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REVIEW ARTICLE

The National Centralized Repository for Alzheimer's Disease and Related Dementia's Biomarker Assay Laboratory: A Resource for the Alzheimer's Disease Research Community

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Abstract

Developments in Alzheimer's disease blood-based biomarkers research have provided critical information as to their use in early detection and assessments of progression in patients. The National Centralized Repository for Alzheimer's Disease and Related Dementias (NCRAD) Biomarker Assay Laboratory (BAL) was developed to provide expertise and access to robust and reliable research-use-only biomarkers (not for medical decision making) for the Alzheimer's disease and related dementias (ADRD) research community. The NCRAD BAL is a quality-focused laboratory delivering biomarker data through the use of highly standardized procedures and highly automated instrumentation with the goal of limiting pre-analytical variability while preparing samples for analysis, including limiting lot-to-lot variability of the assays. As biomarkers become United States Food and Drug Administration (FDA) -approved and move into use in CLIA labs, the NCRAD BAL will bring forward new platforms and assays reflecting the most current research-use-only biomarkers to support the research mandate of the ADRD research community.

KEYWORDS
Alzheimer's disease, ATN, Blood-based Biomarkers, Fluid Biomarkers, NCRAD, Neurodegeneration

Highlights

- The National Centralized Repository for Alzheimer's Disease and Related Dementias (NCRAD) has developed a Biomarker Assay Laboratory to support research of robust and reliable fluid biomarkers for Alzheimer's disease and neurodegeneration.
- NCRAD supports Alzheimer's Disease Research Centers by providing biomarker analyses on samples with clinical and imaging data through the National Institute on Aging (NIA) -funded Alzheimer's Disease Center Fluid Biomarker Initiative.
- The NCRAD Biomarker Assay Laboratory focuses on decreasing variability that will affect study results by utilizing highly standardized and automated procedures, strict monitoring of control measures, and controlling for lot-to-lot and instrument-to-instrument variability.

1 | BLOOD-BASED BIOMARKERS IN THE ALZHEIMER'S DISEASE FIELD

Over the past 20 years, much progress has been made in assessing Alzheimer's disease (AD) neuropathologic change during life and across the full disease course. Initially, assessment of biomarkers for AD only occurred in brain tissue at autopsy.[1] In subsequent years, the development of amyloid and tau cerebrospinal fluid (CSF) tests, as well as positron emission tomography (PET) tracers, provided options for detection in the clinic.[2] Recent advances in blood-based biomarkers (BBBM) have turned the focus towards developing a deeper understanding of how these measurements will be used in clinical research and practice.[3–6] In the past 5–10 years alone, advances in the field of fluid-based biomarkers have resulted in a shift from biomarkers in the CSF, which is more difficult and costly to obtain routinely, to plasma, making these biomarkers more broadly accessible.[7] Central to this progress has been the development of instrumentation and assays that have the sensitivity, selectivity, and precision necessary to measure these biomarkers in blood with reliable results.[8,9]

A pivotal publication in the Alzheimer's field that provided guidelines for the use of BBBM was the National Institute on Aging and Alzheimer's Association (NIA-AA) Research Framework, often referred to as the "ATN framework". It specified that AD could be defined by pathological processes that were seen at autopsy or by biomarkers in vivo, specifically biomarkers for β [A]myloid, [T]au, and [N]eurodegeneration.[10] At the time of publication of the ATN framework, the leading BBBMs were beta amyloid 42/40 ratio (Aβ 42/40) for measurement of β amyloid, phosphorylated tau 181 (P-tau181) for measurement of tau, and neurofilament light chain (NfL) for measurement of neurodegeneration.

