

NACC-ADRC Biomarker Best Practices Guidelines

2025 Update

Preanalytical Standards for Blood and CSF Biomarkers

National Alzheimer's Coordinating Center

Timothy Van Meter, PhD, Thomas Karikari, Co-Leads

NACC-ADRC Workgroup on Biofluid Biomarker Best Practices

Overview: Why These Guidelines Matter

Purpose

Standardize preanalytical handling of biofluid biomarkers across 37 ADRCs to ensure data quality and comparability in multicenter research

- ✓ Updated from 2014 NIA guidelines
- ✓ Focus on CSF and blood biomarkers
- ✓ Evidence-based recommendations

Clinical adoption of biomarkers as tools to improve patient care requires consistent evidence.

NACC-ADRC Network: Scale of the Resource

55,000+

Research Participants

21,395

Alzheimer's Disease

37

US-Based ADRCs

16

CSF Biomarkers Studied

28

Plasma/Serum Biomarkers

(Data taken from September 1, 2025 update of UDS data base, NACC)

Overview: Why These Guidelines Matter

UDS MCI and Dementia resources

Primary etiologic diagnosis for cognitively impaired subjects (MCI & Dementia)_n

Cognitively normal	20356
Cognitively Impaired non-MCI	2328
Alzheimer's disease	21395
Lewy body disease including Parkinson's disease	2085
Vascular brain injury ² or stroke	1425
FTLD - with bvFTD	1489
FTLD - with PPA	875
FTLD - Other	811
Traumatic brain injury	148
Other/unknown ¹	4092
Total	32320

1. Medical illness, psychiatric illness, depression, et al.
Includes probable and possible Vascular dementia diagnoses

NACC-ADRC Network: Scale of the Resource

55,000+

Research Participants

21,395

Alzheimer's Disease

37

US-Based ADRCs

16

CSF Biomarkers Studied

28

Plasma/Serum Biomarkers

Biofluid Biomarker data

MRI

PET Scans

Genotypes (APOE)

DNA

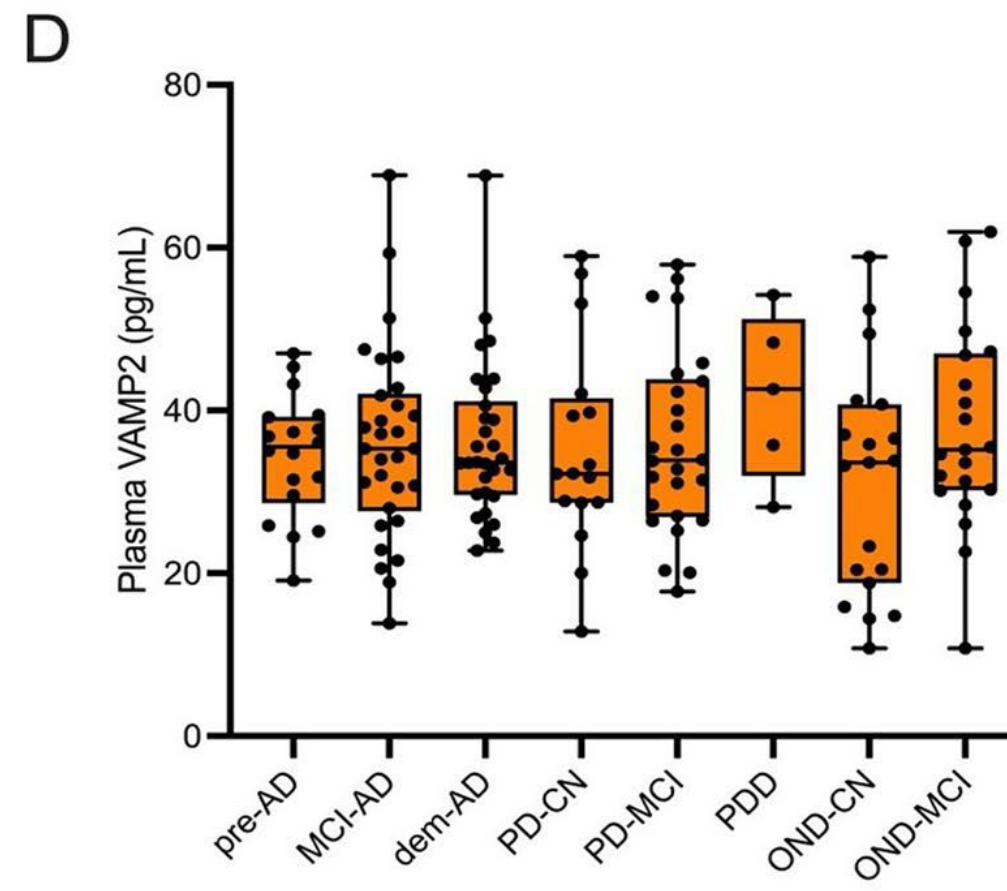
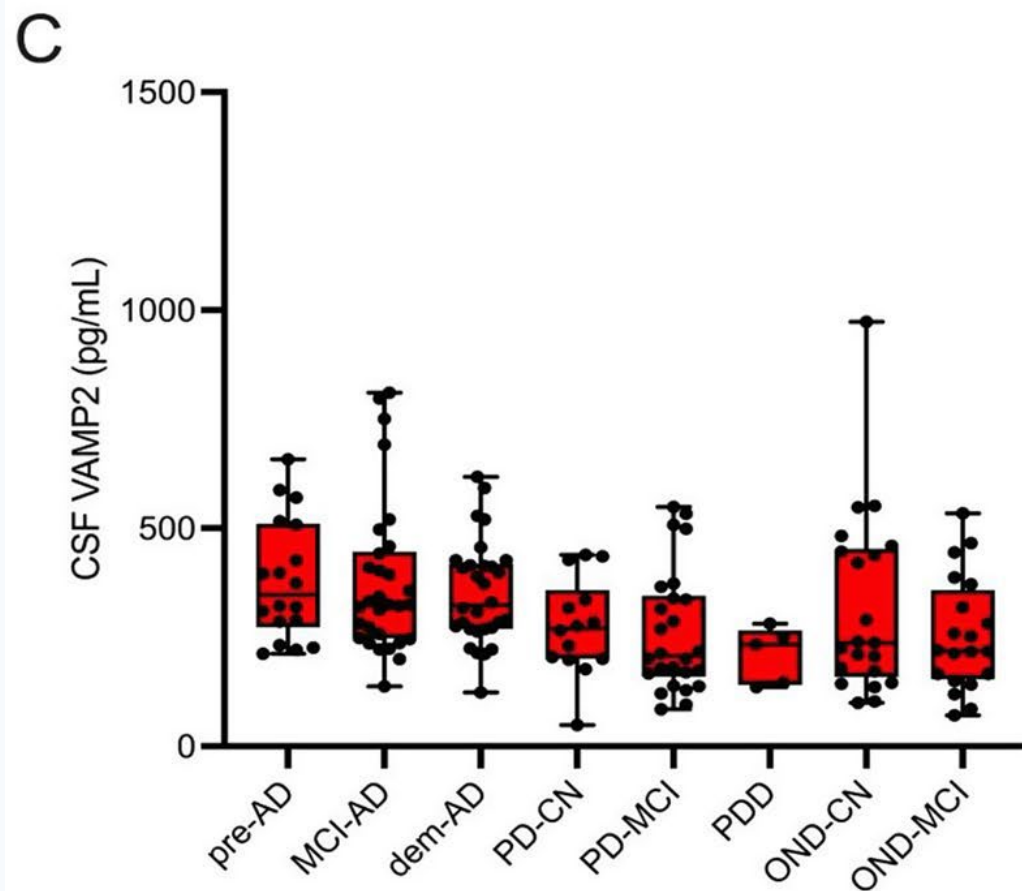
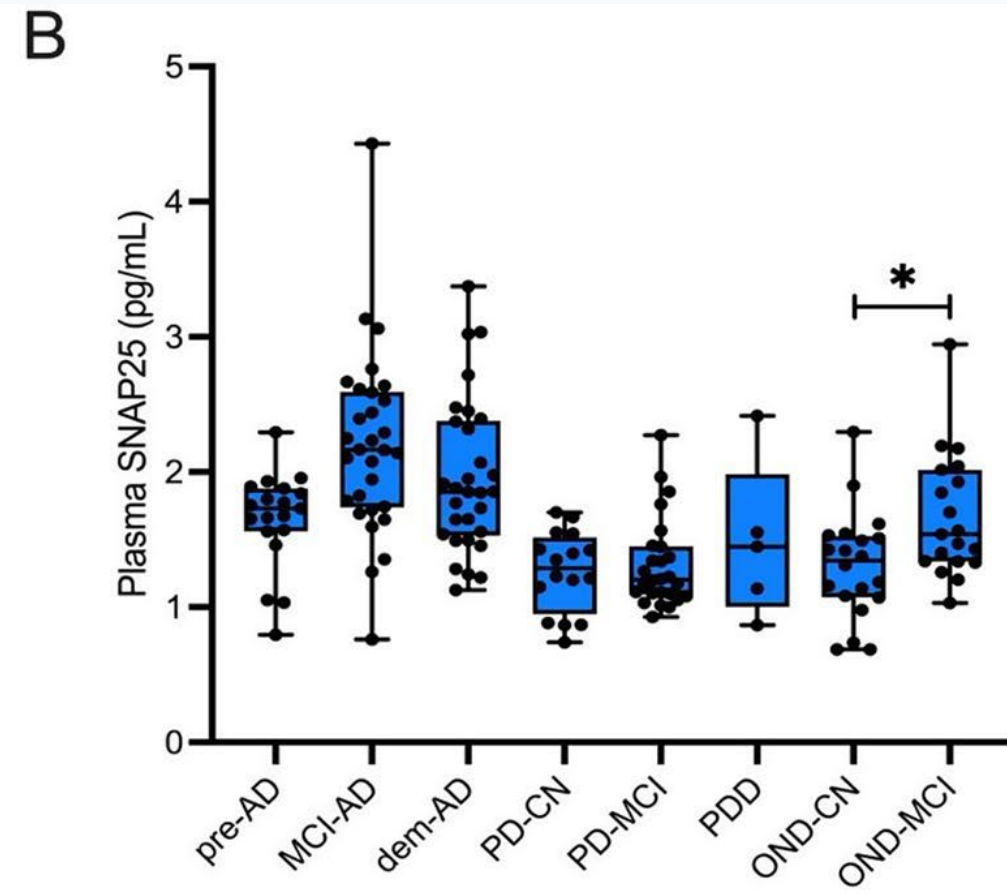
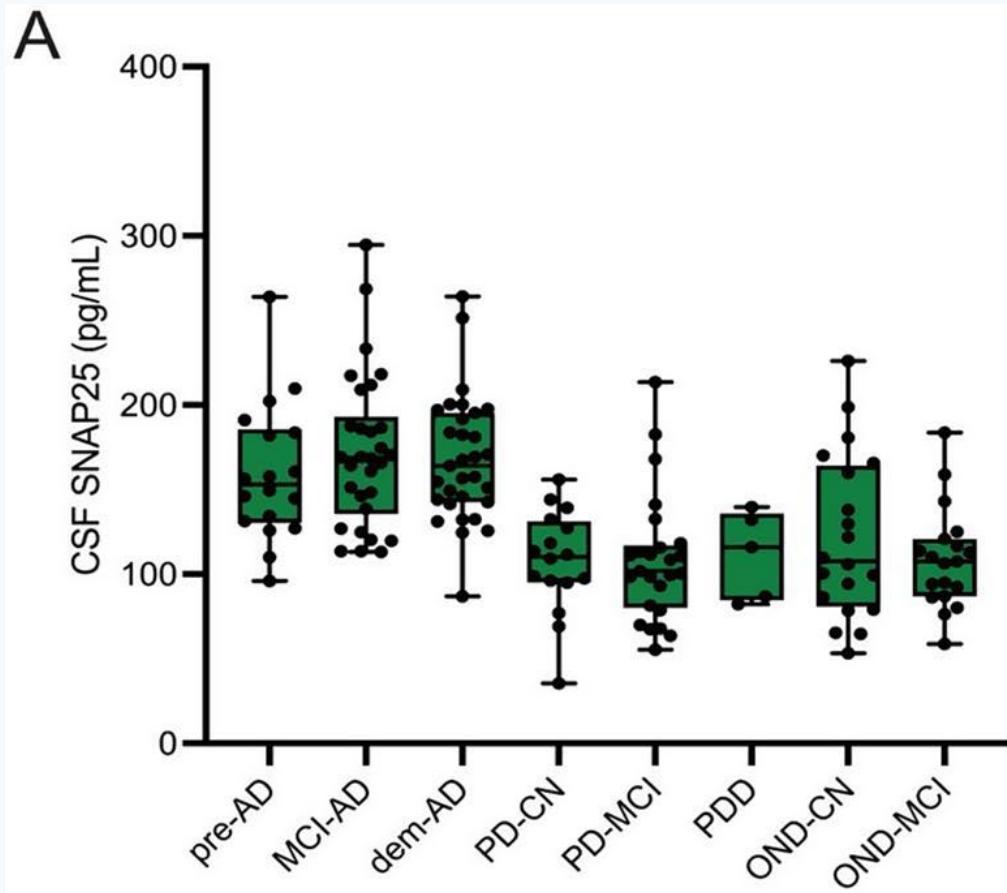
Neuropsychological test scores

