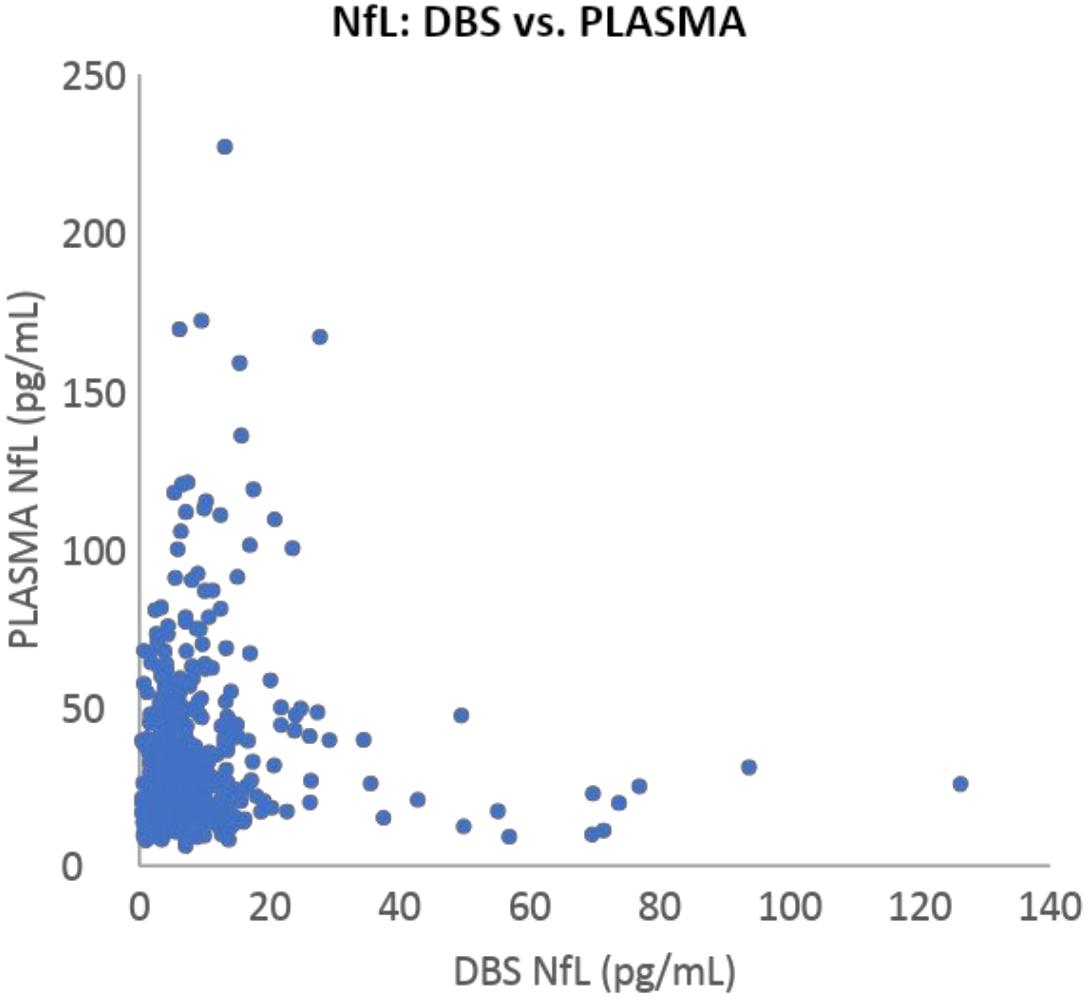
- % Recovery of NfL with DBS protocols
  - UMN: 140.37% (1.42 vs. 1.01 pg/ml)
  - Published method: 6.24% (0.06 vs. 1.01 pg/ml)

- % Recovery of GFAP with DBS protocols
  - UMN: 60.31% (4.54 vs. 7.03 pg/ml)
  - Published method: 40.64% (3.056 vs. 7.03 pg/ml)