Both tau and NfL are structural proteins in the neuron.[11,12] Tau is a protein associated with microtubule stabilization and typically found in the axon. It is hyperphosphorylated and mislocalized in AD, leading to neurofibrillary tangles.[1] Increased NfL levels in both blood and CSF compared to age-matched controls can be indicative of axonal injury or degeneration. An increase in NfL concentrations can occur in many diseases, so it is not specific to AD.[13] Beta-amyloid is a protein resulting from the cleavage of the amyloid precursor protein (APP). Cleavage occurs at differing locations on the protein, and longer peptides, such as Aβ 42, aggregate and form plaques in individuals with AD. Aβ 42 is not typically utilized without normalization to Aβ 40, as the Aβ 42/ Aβ 40 ratio has been shown to change with the development of amyloid pathology.[14]

The AD biomarker field has moved quickly since the publication of the ATN framework, and phosphorylated tau 217 (P-tau 217) has replaced P-tau181 as the leading tau biomarker. Ongoing research continues to identify the best tau marker, including study of eMTBR-tau243.[9,15] In addition, AD research continues to refine BBBMs and their meaning as evidenced by the update to the ATN framework in which ATN was expanded to ATNIVS to include inflammation (I), vascular (V), and α-synuclein (S).[16] The expansion into inflammatory biomarkers for AD and neurodegeneration has led to interest in glial fibrillary acidic protein (GFAP), found in astrocytes, and increases in response to astrocytic injury or reactivity of astrocytes.[17] Blood levels of GFAP are elevated in patients with AD, but this is not specific to AD. Vascular and α-synuclein BBBMs are limited and are an area of ongoing research.

2 | DEVELOPMENT OF BIOMARKER LABS TO SUPPORT AD RESEARCH

Alzheimer's Disease Research Centers (ADRCs) across the United States have been key to the recruitment and assessment of research participants across the spectrum of cognitively normal (CN), mild cognitive impairment (MCI), subjective cognitive impairment (SCI), and dementia. As part of enrollment in the ADRCs, participants undergo uniform cognitive and clinical assessment, as implemented in the Uniform Data Set (UDS), which is maintained at the NIA-funded National Alzheimer's Coordinating Center (NACC). Biological samples such as CSF and whole blood are collected at the research visit. From these samples, a variety of derived samples can be obtained, including plasma, serum, DNA, RNA, and cells. These samples are integral to the research of many investigators within the ADRCs.

Since the early 2000s, the National Centralized Repository for Alzheimer's Disease and Related Dementias (NCRAD) has been a key partner to the ADRCs. NCRAD has been funded by the NIA to assist the ADRCs with their sample-sharing efforts. This began with the banking of a DNA sample at NCRAD from the well-characterized ADRC participants. The DNA at NCRAD was linked to the rich UDS at NACC, making these samples invaluable to the growing field of AD genetics.

In 2019, NCRAD developed a new partnership with the ADRCs focused on the central banking of a wider range of blood-based samples, including plasma, serum, RNA, and peripheral blood mononuclear cells (PBMCs). The focus of this NCRAD-ADRC collaboration was to support the rapidly expanding demand from the research community for high-quality samples paired with extensive clinical and imaging data (Uniform Data System [UDS]). This new initiative was called the Alzheimer's Disease Centers Fluid Biomarker (ADCFB) Initiative.

In response to the growing demand for access to BBBMs, NCRAD established the Biomarker Assay Laboratory (BAL) in 2021. The NCRAD BAL provides robust (highly standardized) and reliable (low variability) biomarker measurements for the commonly used blood and CSF tests. Like NCRAD, the BAL is designed to support the ADRCs as well as the broader research community in the study of Alzheimer's disease and related dementias (ADRD). While some ADRCs have a local biomarker lab, a partnership with NCRAD allows the in-house ADRC labs to utilize their instruments to focus on novel biomarker research rather than commercially available biomarkers with established clinical research utility. NCRAD BAL receives most samples for analysis from the ADRCs and investigators in the United States, but is able to analyze samples from across the globe.

TABLE 1 Assays offered in the NCRAD BAL.