Demographics

Comorbid conditions

Copathologies

(Data taken from September 1, 2025 update of UDS database, NACC)



Advancing Clinical Research

- Data needed on large well-characterized cohorts
- Multiple comparable data points
- Up to date clinical, pathology and imaging data collected.
- Sources of preanalytical variance controlled and minimized.
- Case in point:

Differential diagnosis

Which thresholds to use?

***32% of AD cases had synucleinopathy**

Guideline Development Methodology

ADRC Biomarker Steering committee convened 2 closely related workgroups

- 1. Biofluid Biomarker Best Practices Workgroup**
- 2. Data variables and Data Quality Workgroup**

Biofluid Biomarkers Best Practices Workgroup Activities 2023-2025

1. Surveyed all 37 ADRC centers for current biomarker practices and platforms
2. Comprehensive literature review of preanalytical factors
3. Expert consultation with key opinion leaders
4. Integration with SABB and GBSC standardization efforts (Global harmonization)



Biomarker Inclusion Criteria

Multi-Center Usage

Biomarker studied by at least 2 ADRCs

Validation Evidence

Large cohort studies (>200 subjects) with statistical validation demonstrating alteration in AD or related conditions

Guideline Development Methodology

Workgroup Survey questions: What information was asked for?

- Q1. Frequency of use for individual CSF or plasma biomarkers (which biomarkers)
- Q2. Tracking of sample use (barcoding and type, other)
- Q3. Labeling practices (standard info needed)
- Q4. Freeze thaws
- Q5. Are Centers in the position to reduce the # of freeze thaws?
- Q6. How much effort is underway to create redundancies in biorepositories?
- Q7. Are power outage-related freeze-thaws being tracked?
- Q8. Long term storage temp
- Q9. Backup power
- Q10. Inventory software types
- Q11. Storage tube types
- Q12. Aliquot volumes
- Q13. Remote freezer monitoring 24/7 with staff notifications of deviations
- Q14. Centrifuge time/ temperature

Results of 2024 ADRC Survey: Biomarker Assay Utilization

**37 ADRC centers surveyed by questionnaire via NACC
Presented for feedback at ADRC and AAIC meetings**

Criteria discussed by the Workgroup:

- 1. Weight of evidence: Large cohort studies assessing the target, multiple platforms**
- 2. Assay QC well qualified and transparent**
- 3. Clinical ranges are understood (what should the results look like?)**
- 4. Used by multiple ADRCs, expected to have increasing utilization in ADRC-supported studies in the near future.**

Final Guideline Criteria:

- Utilized by at least 2 ADRCs
- Validated in large cohort studies.

CSF: > 16 biomarkers in widespread use (e.g., Abeta1-42/40, NRGN, sTREM2)

Blood (Plasma or Serum): > 40 proteins or specific modifications (pS217-tau)

Blood Biomarkers Currently Studied at ADRCs
b-amyloid (Ab38, Ab40, Ab42)
Brain Derived Neurotrophic Factor (BDNF)
Cytokine and chemokine panels
Glial Fibrillary Acidic Protein (GFAP)
Intercellular Adhesion Molecule 1 (ICAM-1, CD54)
Neurofilament Light Chain (NEFL, NfL)
Neurogranin (NRGN)
Neuron Specific Enolase (NSE, ENO2)
Synaptosome Associated Protein-25 (SNAP-25)
Synuclein alpha (SNCA)
Synuclein Alpha (SNCA, p-Ser129-SNCA)
Synuclein Beta (SNCB)
TAR DNA Binding Protein 43 (TDP-43)
Tau, Brain Derived Isoform or total (BD-Tau)
Tau, Microtubule Binding Region fragment (MTBR)
Tau, Phosphorylated at T181, S202/205, S217, T231 (pTau181, pTau217, etc.)
TREM1, soluble
Vascular Cell Adhesion Molecule (VCAM-1)
Vascular Endothelial Growth Factor (VEGF)
VILIP-1
YKL40 (Chitinase like 3)

ADRC Survey Results- Completed 2024

***Note:** Not a focus in this update:
Other biofluids, such as urine and saliva
RNA, DNA, extracellular vesicles

Internal Assays (Performed “In house”)

Biomarker	Frequency	% Centers
Ab40	15	48.40%
Ab42	15	48.40%
GFAP	13	41.90%
NfL	13	41.90%
NRGN	13	41.90%
pT181-Tau	13	41.90%
pS217-Tau	14	45.20%
pT205-Tau	1	3.20%
pT231-Tau	10	32.30%
TotalTau	6	19.40%
BD-Tau	1	3.20%
MTBR-Tau	1	3.20%
sTREM2	1	3.20%
Secernin	1	3.20%
UCHL1	1	3.20%
Vilip1	1	3.20%

External Assays (Outsourced)

