STABILITY OF BLOOD BASED BIOMARKERS USED IN DEMENTIA RESEARCH

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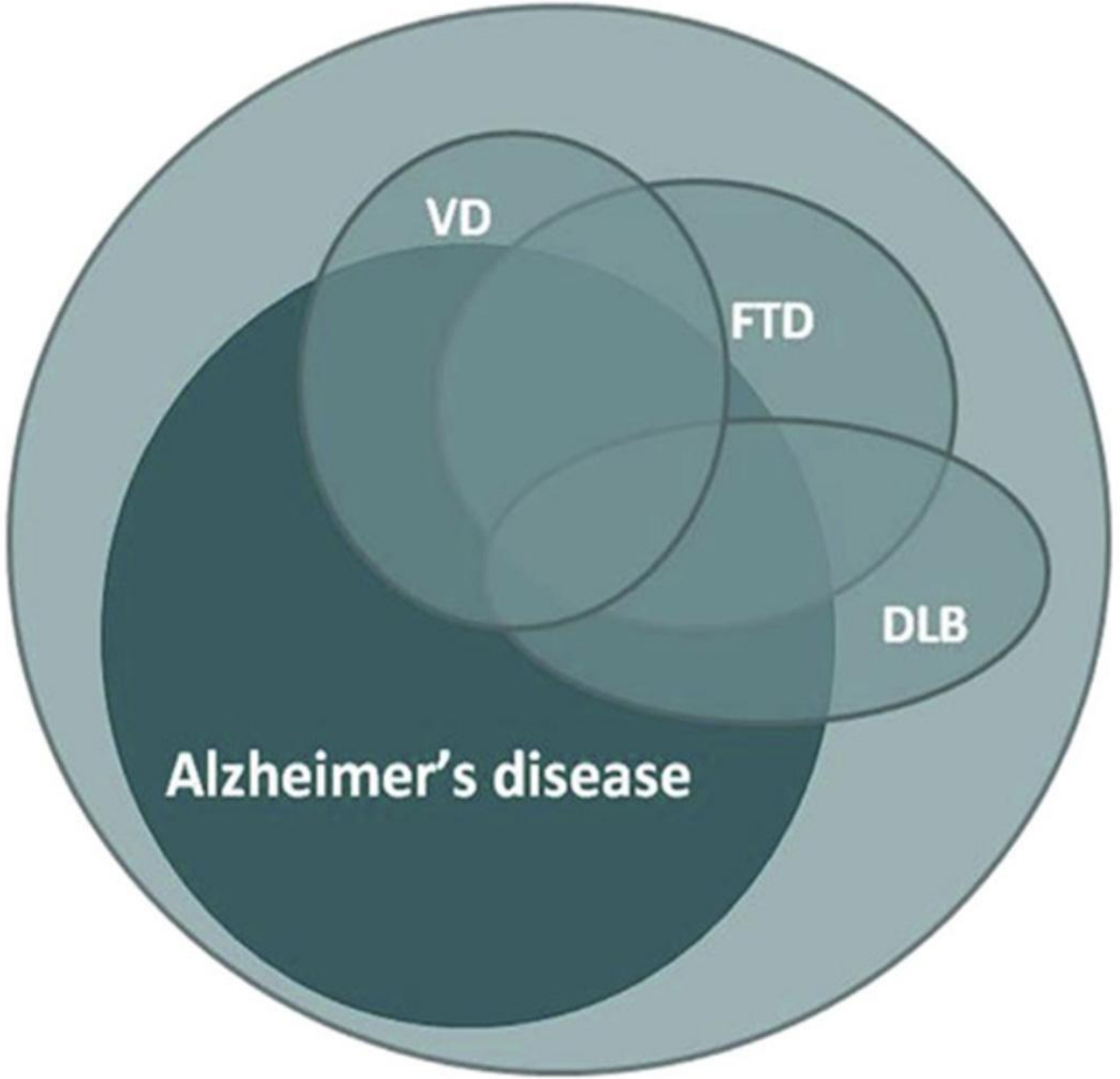
INTRODUCTION

- Among the various neurodegenerative conditions, Alzheimer's disease (AD) is the major cause of dementia worldwide
  - Very difficult to diagnose based on clinical presentation alone

- Since cerebrospinal fluid (CSF) is in direct contact with the central nervous system biochemical changes in the CSF have been useful as biomarkers for AD related dementias
  - Imaging studies and CSF based biomarkers have been very useful in AD diagnosis
  - However, difficulty in obtaining CSF and specialized equipment needed for imaging limit the applicability of these methods to large scale population-based studies