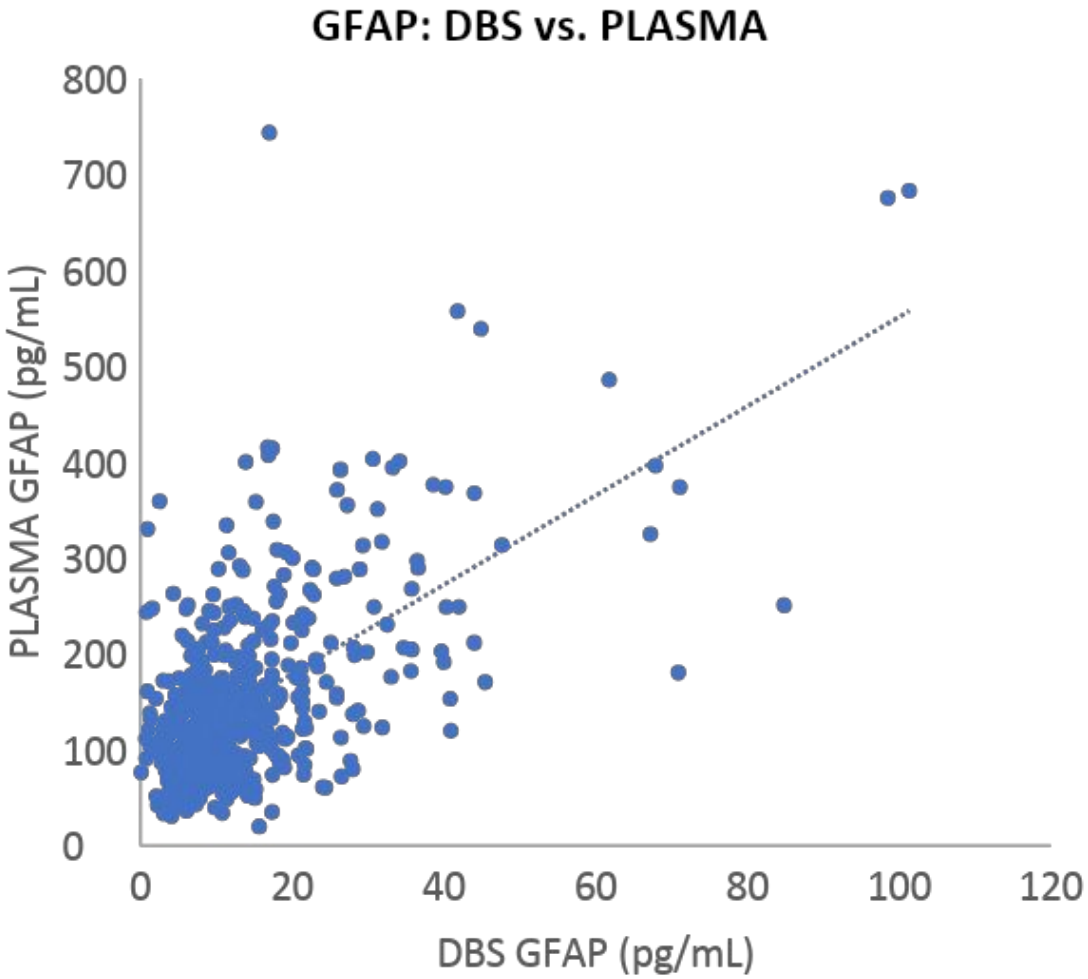


Alzheimer's Dement. *(2021)*;13:e12145.