Biomarker	Frequency	% Centers
Ab40	10	32.30%
Ab42	10	32.30%
GFAP	6	19.20%
NfL	7	22.40%
NRGN	1	3.20%
pT181-Tau	4	12.80%
pS217-Tau	6	19.20%
pT205-Tau	1	3.20%
pT231-Tau	6	19.20%
TotalTau	6	19.20%
BD-Tau	1	3.20%
MTBR-Tau	1	3.20%
sTREM2	3	9.60%
Secernin	1	3.20%
UCHL1	1	3.20%
Vilip1	2	6.40%
SNAP25	2	6.40%
SNCA	1	3.20%
pSer129-SNCA	1	3.20%
SNCB	1	3.20%
YKL40	2	6.40%
pS202-Tau	1	3.20%
pS212-Tau	1	3.20%
pNfH	1	3.20%
NfH	1	3.20%
APOE	1	3.20%
Cytokines	1	3.20%
SAA	1	3.20%
NPTX2	1	3.20%

Studies needed to support standardization

Highlights:

- **ATN Biomarkers most Common**
- **Expansion of novel Tau epitopes**
- **Synaptic biomarkers of high interest**
- **Inflammation biomarkers**
- **Few vascular biomarkers represented**

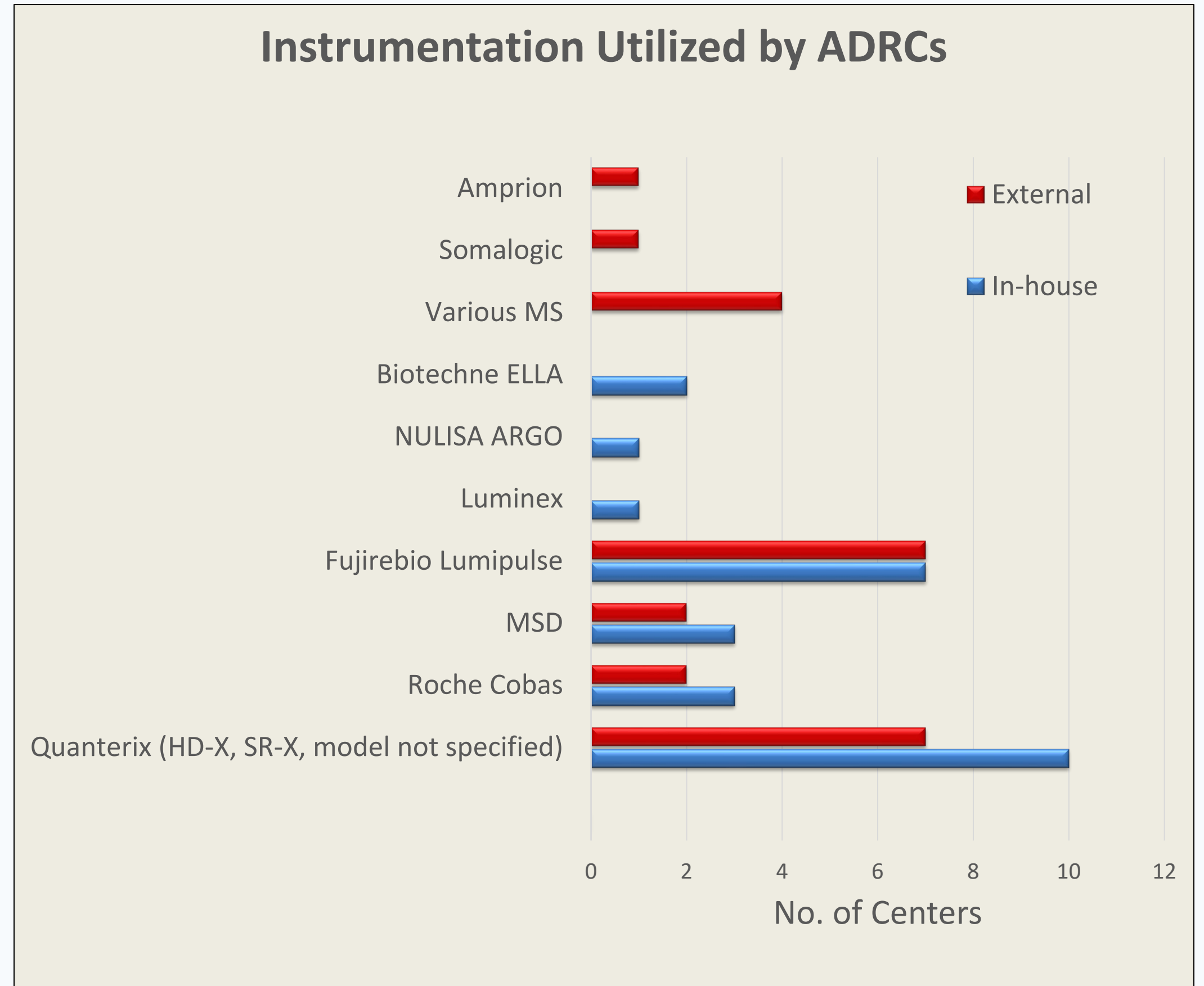
Results of 2024 ADRC Survey Biomarker Assay Utilization

- Survey responses received

- 31 of 37 ADRCs responded
- 14 centers submitted SOPs overall
- 14 centers submitted blood/plasma SOPs
- +8 centers submitted CSF-specific SOPs

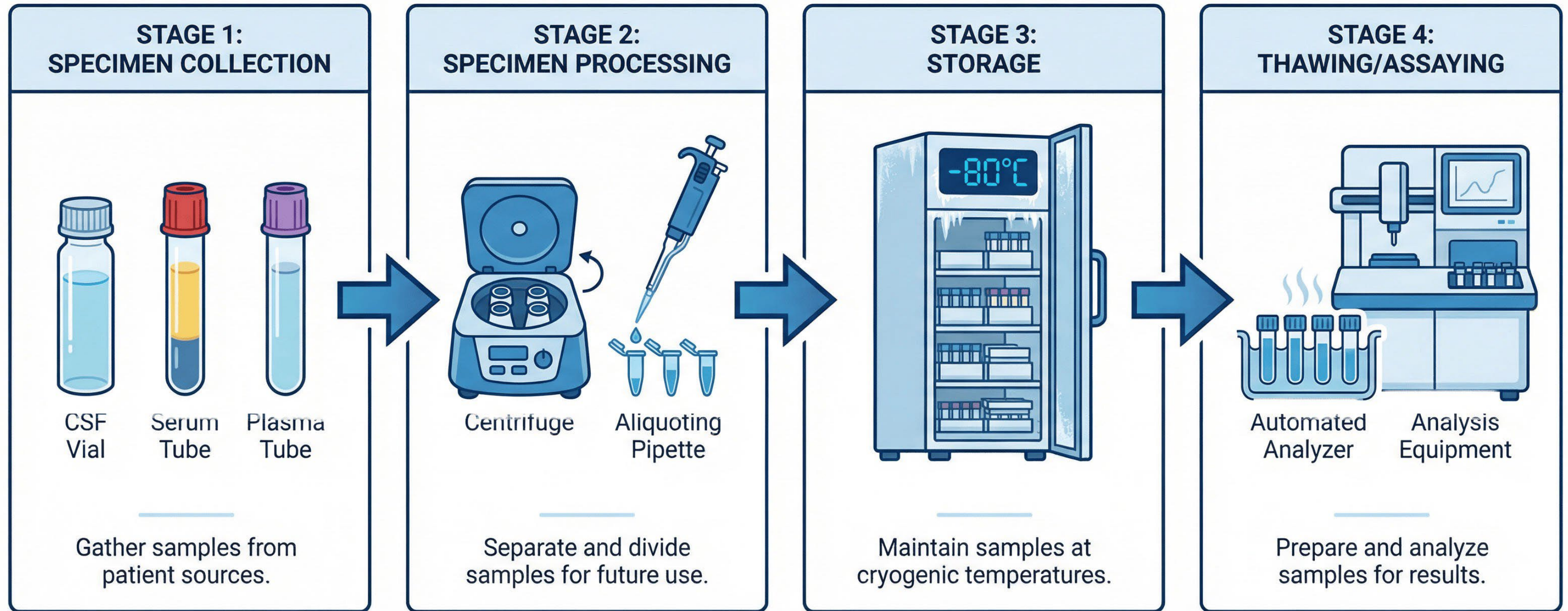
- Instrumentation:

- Discovery and characterization assay instrumentation:
 - e.g., Somalogic high plex and NULISA
- Validation Instruments:
 - MS/IP-MS, MSD
- Clinical instruments:
 - Roche COBAS