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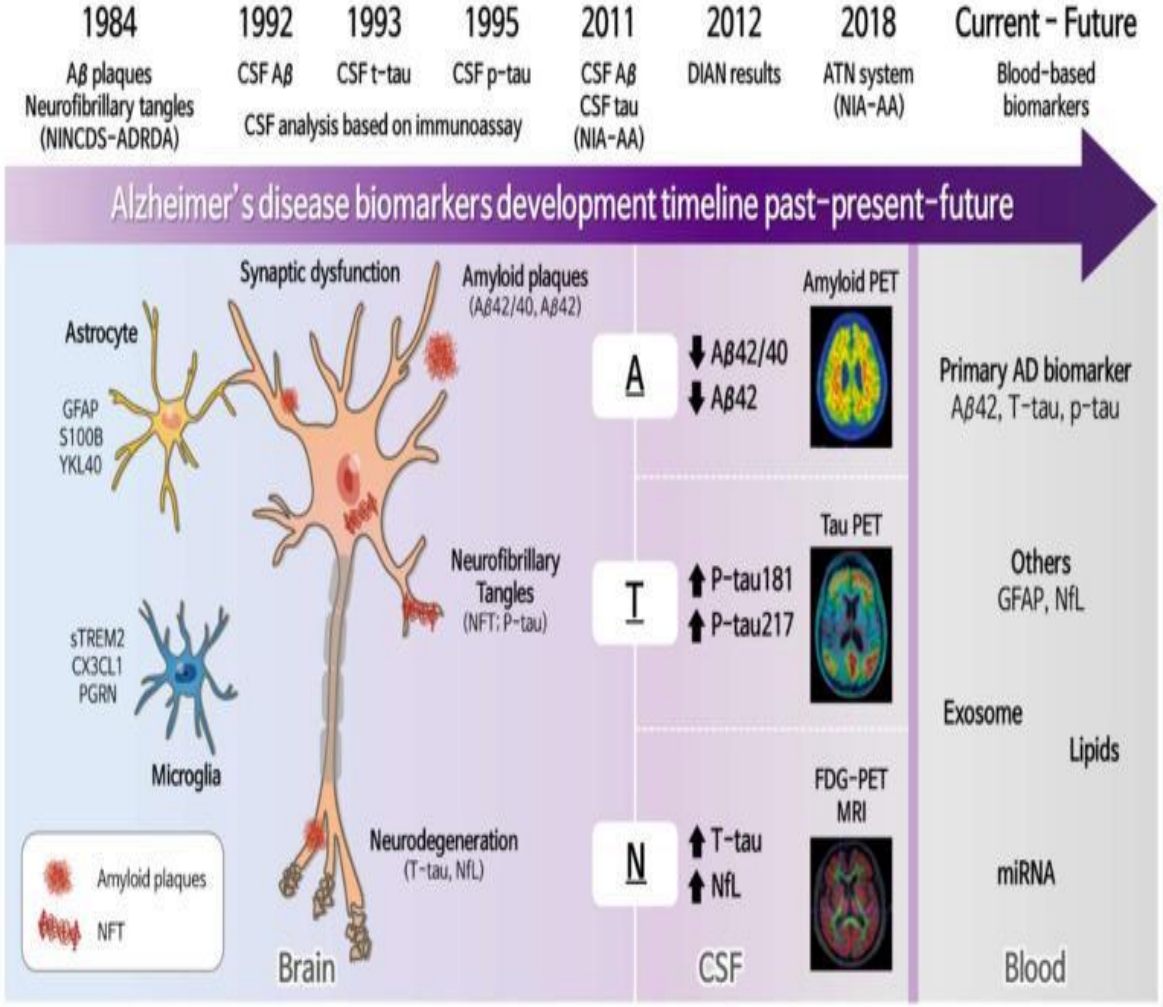
Alzheimers Dement. 2014 Jan; 10(1): 115–131.

BIOMARKERS RELATED TO SPECIFIC BIOLOGICAL PROCESSES

- Beta Amyloid deposition **(A)**
  - Aβ42/Aβ40 ratio
- Phosphorylated Tau Protein subunits **(T)**
  - P tau-181, p tau-217, p tau-231
- Markers of neurodegeneration **(N)**
  - Neurofilament Light Chain (NfL)
- Markers of neuroinflammation/astrocyte activation
  - Glial Fibrillary Acidic Protein (GFAP)



<u>Biomedicines.</u> 2022 Apr; 10(4): 850.

BIOMARKERS IN POPULATION BASED STUDIES: HEALTH AND RETIREMENT STUDY

- *What was missing from the existing cognitive biomarker literature is replication in representative population-based samples of older adults, including individuals from racial / ethnic minorities.*
  - HRS is conducting a pilot to test promising A/T/N biomarkers.