Assay kits	Biofluid	Platform	Biomarkers	Qualification status
NF-Light Advantage Kit	Plasma	Quanterix Simoa HD-X	NfL	Complete
Neurology 2-Plex B (N2PB)	Plasma	Quanterix Simoa HD-X	NfL, GFAP	Complete
Neurology 4-Plex E (N4PE)	Plasma	Quanterix Simoa HD-X	NfL, GFAP, Aβ 40, Aβ 42	Complete
P-tau 181 v2.1 Advantage Kit	Plasma	Quanterix Simoa HD-X	P-tau 181	Complete
P-tau 217 Alzpath Kit	Plasma	Quanterix Simoa HD-X	P-tau 217	Complete
Lumipulse G β-Amyloid 1-42 Kit	Plasma	Fujirebio Lumipulse	Aβ 42	Complete
Lumipulse G β-Amyloid 1-40 Kit	Plasma	Fujirebio Lumipulse	Aβ 40	Complete
Lumipulse G P-tau 181 Kit	Plasma	Fujirebio Lumipulse	P-tau 181	Complete
Lumipulse G P-tau217 Kit v2	Plasma	Fujirebio Lumipulse	P-tau 217	Complete
Lumipulse G β-Amyloid 1-42 Kit	CSF	Fujirebio Lumipulse	Aβ 42	Complete
Lumipulse G β-Amyloid 1-40 Kit	CSF	Fujirebio Lumipulse	Aβ 40	Complete
Lumipulse G P-tau 181 Kit	CSF	Fujirebio Lumipulse	P-tau 181	Complete
CNS NULISAseq Panel	Plasma	Alamar ARGO HT	120 CNS disease-related biomarkers	Complete
Inflammation AQ NULISAseq Panel	Plasma	Alamar ARGO HT	250 inflammation biomarkers	Complete

Note: Three different platforms are available for use with qualified assays. The newest option is the Alamar ARGO HT for use with NULISAseq technology.
Abbreviations: BAL, Biomarker Assay Laboratory; CNS, central nervous system; CSF, cerebrospinal fluid; GFAP, glial fibrillary acidic protein; NCRAD, National Centralized Repository for Alzheimer's Disease and Related Dementias; NfL, neurofilament light chain.

3 | NCRAD BAL

3.1 | Purpose and development

The purpose of the NCRAD BAL is to provide high-quality, research-use-only, biomarker data for investigators and clinicians. Quality and consistency are the foremost principles underlying the NCRAD BAL. The AD biomarker field moves extremely quickly; therefore, both the best-in-class biomarkers and instrumentation or assays for these biomarker shifts significantly from year to year. To stay current, the NCRAD BAL was conceptualized to be able to quickly pivot and shift assays or platforms with the changes in the field while maintaining its focus on quality and consistency. To accomplish this goal, the lab established new assay qualification protocols to ensure appropriate assessments of the assays/platforms prior to implementation, as well as comparability studies when other assays or platforms are being used for the same biomarker. These protocols provide the necessary data and analysis to maintain comparability with previous platforms/assays wherever possible.

The NCRAD BAL is a quality-focused laboratory delivering biomarker data through the use of highly standardized procedures and highly automated instrumentation. Key instrumentation in the lab consists of two Tecan Fluent 1080 automated liquid handlers, two Quanterix Simoa HD-X, a Fujirebio Lumipulse G1200, and an Alamar ARGO HT for use with NULISAseq technology. The lab is currently equipped and staffed to analyze approximately 15,000 samples a year. Current key assays include plasma assays for NfL, GFAP, P-tau217, Aβ 40, and Aβ 42 on the Quanterix platform; Aβ 40, Aβ 42, on the Fujirebio platform; and CSF P-tau181, Aβ 40, and Aβ 42 only on the Lumipulse (Table 1). To date, the NCRAD BAL has analyzed over 28,687 plasma samples, 2,441 CSF samples, and generated over 129,500 biomarker results for researchers. The largest portion of the samples, 53%, has been analyzed for ADRCs participating in the ADCFB (Figure 1). 91.5% of the samples analyzed in the NCRAD BAL are plasma samples; the NCRAD BAL recently expanded its operations to include CSF analysis.