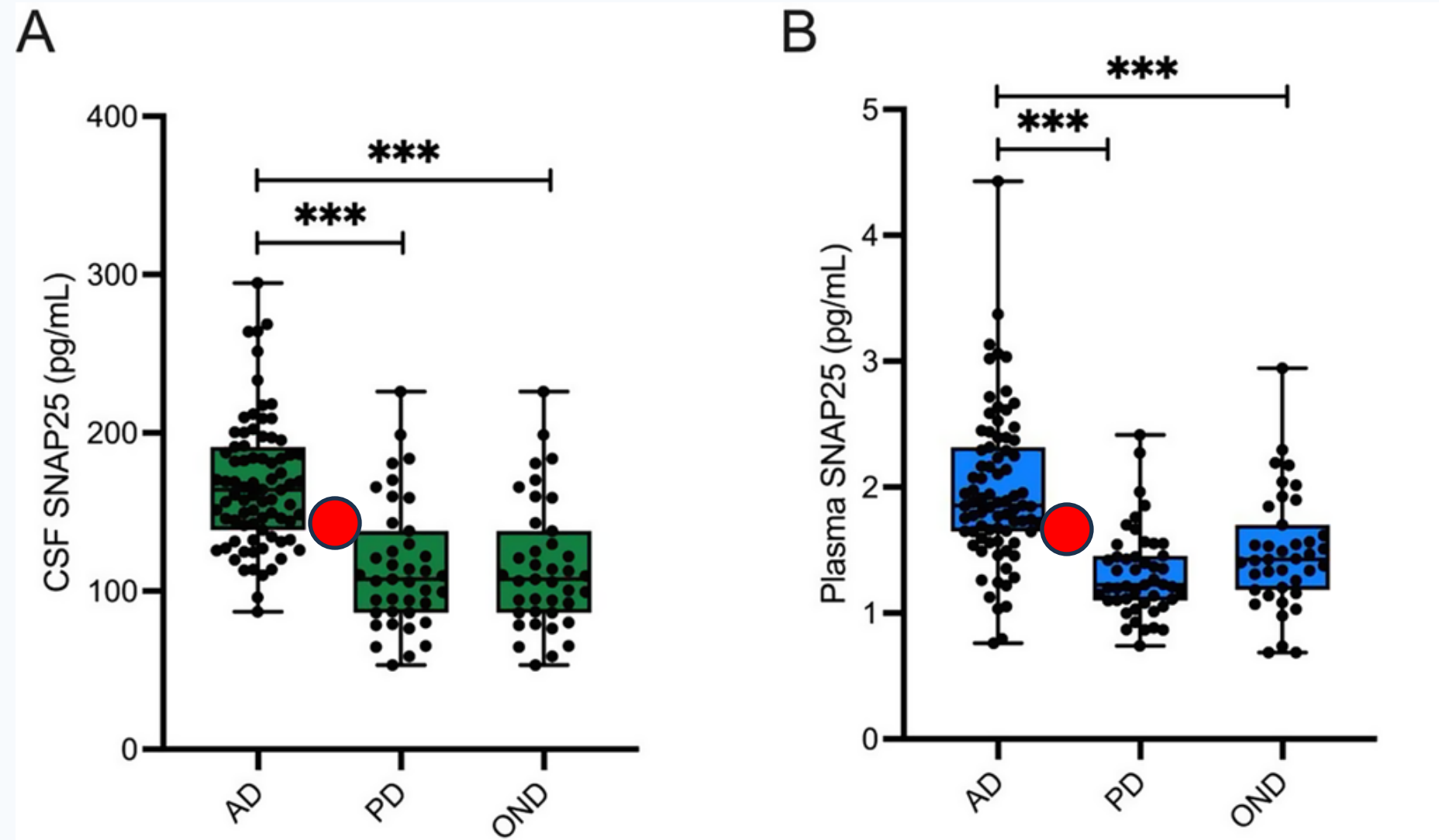
Standardized Biofluid Processing Workflow

Goal: Minimizing preanalytical variance across specimen types



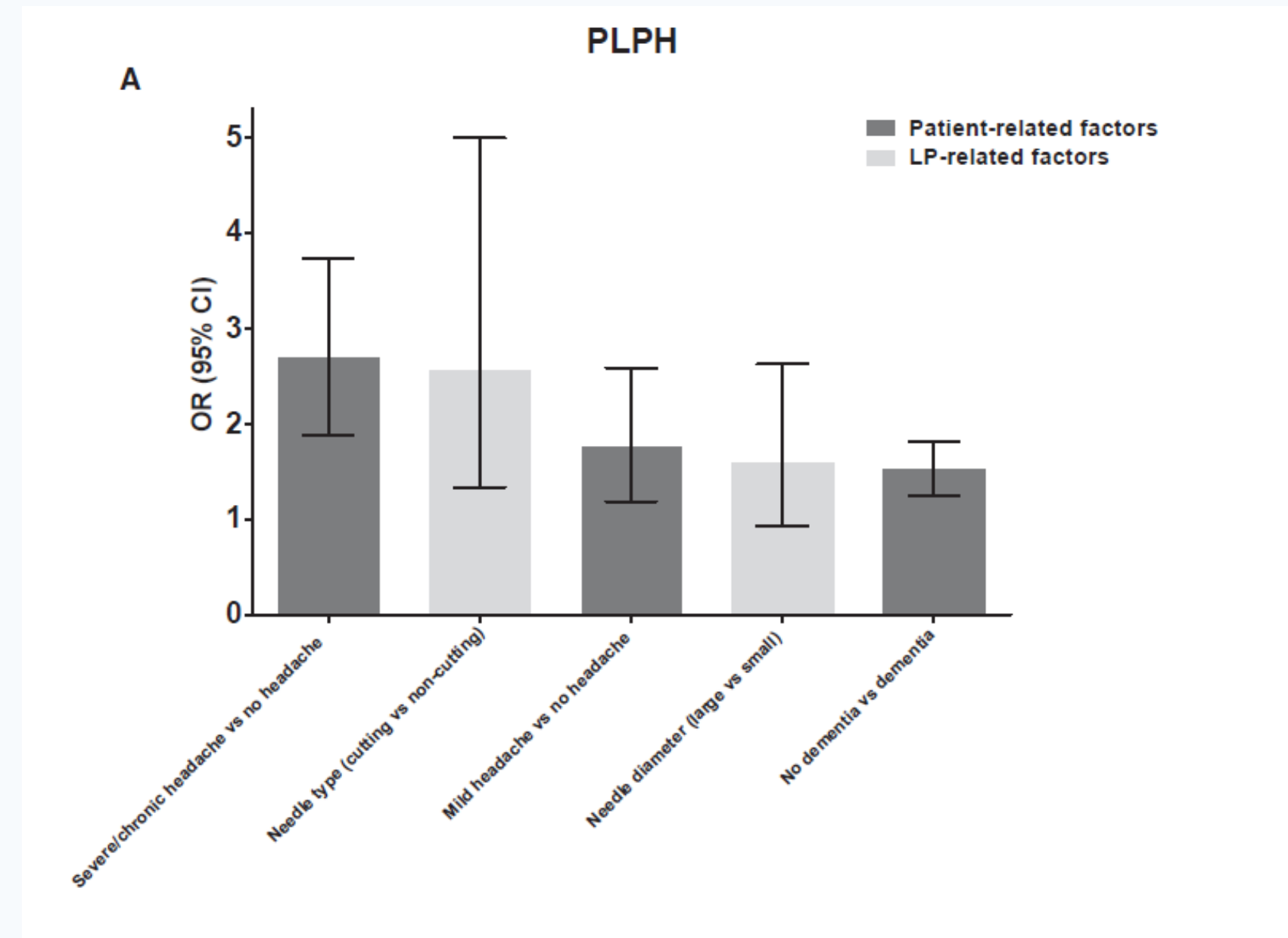
CSF Collection: Critical Preanalytical Variables

Preanalytical handling significantly impacts biomarker measurements. Key factors with documented effects:



Assay precision is affected by all preanalytical variables

Gaetani et al. *Alzheimer's Research & Therapy* (2025) 17:115



S. 116 Engelborghs et al. / *Alzheimer's & Dementia: Diagnosis, Assessment & Disease Monitoring* 8 (2017) 111-126

☐ CSF Collection: Critical Preanalytical Variables

Preanalytical handling significantly impacts biomarker measurements. Key factors with documented effects:

Biomarker	Critical Factor	Documented Effect
A β 42	Tube material & fill volume	Adsorption to recipient material; some studies find no fill volume impact
A β 42	Transportation position	Lower levels with horizontal vs. upright transport
A β 42	Tube filling	Higher levels with maximal tube filling
A β 42	Tube type	Variation by tube type; low-bind polypropylene reduces variation
A β 42	Mixing method	Higher levels with no mixing vs. roller/inversion for fresh CSF
A β 42	Blood contamination	0.25% blood contamination decreases A β 1-42 in frozen samples
Various (NRGN, SNCB, SNCG)	Time of day	6.2-12.2% diurnal variation (not GFAP, NfL, pTau/Tau)
A β , pTau, tTau	Storage temperature	Optimal storage at -80°C