- Priorities:

  (1) highly reliable and replicable in blood (plasma/serum);

  (2) have validated correlations with AD/ADRD neuropathology from cerebrospinal fluid (CSF) or autopsy measures;

  (3) are found in higher concentrations in people with cognitive impairment and AD/ADRD;

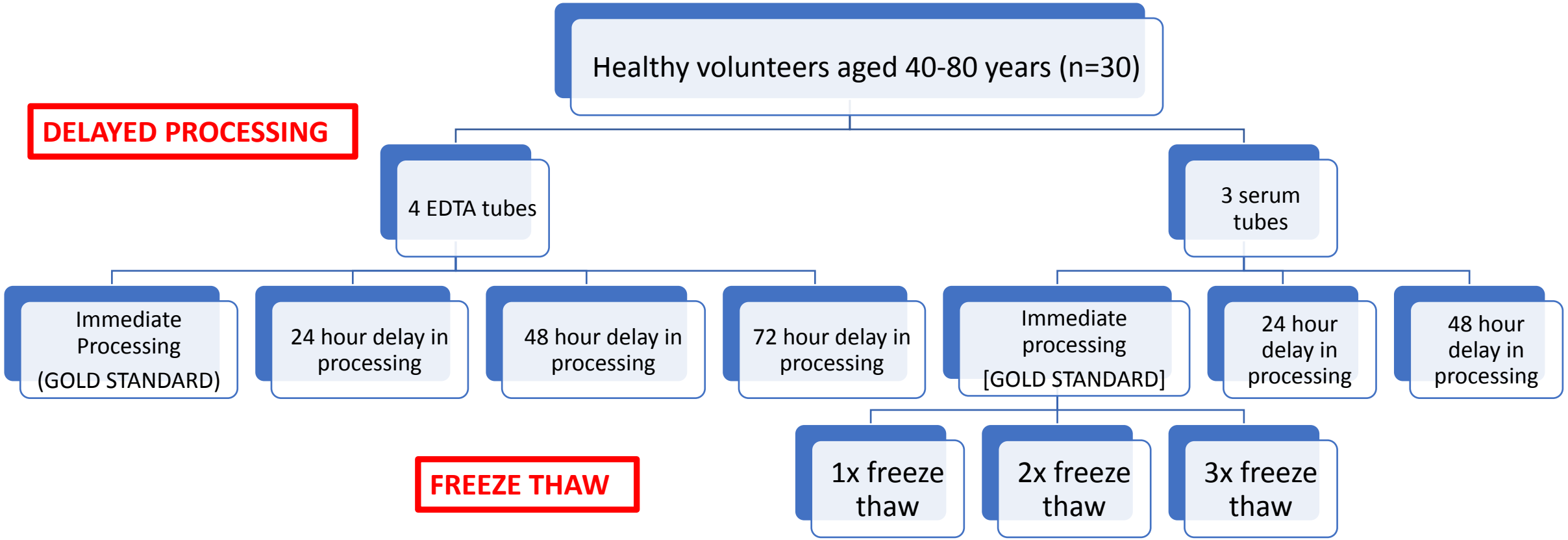
- Final list based on consultation with dementia experts at the NIA Intramural Research Program

CHALLENGES FOR IMPLEMENTING BLOOD BASED BIOMARKERS IN POPULATION STUDIES

- Pre-analytical variation
  - Delayed time interval between biospecimen collection, processing and storage
  - Freeze thaw effects

- Harmonization of biomarker measurements across different studies

STUDY DESIGN TO EVALUATE ANALYTE STABILITY



BIOLOGIC VARIATION

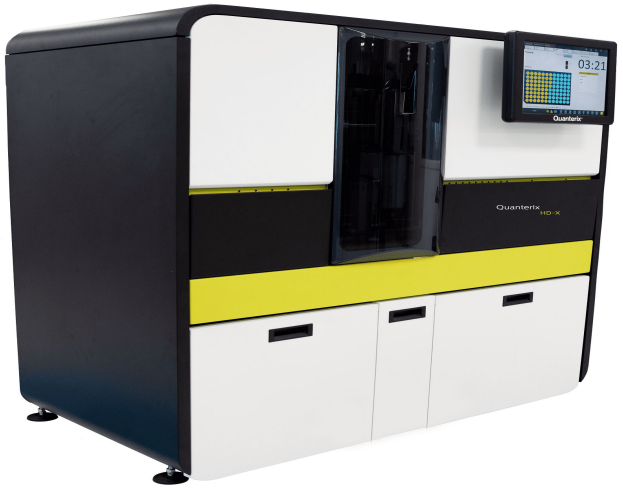
In addition, we also collected blood from the same people after 2 weeks to estimate biological variability in biomarkers of neurodegeneration.

