Standardization of Biomarker Measurements Across ADRCs

National Centralized Repository for Alzheimer's Disease and Related Dementias

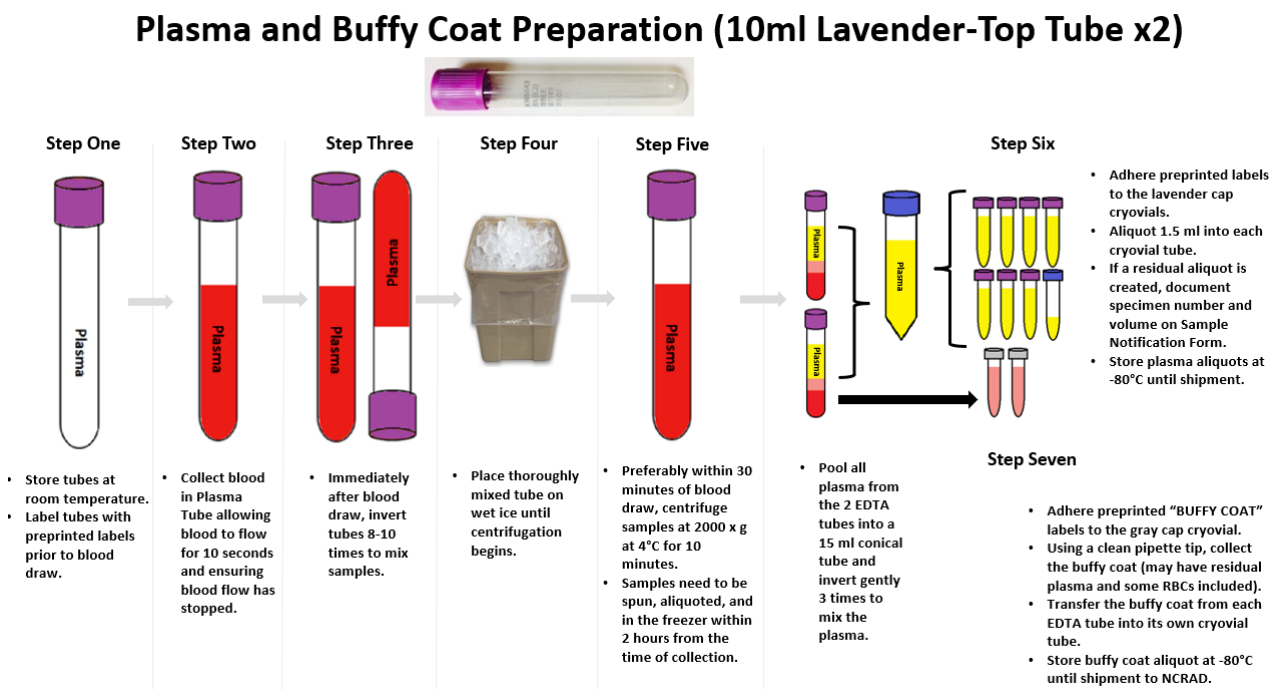
Kristen A. Russ, PhD
NCRAD Biomarker Assay Lab Director
Assistant Research Professor, MMGE, IUSM

Standardization and quality of biomarker measurements starts with standardizing specimen collection and handling procedures

NCRAD

NCRAD Protocol Development and Standardization

- Manual of Procedures
  - Includes descriptive and pictorial instructions
  - Training videos available
- Sites have in person or virtual training
  - NCRAD staff walk through the collection and shipping procedures with the team



NCRAD Protocol Development and Standardization

- Sample Verification
  - Provides verification of sample characteristics against the information provided by the site for participants with buffy coats provided
- Blinded Samples
  - All samples provided to labs (including the Biomarker Assay Lab) are received blinded.
- Majority of collection materials are provided in collection kits
- NCRAD receives documentation from sites if samples have non-conformance

NCRAD

Standardization of biomarker measurements and data handling within the NCRAD Biomarker Assay Laboratory (BAL)