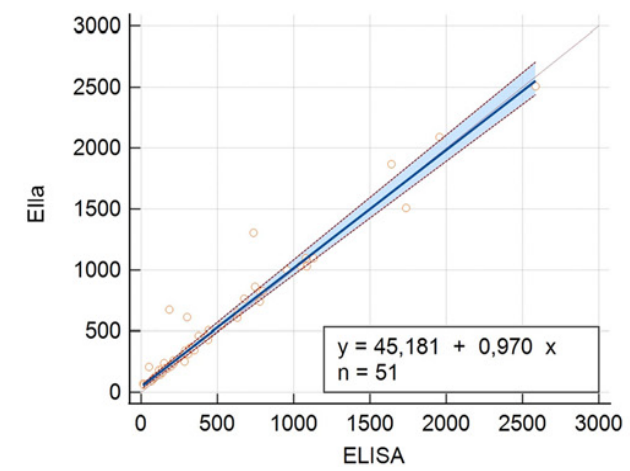
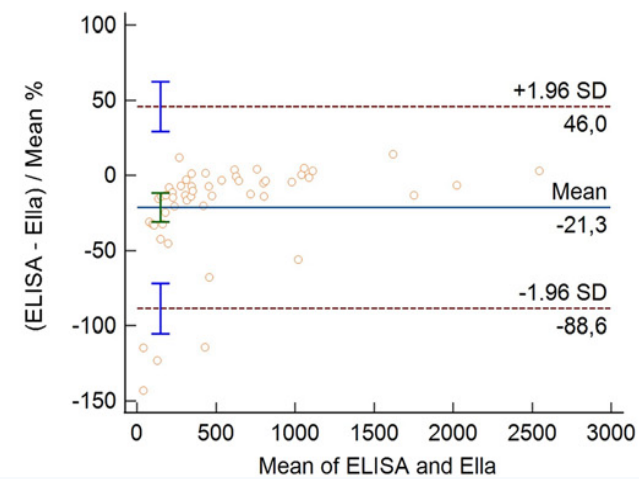
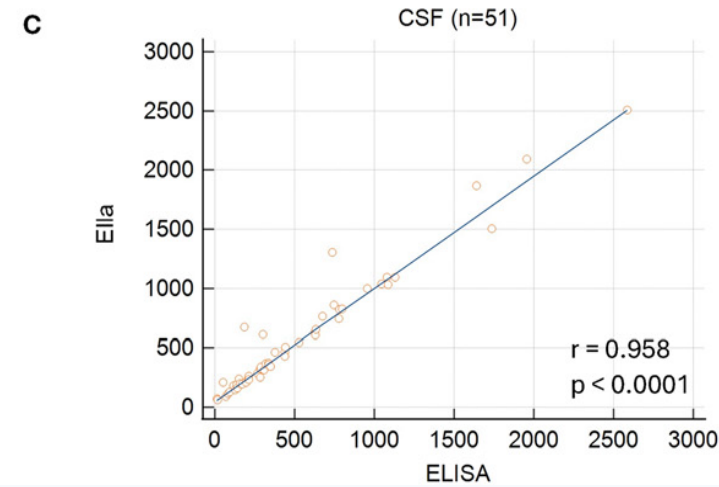
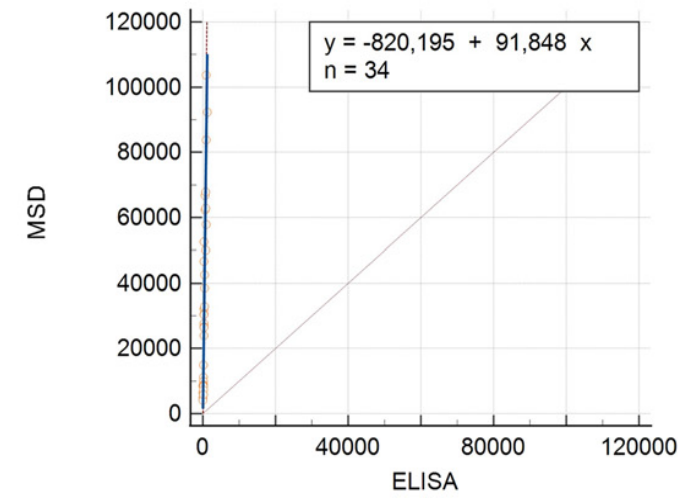
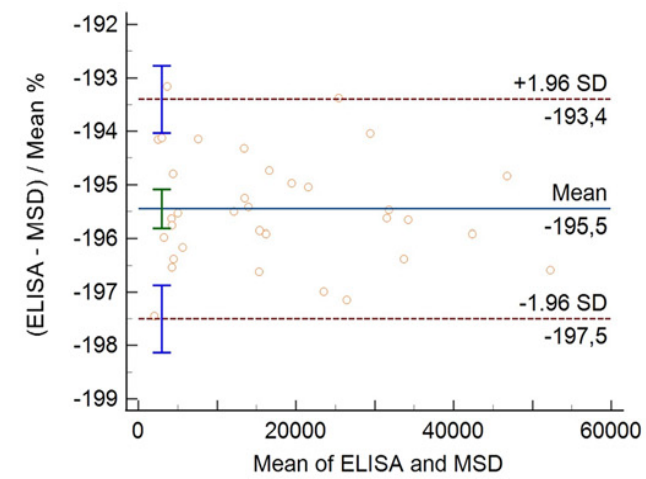
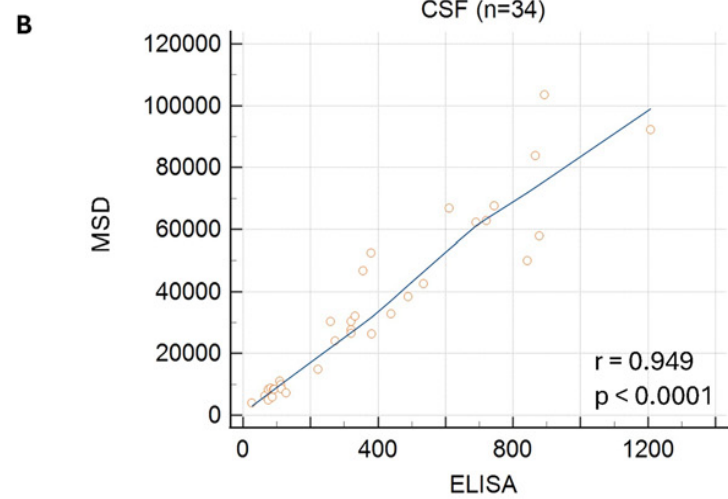
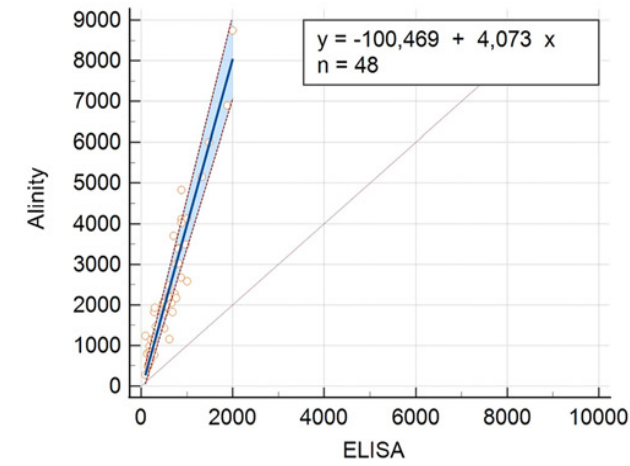
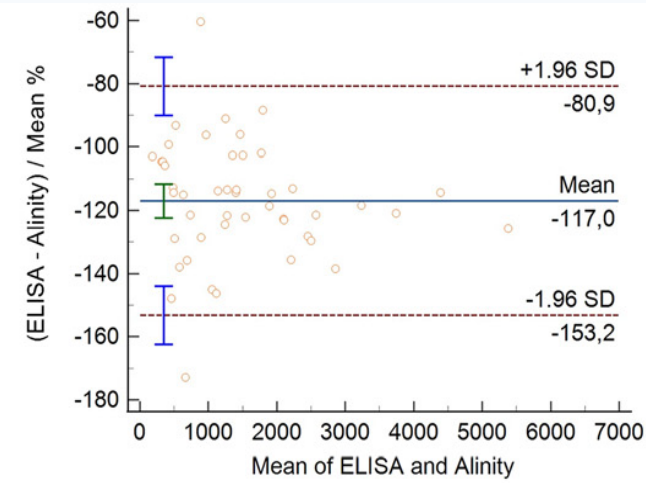
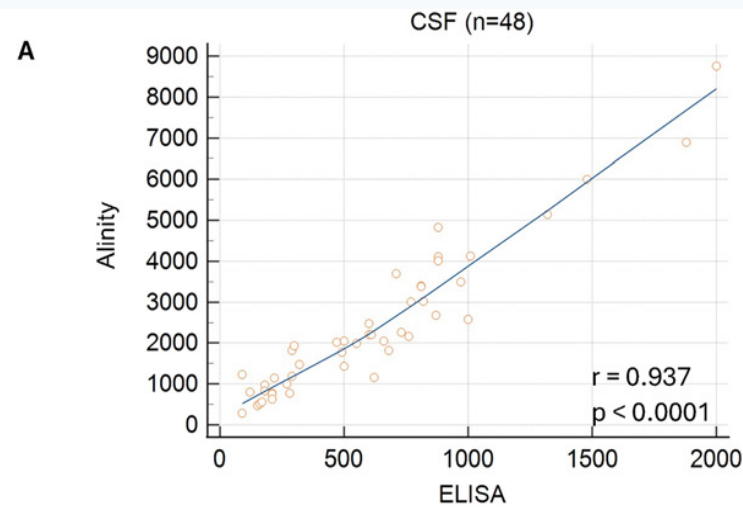
Van Meter et al. Alz & Dem., Preanalytical guidelines for blood and CSF Biomarkers 2025: Recommendations from the NACC ADRC Biofluid Biomarker Best Practices Workgroup. 2026, In press.