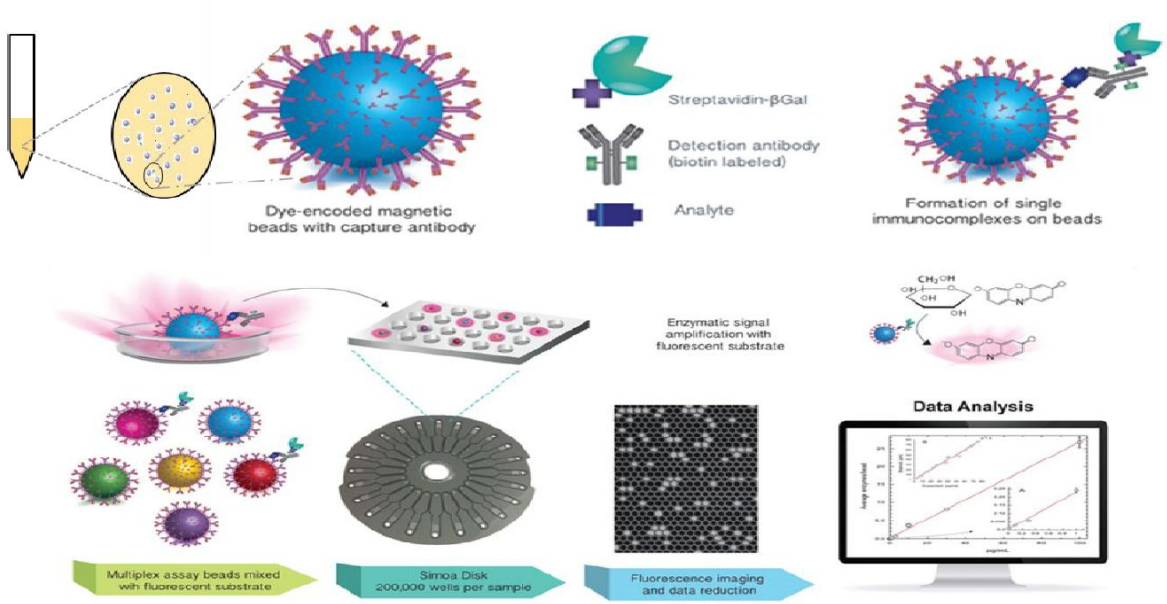
LABORATORY METHODS

- Aβ 42, 40, neurofilament light (NfL) and Giliary Fibrillary Acidic Protein (GFAP) measured using the Quanterix assay on a HDX instrument (N4PE assay)