HRS: DBS vs. PLASMA RESULTS

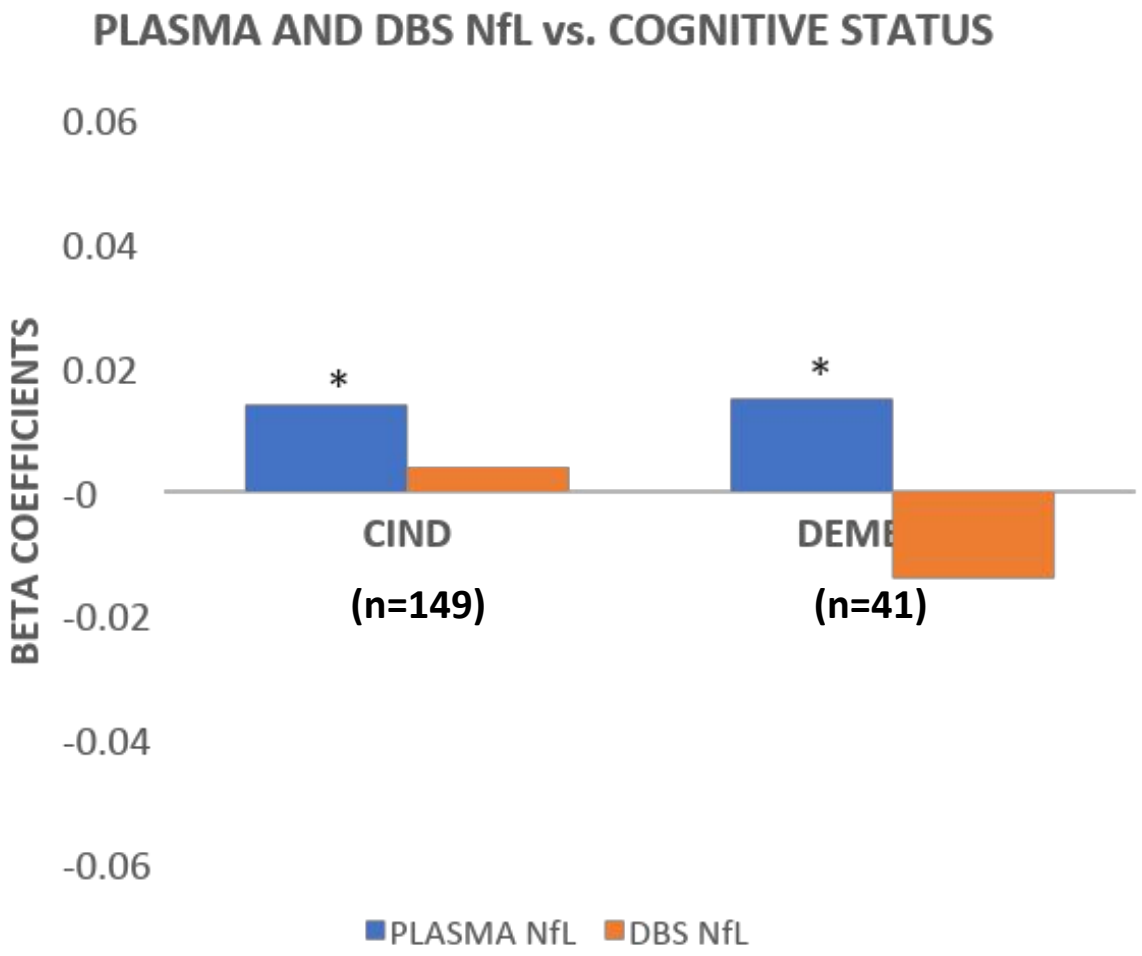


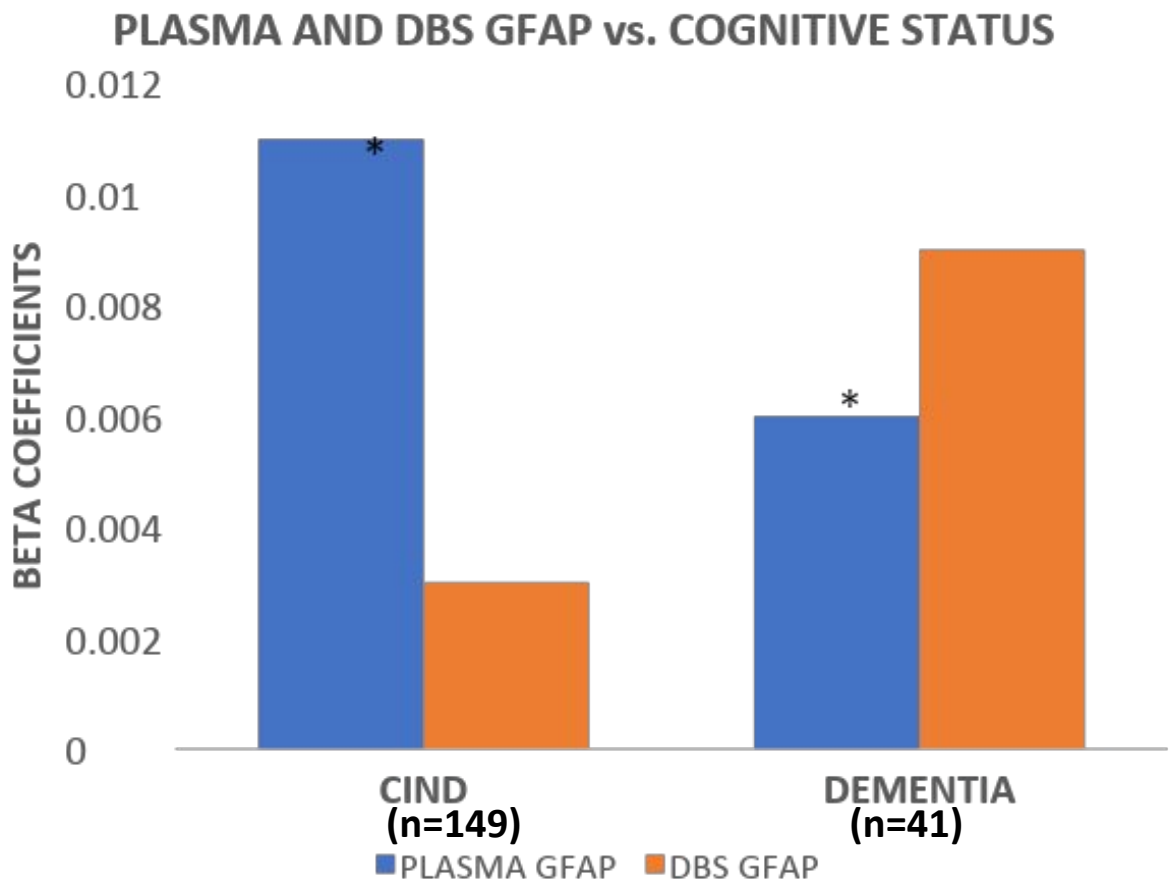
Average CV DBS: 8%



Average CV DBS: 14%

HRS: DBS BIOMARKERS VS. COGNITIVE STATUS





CONCLUSIONS AND FUTURE DIRECTIONS

- DBS values for all biomarkers are markedly lower than the corresponding plasma values
  - All values are well within the analytical range of the highly sensitive Simoa assays
  - Biomarker recovery from DBS is reasonable (>60%) for all biomarkers.

- DBS NfL did not correlate well with plasma NfL
  - This result is in contrast to published studies that demonstrate a reasonable correlation between DBS and plasma NfL (0.76-0.80)

- DBS GFAP shows reasonable correlation with plasma GFAP values
  - Consistent associations with cognitive status

- In HRS, DBS GFAP could be considered as an alternative to plasma GFAP when venous blood samples are not avaialble..

ACKNOWLEDGEMENTS

- Catherine Lilyerd Eileen Crimmins
- Alec Victorsen Jessica Faul
- Amha Melka
- Shweta Sharma

- FUNDING: Biomarker Network

RESULTS: PHOSPHORYLATED TAU-181