Specimen Preparation

- BAL technicians are blinded to any information not necessary to track samples as they move through the analysis procedures
  - Site ID, collection date, kit number etc. are not available to the BAL technicians
- NCRAD staff handles creating balanced plate designs to enable BAL to remain blinded
  - Plates/runs are balanced on age, biological sex, and longitudinal samples
  - Samples are provided to BAL in a balanced order

Specimen Analysis in the NCRAD BAL

BAL Laboratory Equipment

- Automated liquid handling
  - Tecan Fluent (2)
    - Utilized to prepare Quanterix HD-X plates
      - Lower variability when performing assay preparations compared to onboard processes
    - Regularly monitored using a colorimetric method to ensure consistent and precise readings across all pipettors
    - Regular preventative maintenance as recommended by the manufacturer

Specimen Analysis in the NCRAD BAL

BAL Laboratory Equipment

- Immunoassay platforms
  - Quanterix HD-X (2)
    - For use with plasma
  - Fujirebio Lumipulse G1200
    - For use with plasma or CSF
  - Alamar NULISA
    - For use with plasma or CSF

NCRAD

Assay Selection and Characterization

- Qualification Studies
  - Utilizes local sample collection
    - 5 samples spanning high, medium, and low concentrations
  - 2 days to capture intra and inter-day variability
    - Precision
    - Dilutional Linearity
    - Parallelism
  - Passing Criteria
    - Must have average total CV ~10%
    - Performance must be consistent with the characterization provided by the manufacturer
  - Comparability Study
    - If assessing new assay for biomarkers currently offered through the BAL, ~100 samples with amyloid or tau PET data are analyzed on the new assay and compared to existing data

Producing consistent and reliable measurements

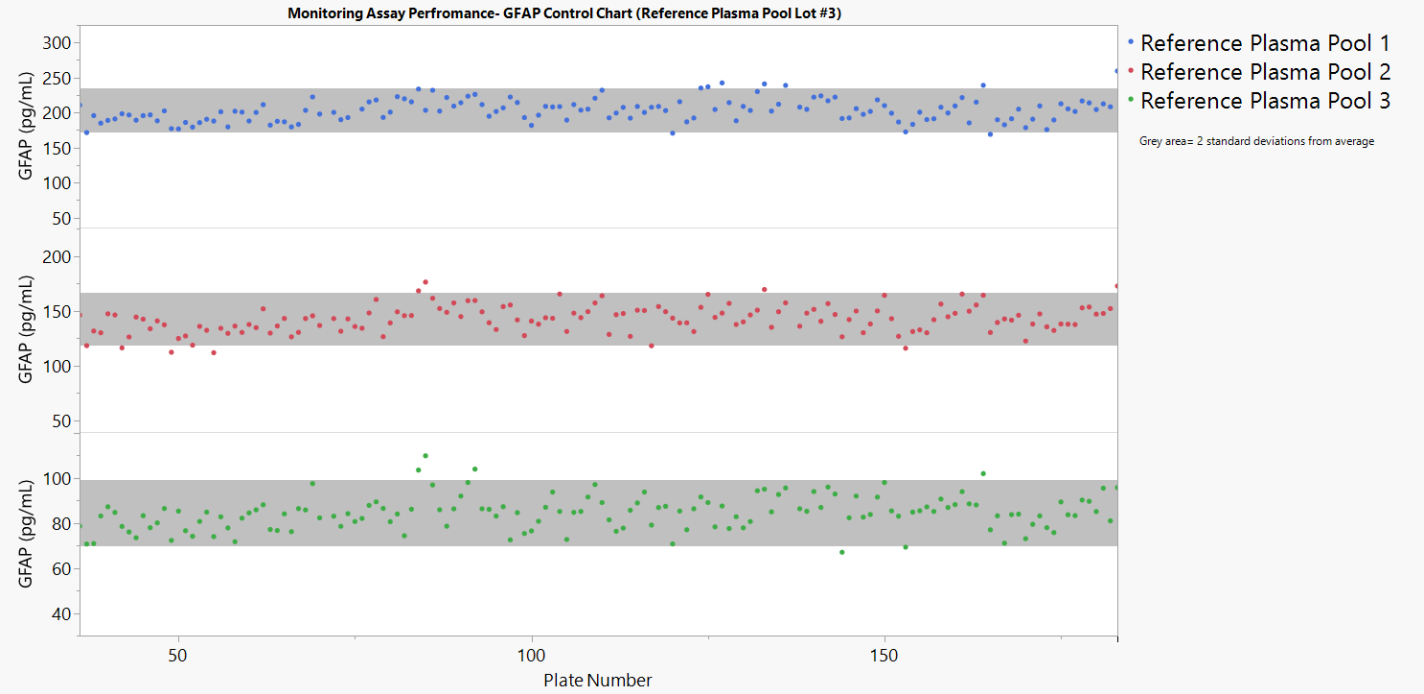
Bridging and control measures are key to consistency of data across time