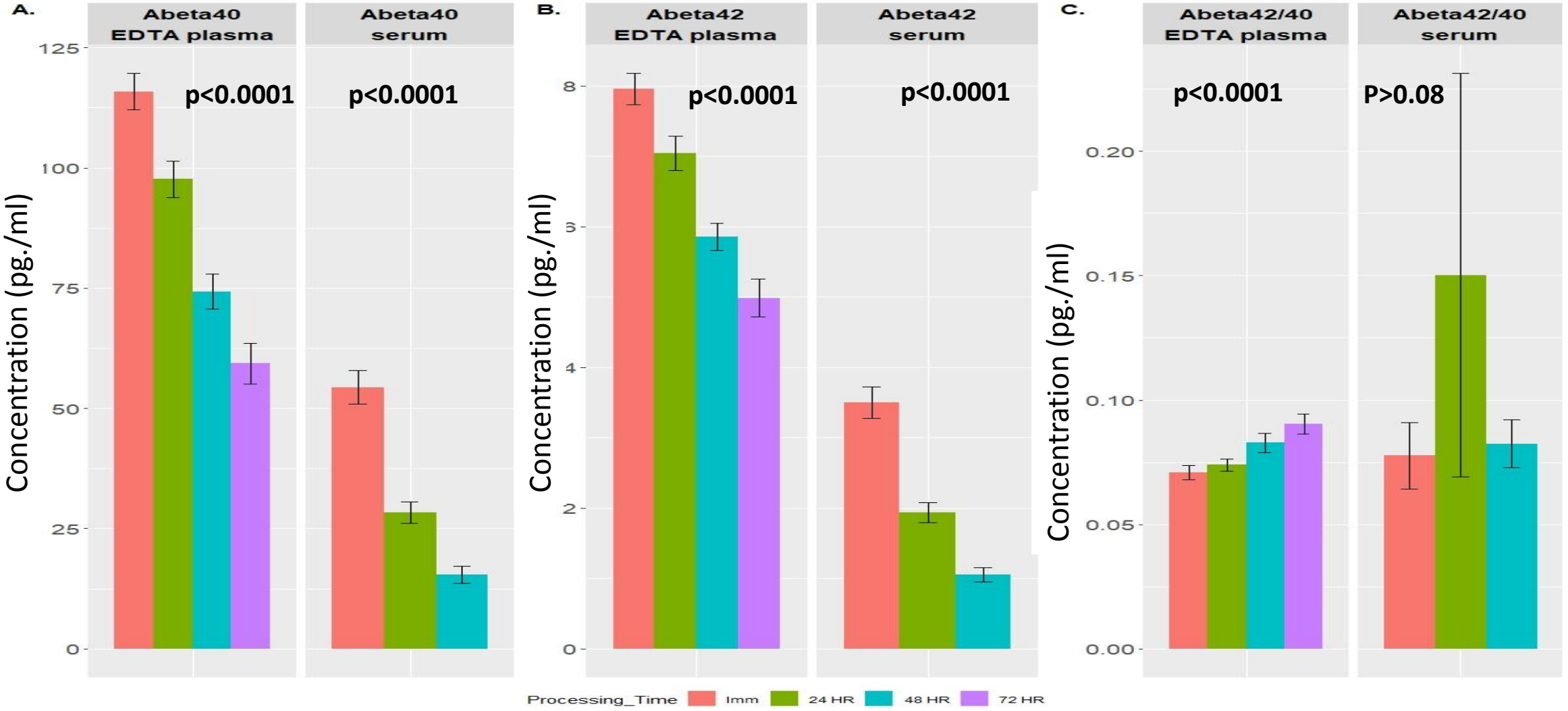
- P-tau-181 measured using the Quanterix assay (Simoa Inc.) on a HDX instrument (ptau 181 V2)