❄️ CSF Storage & Stability: Freeze-Thaw Impact

CSF
A – ELISA
vs Alinity

B – ELISA
vs MSD

C – ELISA
vs Ella



Critical Threshold

A β 42 shows significant degradation after just 2 freeze-thaw cycles

Minimize freeze-thaw cycles for A β measurements in CSF



Optimal Storage

Long-term storage at -80°C
NfL requires refrigeration or freezing; not stable at room temperature

🔹 Blood Biomarkers: Collection & Processing Variables

Factor	Recommendation	Impact
Tube Type (Aβ42, Aβ40)	EDTA plasma preferred	Tube type affects measurement accuracy
Time to Centrifugation	Within 2 hours	Delays alter biomarker stability
Centrifugation Temp	4°C recommended	Temperature affects downstream detection
Hemolysis	Avoid/document	Affects NfL, GFAP, Contactin-1 measurements
pTau217 Tube Type	EDTA plasma preferred	Tube selection critical for accuracy

Biomarker	Storage & Stability	Freeze-Thaw Limit
BDNF	Stable at -80°C	Up to 3 cycles
Cytokines	Stable at -80°C	Up to 3 cycles
NfL, GFAP, Contactin-1	7 days at RT/4°C	Monitor carefully
Neurogranin	Stable at -80°C	Up to 5 cycles
pTau217	Stable at -80°C	Up to 3 cycles

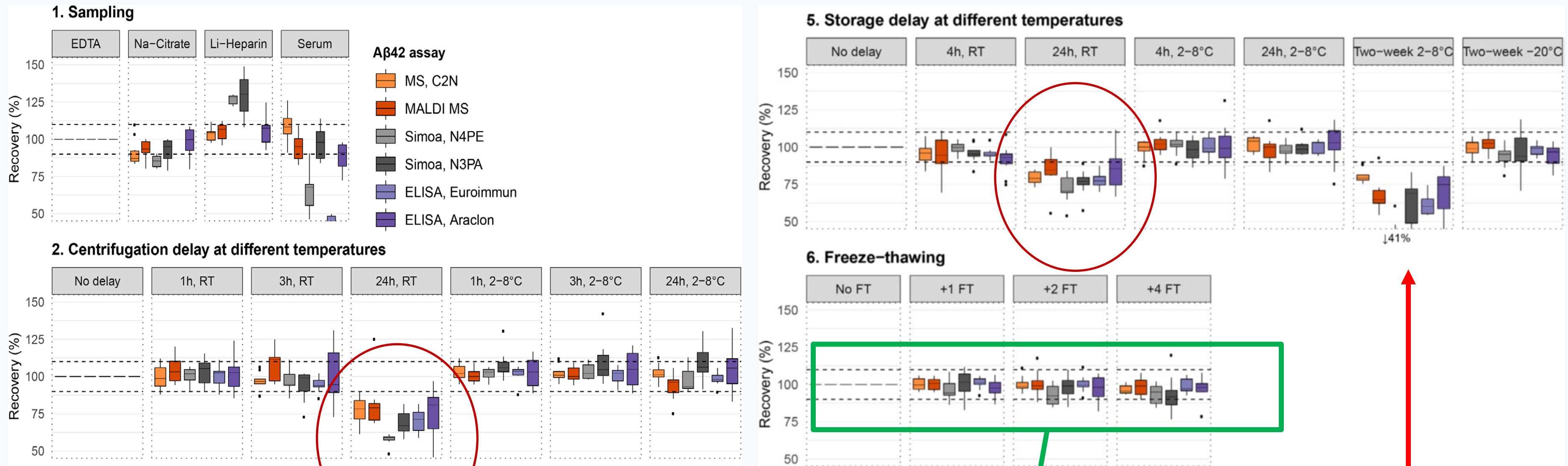
Bear in mind:

These results and recommendations are based on specific assays in widespread use or reported in the literature.

- ***Protein biomarkers are complex proteoforms with varying modifications.***
- ***Degradation of biomarker signals can affect detected levels (epitopes and accessibility to binding agents)***

🚰 Blood Biomarkers: Collection & Processing Variables

Effect of pre-analytical sample handling variations on biomarkers on the A β 42 fragment: A key disease indicator



Ex: A β 42 has been shown to be stable across freeze-thaw cycles...

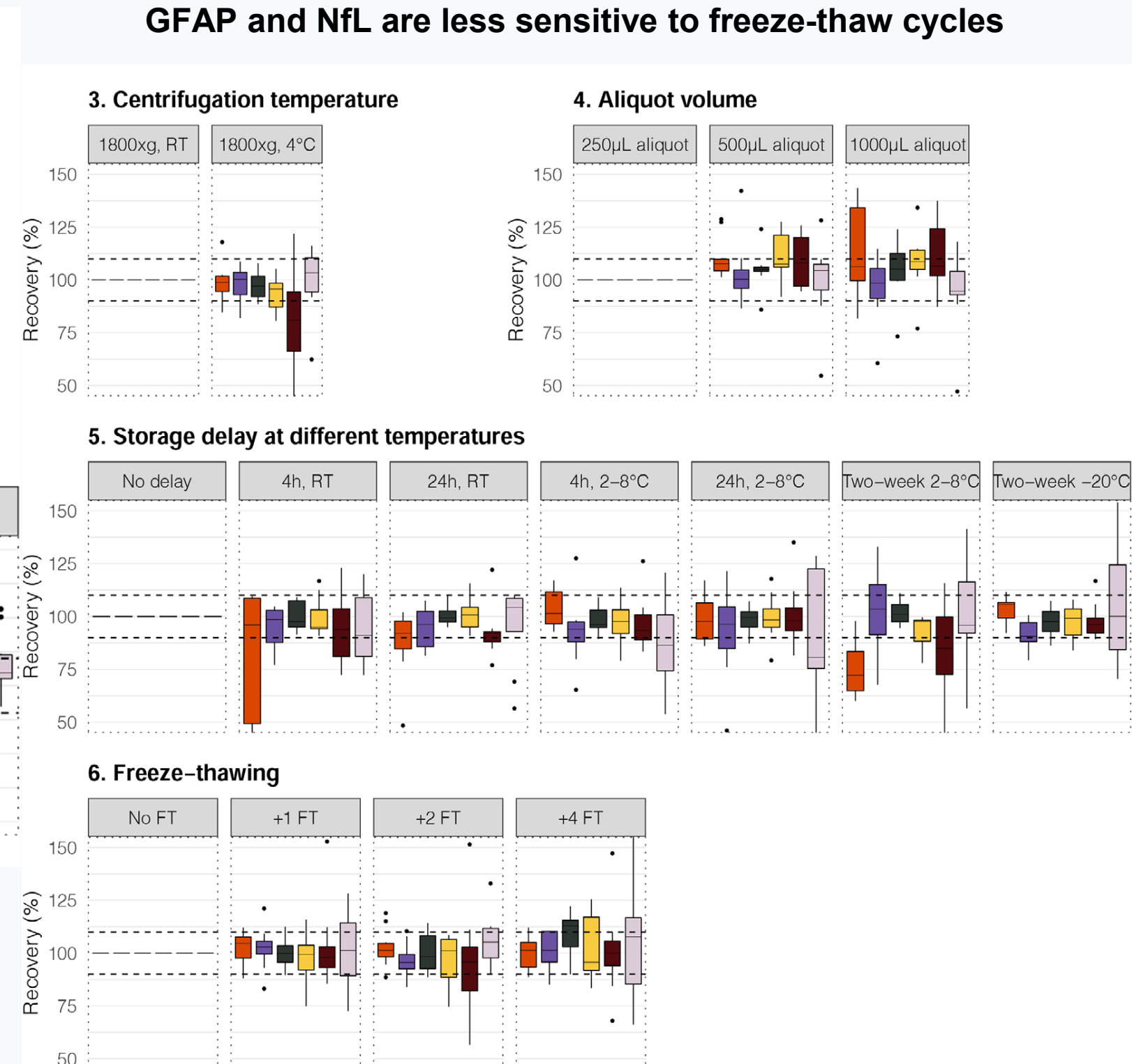
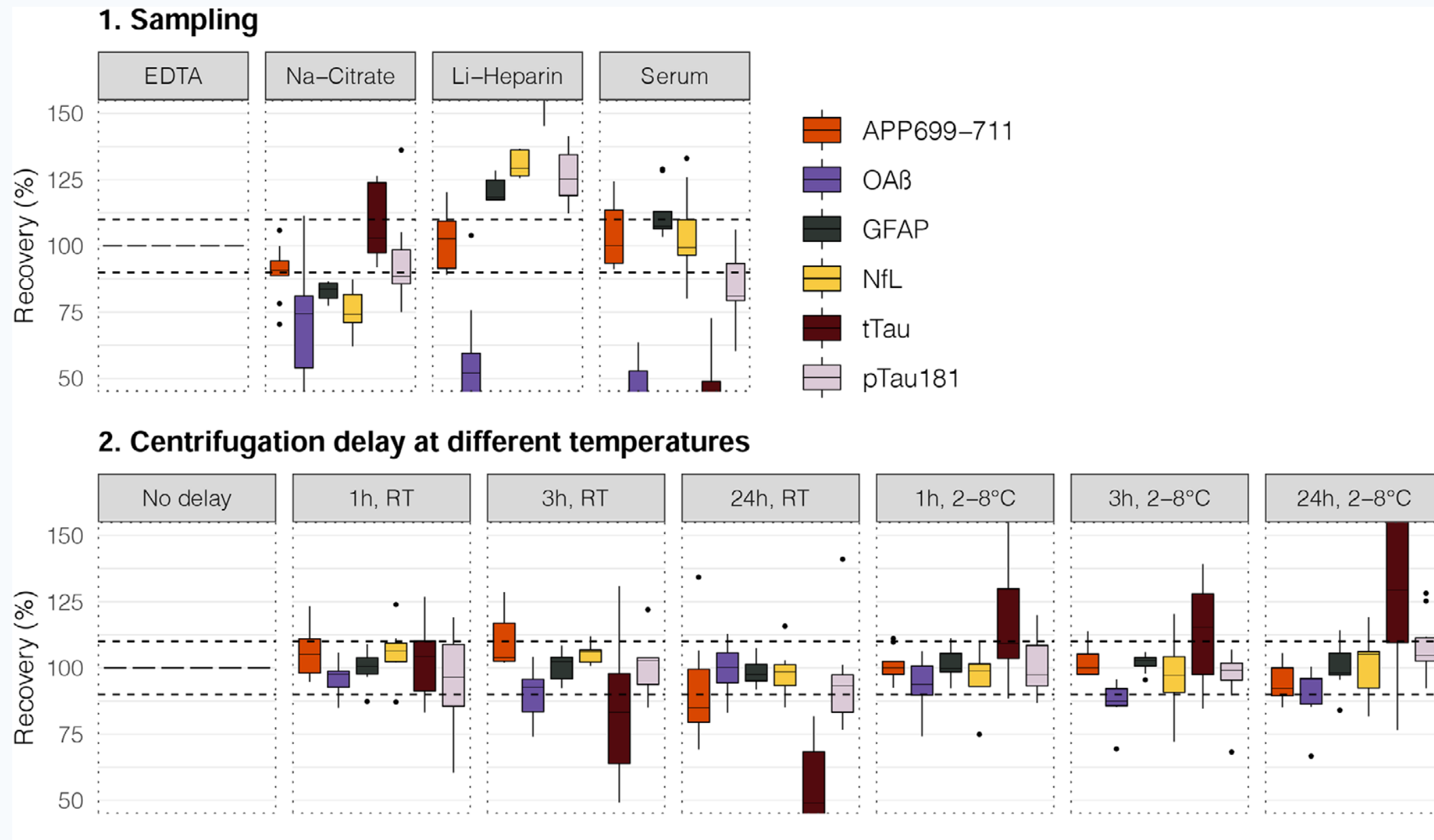
.....But should not be stored at 4 C more than a day!!