- Control measures
  - Identify assay performance outside established quality guidelines
  - Confirms validity of assay results
- Bridging
  - Allows comparison of data between studies
  - Necessary to fully harmonize laboratories

NCRAD

Control Measures

- Assay monitoring within studies and over time at NCRAD
  - Kit QC low and high samples
    - Provided by manufacturer of kits
    - Allows BAL to determine that the assay is working properly from plate to plate
  - Plasma reference pools
    - BAL produces plasma reference pool controls from our local plasma collections
    - High, medium, and low controls
    - Allows monitoring of assay performance within the batch and between batches



Plasma reference pool data points outside +/- 2SD from the mean are investigated to determine validity of the assay plate/run.

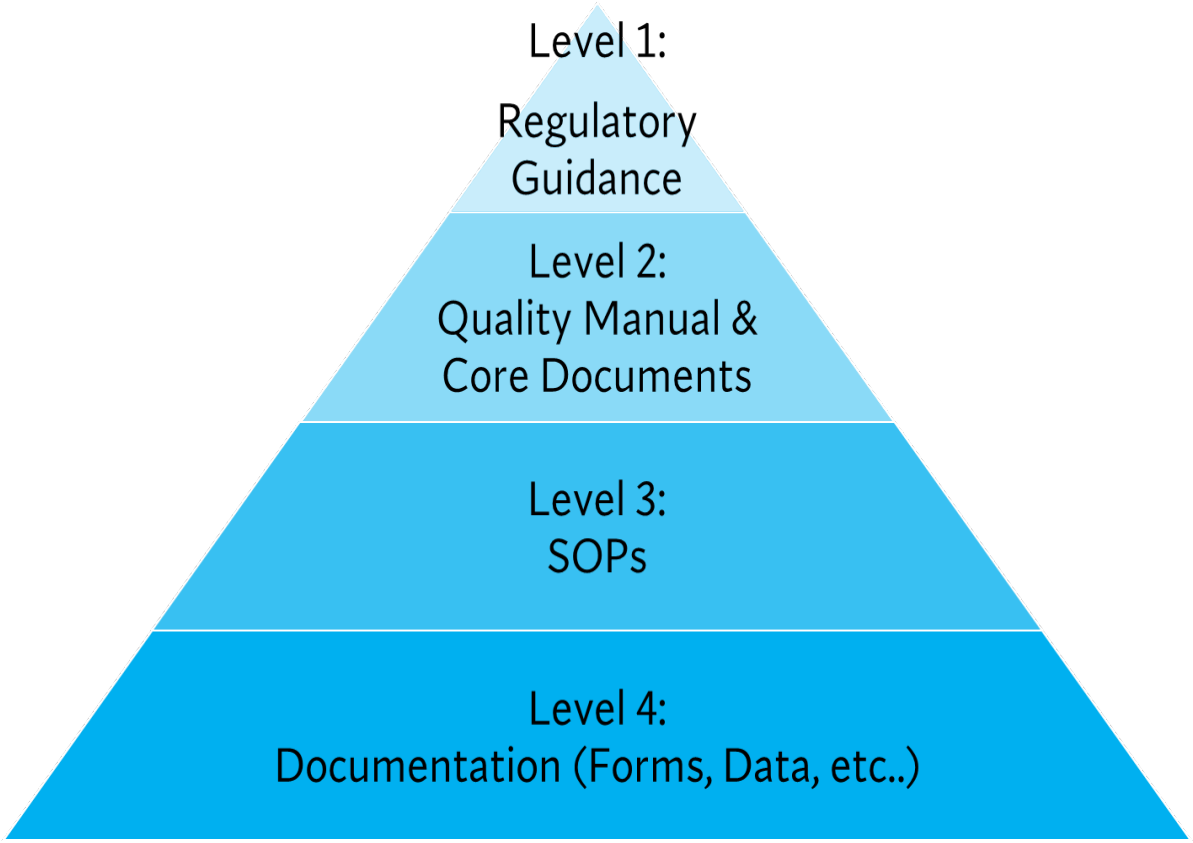
Bridging

Bridging involves detecting and correcting for lot-to-lot or instrument-to-instrument variability

- Enabled by a local collection of plasma from healthy controls and participants with Alzheimer's disease
- Quanterix HD-X Assays
  - 31 samples run on each lot or instrument
  - CV calculated between the lots
    - Under 10% CV considered acceptable
    - Over 10% requires bridging
  - Also assessed after preventative maintenance or instrument repairs
- Fujirebio Lumipulse Assays
  - 31 samples run between changes for immunoreaction cartridge lots
  - 15 samples run between reagent lot changes
  - CV calculated between the lots
    - Under 10% CV considered acceptable
    - Over 10% requires bridging
  - No bridging required to date
  - Also assessed after preventative maintenance or instrument repairs