- Approximately 1000x more sensitive than traditional ELISA assays





RESULTS: DELAYED PROCESSING



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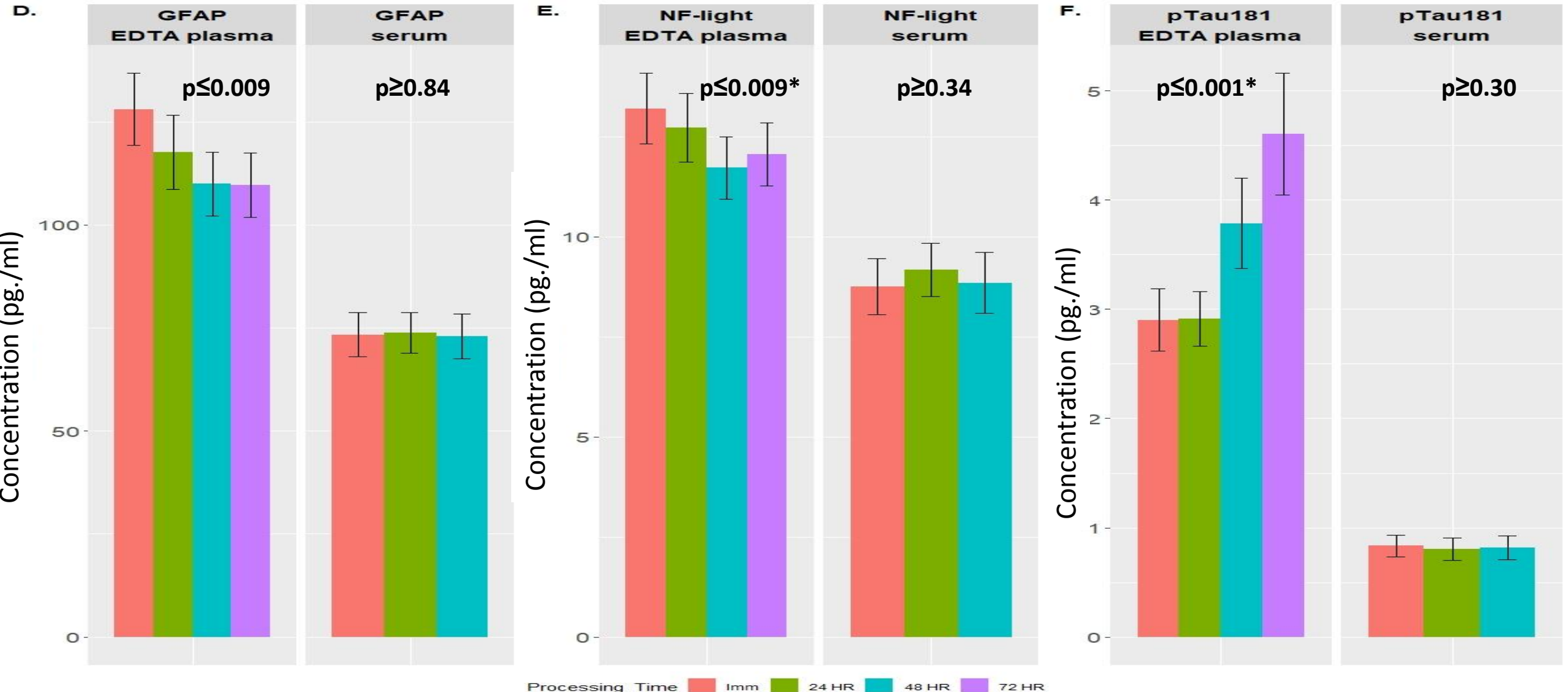
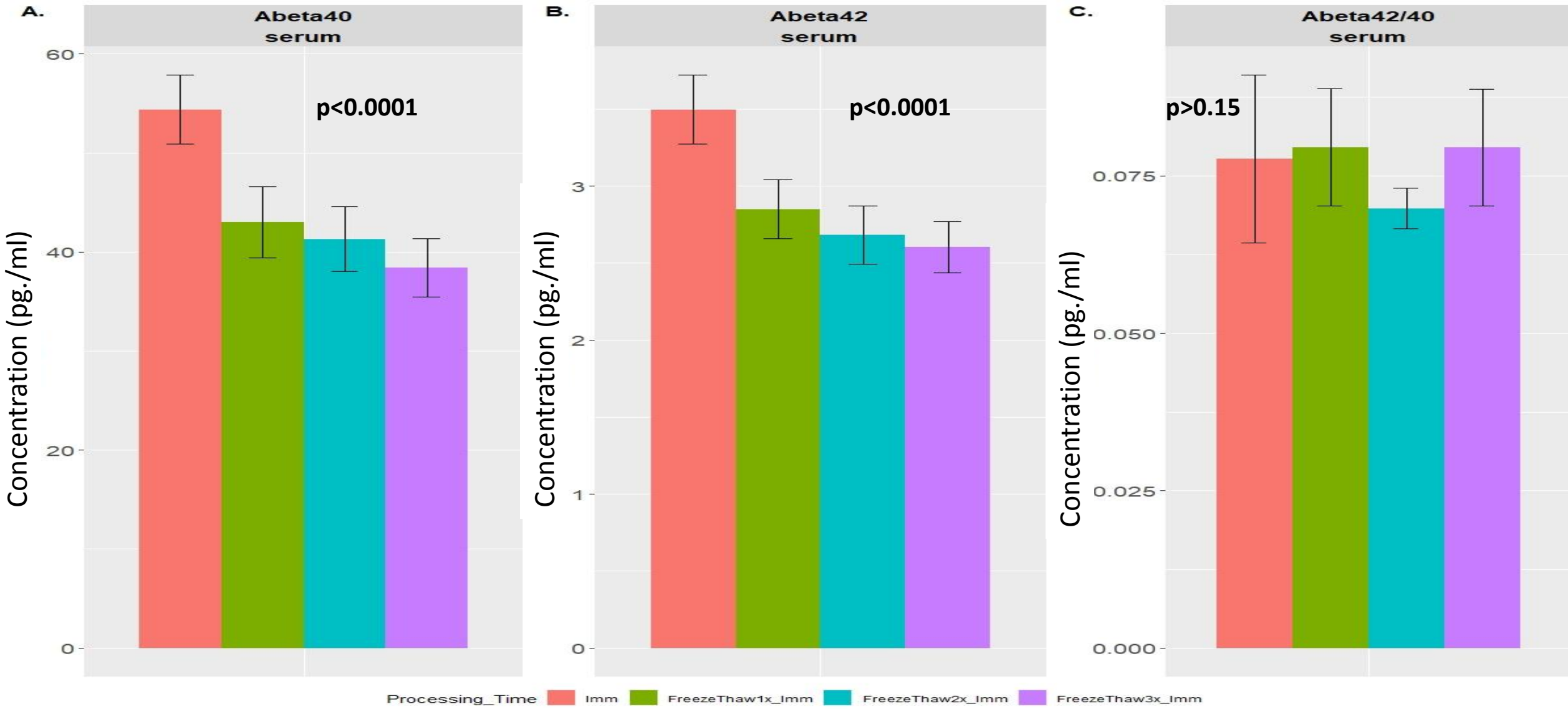