From:

Verberk et al., *Alzheimer's Dement.* 2022;18:1484–1497.

🚰 Blood Biomarkers: Collection & Processing Variables

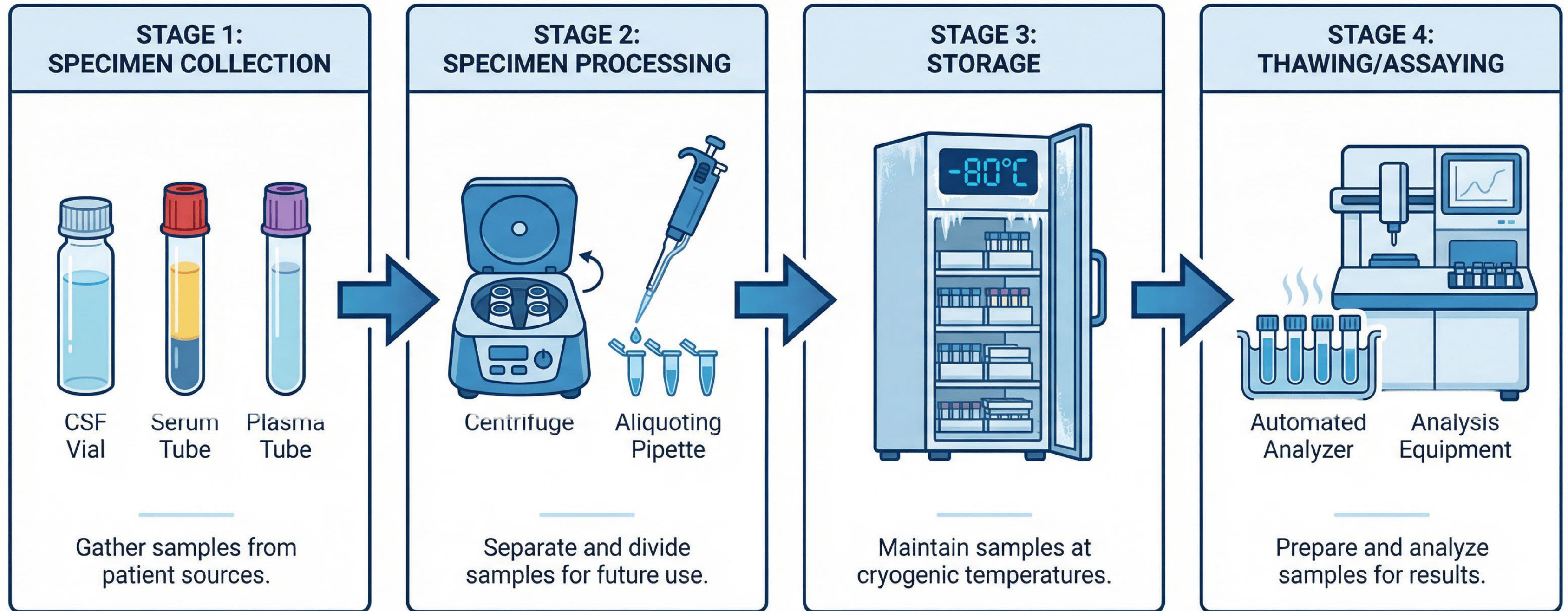
Effect of pre-analytical sample handling variations on biomarkers APP699-711, OA β , GFAP, NfL, t-tau, and p-tau181.



From:
Verberk et al., Alzheimer's Dement. 2022;18:1484-1497.

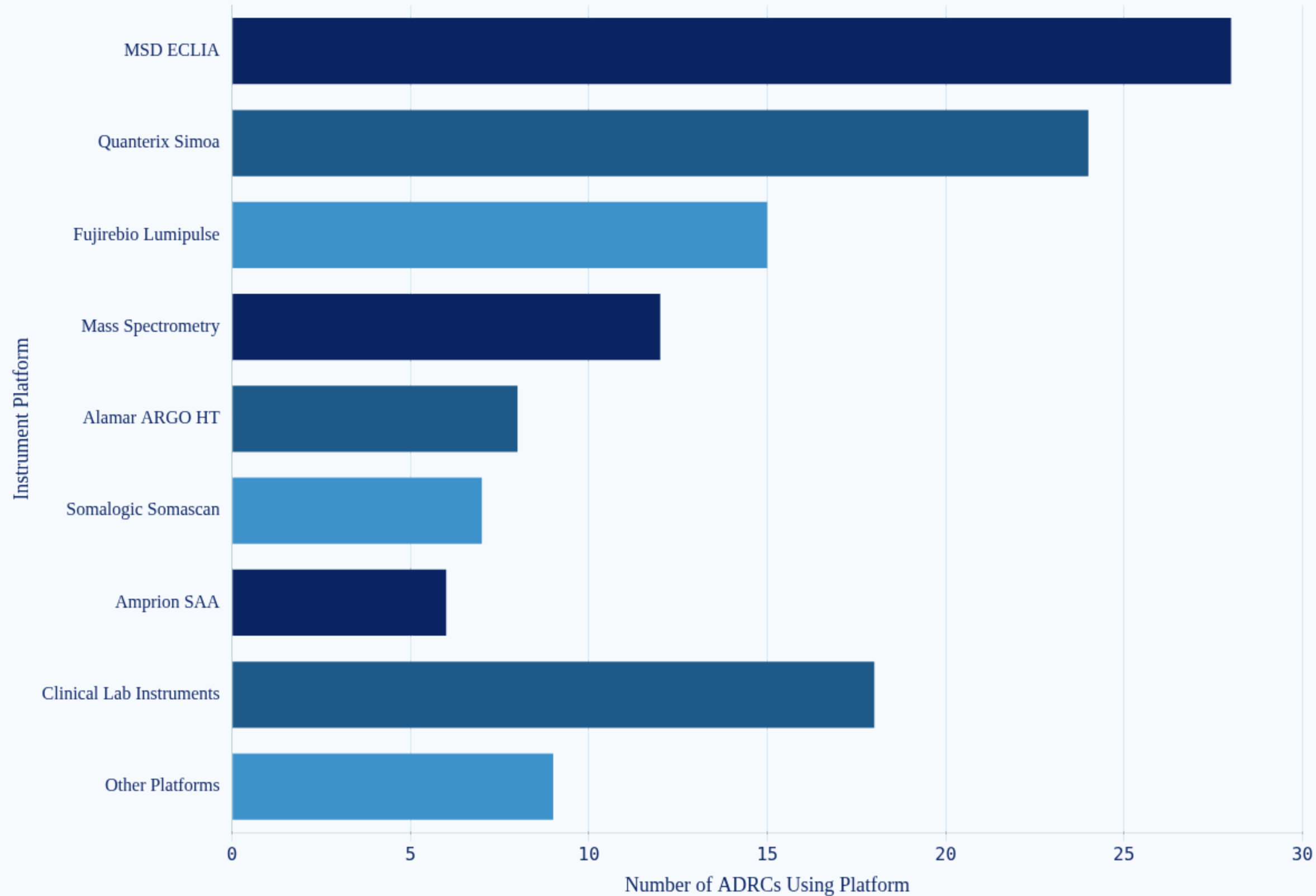
Standardized Biofluid Processing Workflow

Minimizing preanalytical variance across specimen types



Analytical Platforms Used Across ADRCs

Survey of 37 ADRCs



Common Technologies

MSD ECLIA

Most widely adopted (28 centers)