NCRAD

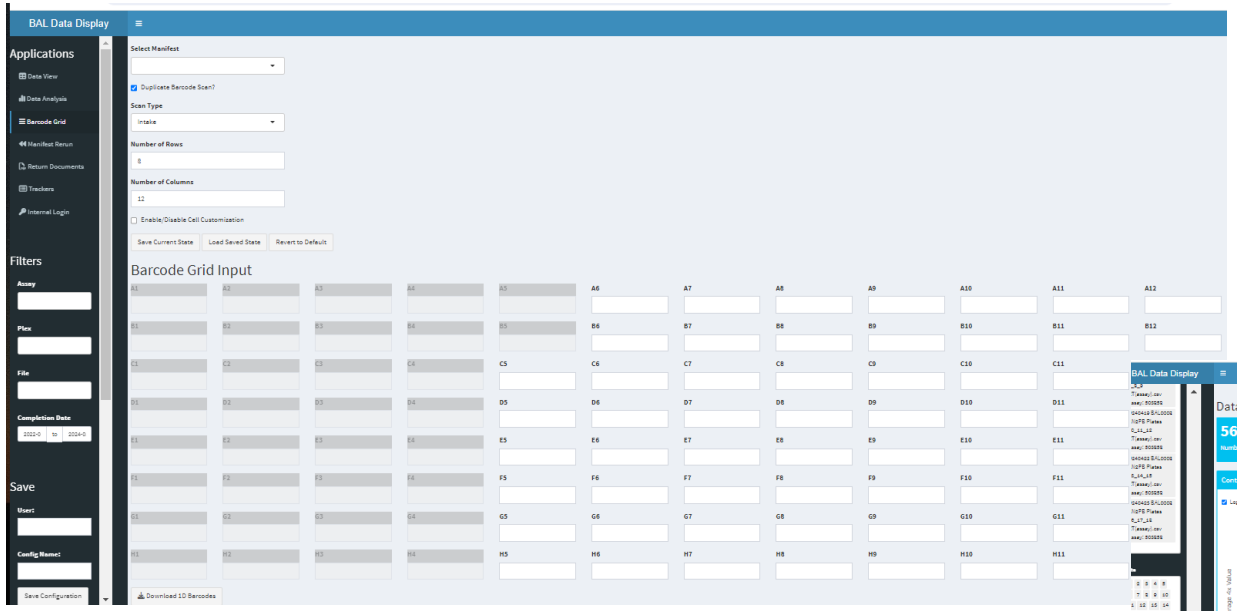
Specimen Analysis in the NCRAD BAL

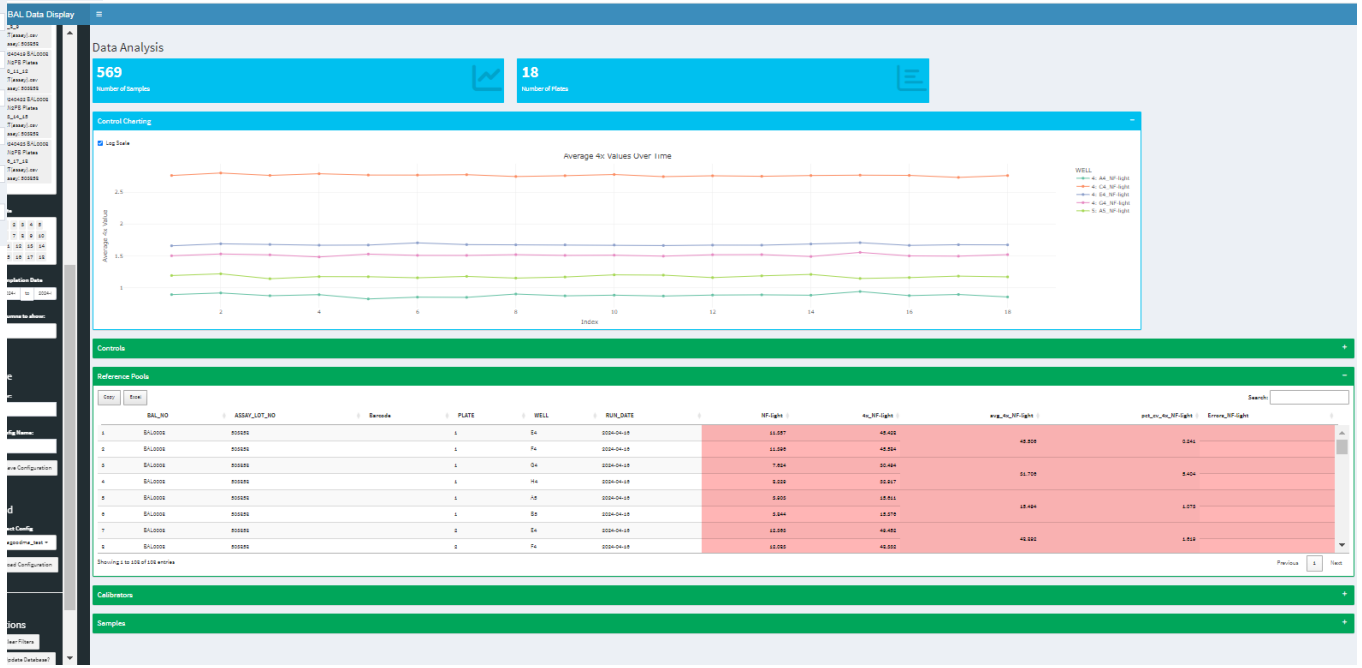


- Highly SOP driven
- Documentation
  - Assay performance monitoring
  - Sample quality monitoring
  - Process deviations logs
- Standardized data handling
  - Many QC checks
    - QC checks built into data templates
    - QC checks performed at various levels prior to data return
      - Technicians, Coordinator, Lab Director
  - Developing new web app to further standardize and remove human element from data handling

NCRAD

Specimen Analysis in the NCRAD BAL





- New web app will further standardize data handing and QC
  - Limits data manipulation
  - Simplifies and decreases time for data analysis and QC
  - Streamlines monitoring of assay performance

NCRAD