RESULTS: FREEZE THAW CYCLES



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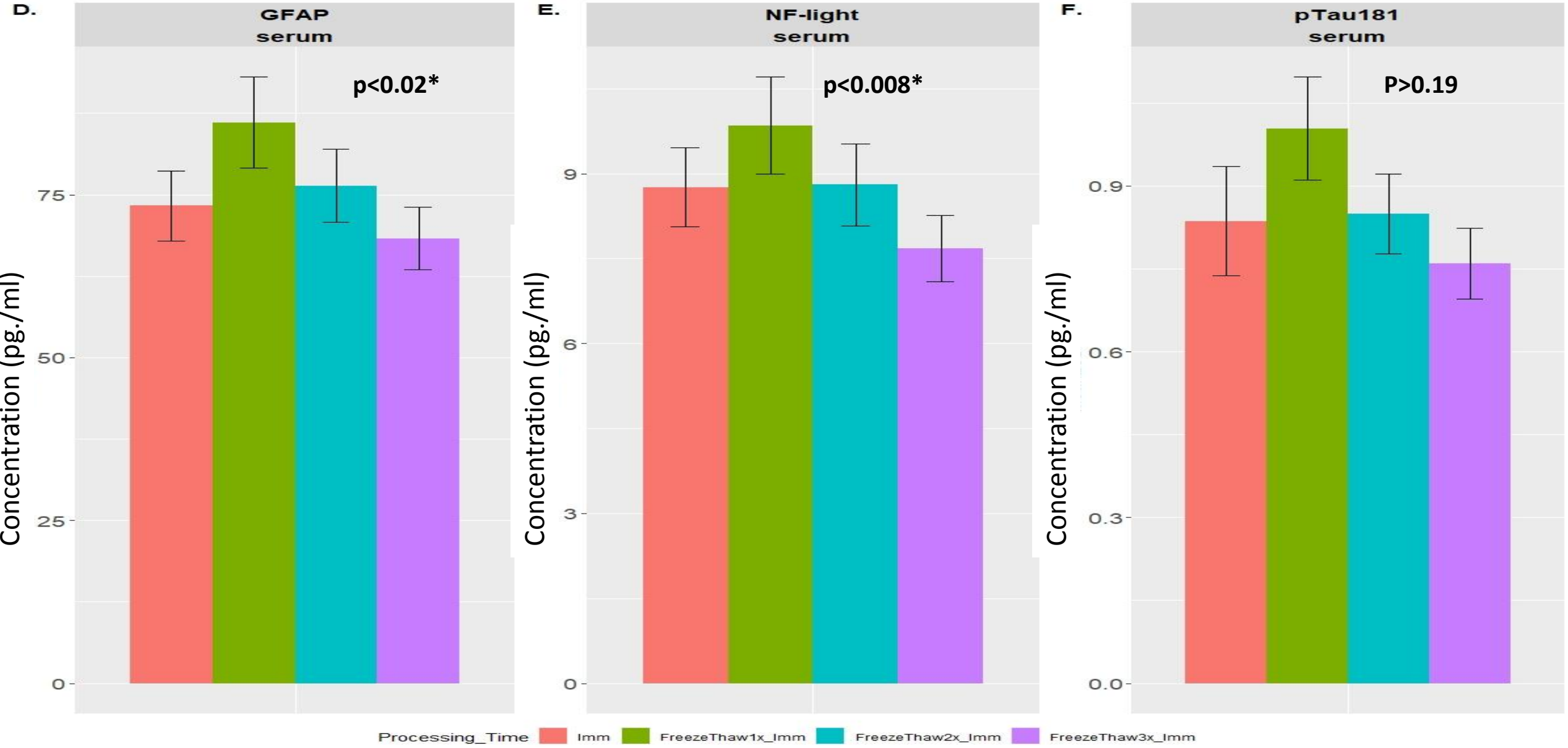
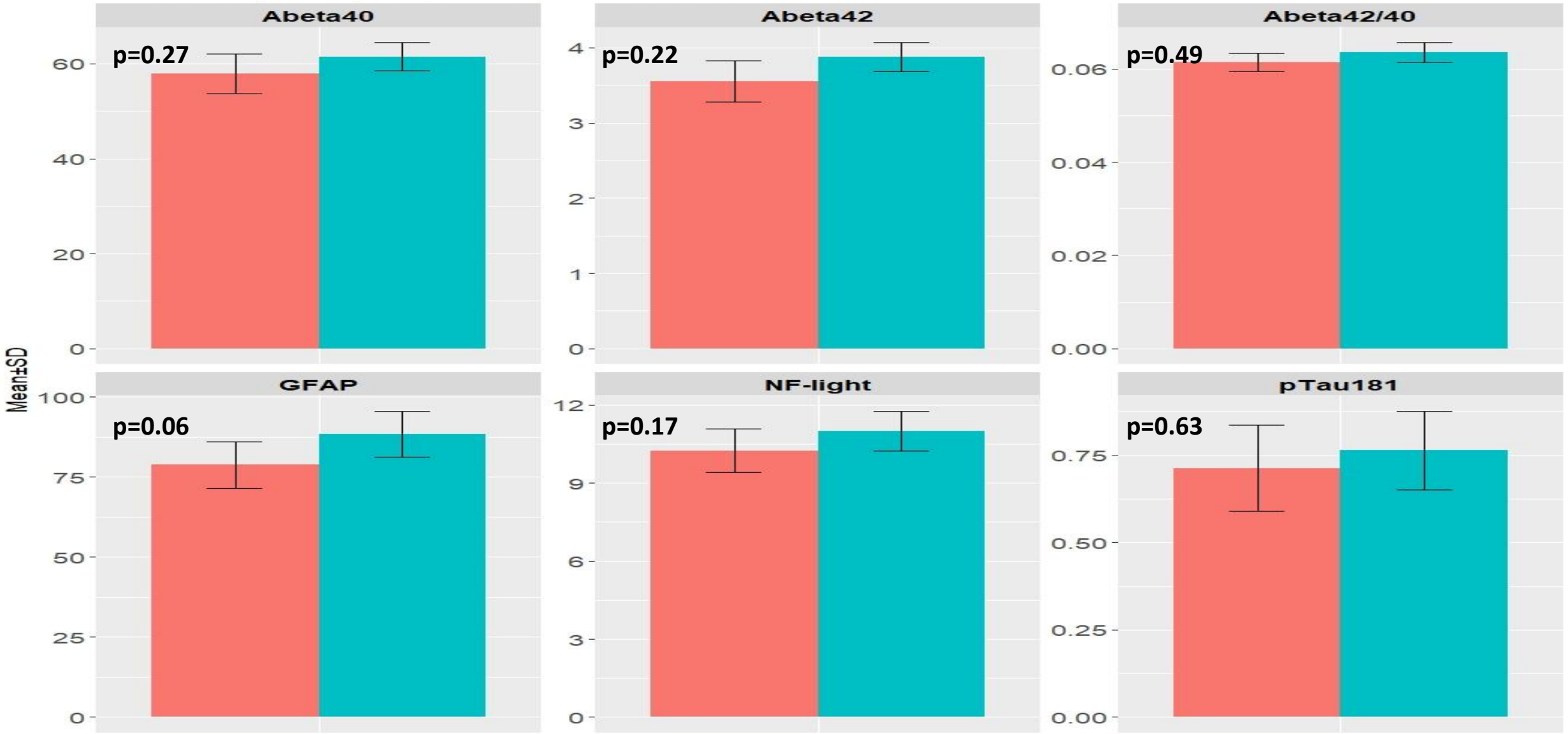