Quanterix Simoa

High-sensitivity assays (24 centers)

Clinical Lab Instruments

Routine clinical testing (18 centers)

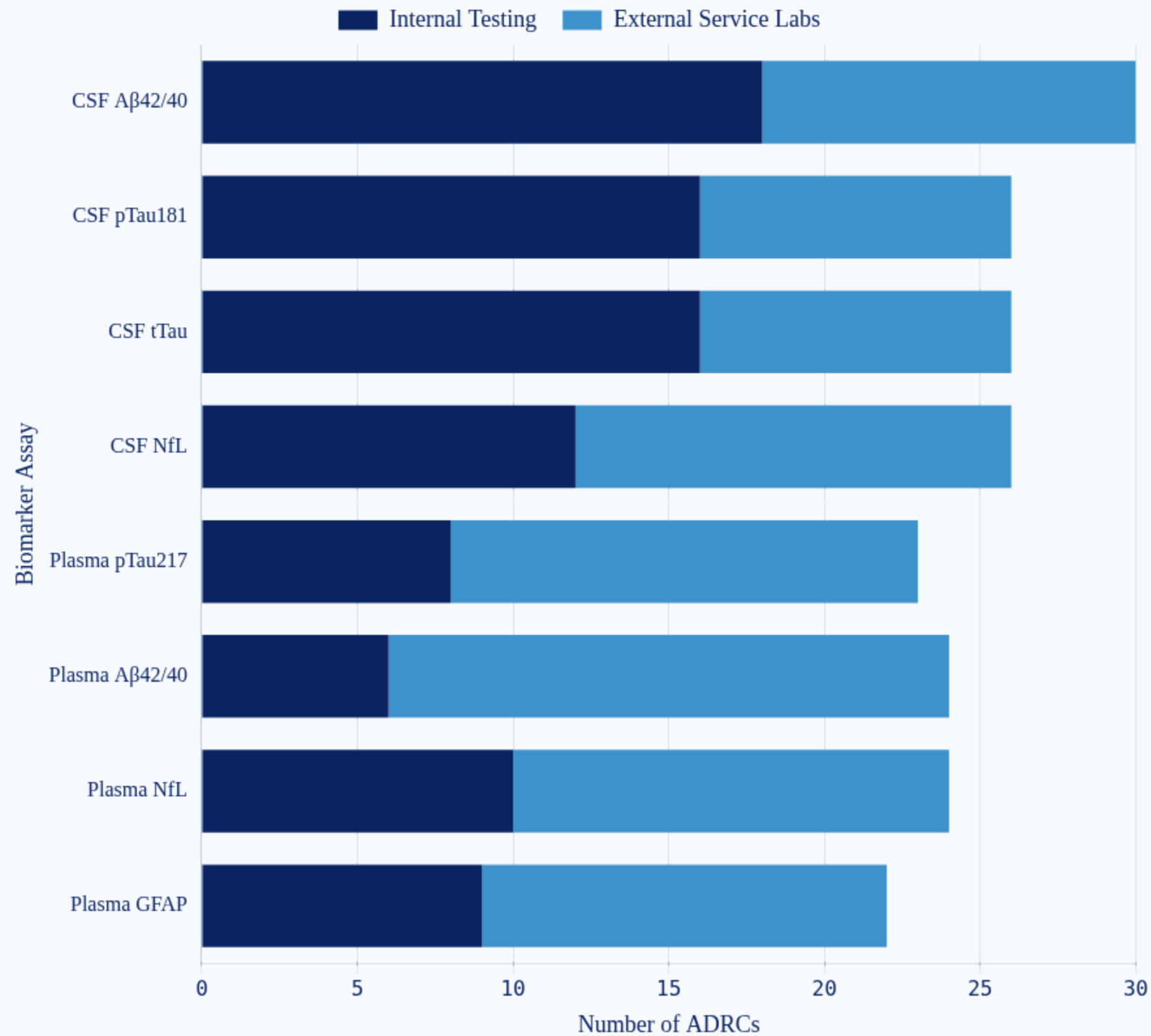
Platform diversity necessitates standardized preanalytical protocols for cross-site data comparability

New platforms in increasing use should be studied in comparable ways.

...And a priority for funding bodies.

Internal vs. External Assay Performance

Resource distribution across ADRC network



Key Observations

- Mix of internal and external testing across all major biomarkers
- CSF assays more commonly performed internally
- Plasma pTau217 and Aβ42/40 heavily rely on external labs*

Standardization critical for both internal protocols and external vendor coordination

Testing Type	Average %
Internal	45%
External	55%

- *Expected to shift in coming years with:**
- more widespread blood testing for preclinical disease (primary and secondary care screening)
 - increasing blood testing with common lab instruments

Core Best Practice Recommendations



CSF Collection

- Use low protein binding polypropylene tubes
- Collect at consistent morning time (0800-1100h)
- Gravity drip method for A β and tau
- Discard first 1-2 mL to reduce blood contamination



Blood Collection

- EDTA plasma preferred for A β and pTau measurements
- Process within 2 hours of collection
- Centrifuge at 2000 x g for 10 min at 4°C
- Document hemolysis and lipemia



Storage

- Long-term storage at -80°C
- Minimize freeze-thaw cycles (≤ 2 for A β 42)
- Use screw-cap tubes with rubber O-rings
- Monitored alarm systems for freezer failures



Documentation

- Record all preanalytical variables
- Document deviations from protocol
- Track sample volumes and locations
- Implement quality control measures

The 2025 guidelines are now a live NACC-ADRC resource on the NACC website

Online link top CSF protocol: ADRC CSF collection guidelines

<https://files.alz.washington.edu/best-practices/csf-collection-processing-storage-guidelines-9.22.25.docx>

Online link for the protocol: ADRC Blood collection guidelines

<https://files.alz.washington.edu/best-practices/blood-collection-processing-storage-guidelines-9.22.25.docx>

These include references and links to many additional useful resources

📌 Future Directions & Emerging Considerations



Additional Biomarkers Under Study

Rapid expansion of novel biomarkers requires ongoing evaluation of preanalytical factors

Examples: Inflammatory markers, vascular biomarkers, disease-specific proteoforms

Guidelines limited to biomarkers with adequate preanalytical validation and multi-center usage



Standardization Across Platforms

Challenge: Platform diversity (MSD, Simoa, Lumipulse, mass spec) necessitates platform-specific protocols

Opportunity: Global reference standards in development (SABB, GBSC initiatives)

Will enable better cross-platform and cross-site data comparability

***Future submission of biomarker information to NACC via standardized processes developed by Data Variables and Data Quality Workgroup**



Expanding Beyond CSF and Blood

Research community exploring additional biofluid types for practical advantages

Examples: Saliva, urine, oral mucosal fluid for subjects with vascular frailty or clotting concerns

Extracellular Vesicles: EVs require specialized protocols (MISEV guidelines) and larger sample volumes

Currently beyond scope but represent important future directions

Key Takeaways

✓ **Standardization is Essential**

Preamanalytical variables significantly impact biomarker measurements—consistent protocols across 37 ADRCs enable reliable multicenter research

✓ **Preamanalytical Control = Data Quality**

Tube selection, timing, temperature, and freeze-thaw cycles are critical control points, maintaining sample integrity

✓ **Comprehensive Documentation**

Recording all preanalytical variables and deviation supports data comparability and enables appropriate statistical adjustments



37

ADRCs with standardized methods

Standardized protocols enabling
future research



44

Biomarkers Standardized

16 CSF + 28 blood



55,000+ Subjects

20,000+ Active subjects enrolled
(AD, FTD, PD, LBD, TBI)

NACC-ADRC Biofluid Biomarker Best Practices Workgroup Members 2023-2025

- Lynn Bekris
- Minerva Carrasquillo
- Kaitlin Casaletto
- Aleksandra Dakic
- Kelley Faber
- Nora Gray
- Mingzaho Hu
- Hesam Jahanian
- Nick Kanaan
- Thomas Karikari
- Argentina Lario Lago
- Jill Morris
- Andy Liu, Duke University
- Angus Nairn
- Ricardo Osorio
- Matthew Perkins
- Jagan Pillai
- Jonathan Reader
- Tom Register
- Clairisa Stayton
- Tim Van Meter
- Rachael Wilson
- Ted Wilson
- Henrik Zetterberg

NIA Representatives

- Cerise Elliot
- Nina Silverberg

NACC Representatives

- Hannah Rosentreter
- Kathryn Gauthreaux
- Jess Welsch
- Heather O'Connell
- Sarah Biber (pre-Aug. '25)

External Expert Reviewers

- Nicholas Ashton, PhD, Banner Health
- Kaj Blennow, Univ. Gothenburg
- Carlos Cruchaga, WashU
- Inge W Verberk, Amsterdam MC
- Charlotte Teunissen, Amsterdam MC
- Henrik Zetterberg, Univ. Gothenburg
- SABB- Standardization of Alzheimer's Blood Biomarkers
- GBSC-Global Biomarker Standardization Consortium

Special thank you to the Biomarker Core Steering Committee for input and guidance throughout the process!

Special thank you to the Alzheimer's Association and SABB, GBSC Committees for collaborative input and guidance in assembling ADRC recommendations.

Workgroup Contacts: KARIKARI@pitt.edu
tvanmeter@brainboxinc.com