RESULTS: BIOLOGICAL VARIABILITY



SUMMARY: PRE-ANALYTICAL VARIATION

- All analytes had substantially lower levels in serum vs. plasma
  - Plasma is the preferred substrate for measurement of all A/T/N markers
- Delayed processing decreases levels of Aβ40, Aβ42, NfL and GFAP
  - More prominent in plasma as compared to serum
  - Change in plasma is minimal for NfL and GFAP (9%-14%)
  - Aβ42/40 is relatively more stable to delayed processing than the individual analytes
- Delayed processing increases levels of ptau181
  - ptau181 stable in serum
- Increasing number of freeze thaw cycles reduces analyte concentration by 9% to 24%
  - Minimize number of freeze thaw cycles when measuring neurodegenerative biomarkers

CONCLUSIONS

- Blood based biomarkers for neurodegenerative diseases show great promise
  - Increasingly used in population studies to evaluate utility as prognostic markers for dementia

- Attention to pre-analytical sources of variation will improve biomarker comparison across studies and improve generalizability of observed associations

- Longitudinal change in biomarkers may be useful in early diagnosis of dementia

COMMON LABORATORY METHODS FOR BIOMARKER ANALYSIS

OLDER METHODS

- ELISA
- Luminex
  - Limited analytical sensitivity
  - Poor antibody specificity

NEWER METHODS

- Simoa assays: Quanterix platform
- Mesoscale Discovery
- ELISAs
  - High analytical sensitivity: Higher sample dilution allows dilution of matrix effects
  - High antibody specificity