3.2 | Controlling pre-analytical variables

Understanding and controlling sources of variability is key to any laboratory's ability to generate consistent and comparable data. Pre-analytical variability can occur during the collection or preparation of the samples for analysis.[18,19] To limit variability during sample collection, NCRAD coordinating staff and the NCRAD BAL laboratory team participate in discussions with studies to provide guidance for collection protocols, train study staff in collection procedures, and provide standardized collection kits to help limit variability in collection supplies, sample storage tubes, and specimen labeling systems. More information about NCRAD protocols to ensure the collection of high-quality specimens is reviewed in Edler et al. in this issue.[20]

Once samples arrive at the NCRAD BAL, a thorough inventory of the specimens, specimen tube types, specimen quality, and quantity is recorded to ensure that any variability can be tracked alongside information about storage conditions and freeze/thaw cycles, which can influence biomarker results. In addition, prior to arriving within the BAL, samples are randomized into sets of 31 samples to minimize potential run order bias. The randomization includes age and sex at a minimum but can incorporate additional study-specific items, such as diagnosis, when necessary. If longitudinal specimens are sent to the NCRAD BAL, all specimens from a single participant are run on the same plate or as part of the same day's analysis. To further reduce possible laboratory bias, the technicians in the NCRAD BAL are blinded

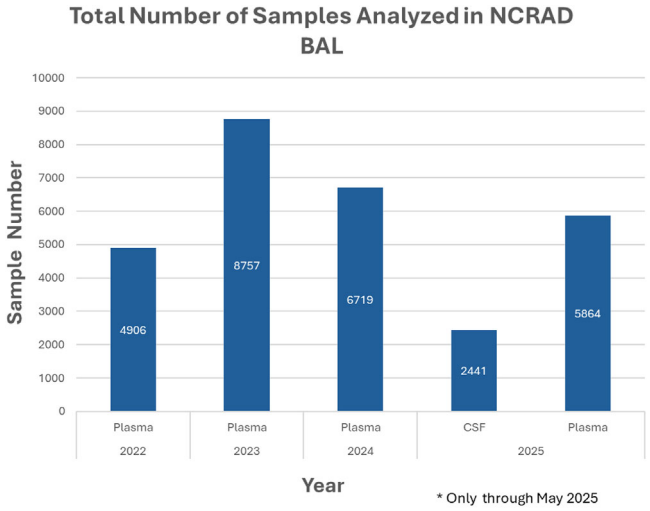

FIGURE 1 Number of samples analyzed by the NCRAD BAL. This reflects studies that have been completed through May 2025 and have had data returned. Several studies, like ADCFB, are ongoing. ADCFB, Alzheimer's Disease Centers Fluid Biomarker Initiative; BAL, Biomarker Assay Laboratory; NCRAD, National Centralized Repository for Alzheimer's Disease and Related Dementias

to any information about the specimens that could identify characteristics like disease state that could influence the interpretation. All blinding and randomization are performed outside of the BAL within the NCRAD Distributions team. Therefore, a third party not associated with the NCRAD BAL provides the plate layout to the team handling the samples.

3.3 | Standardized and controlled procedures

To ensure consistency in the lab, all major laboratory processes (assay qualification, bridging, sub-aliquoting, specimen preparation, assay procedure) follow standard operating procedures (SOPs). SOPs ensure that there is limited day-to-day or technician-to-technician variability in standard processes. To further limit technician-to-technician variability, all liquid handling procedures in the lab are handled by automation. For example, all specimen preparation for assays that require dilutions is pipetted by a Tecan Fluent 1080, which not only receives yearly preventative maintenance and assessments by the manufacturer but also receives biweekly testing to ensure all pipettes are performing to NCRAD BAL standards. In addition to limiting variability within the lab, strong record-keeping is practiced. Daily logs detailing key steps in the SOPs are maintained. These logs require technicians to initial and log the timing of all steps leading to assay completion, including any deviations from standard timing or issues that may arise, to ensure that any information required to properly interpret the data is available.

3.4 | Controlling for variability attributed to assay lots and instruments

One of the largest factors affecting the comparability of data, aside from lab-to-lab procedural differences, is lot-to-lot variability. As the development of plasma biomarker reference materials is ongoing, lot-to-lot variability can greatly affect the data being generated over time. For this reason, most labs or studies either hold specimen analysis to the end of the study or must run selected specimens in each analysis batch to limit the effects of the changing assay lots.

To counter lot effects, the NCRAD BAL has a local collection of plasma from AD and CN consented patients from a local medical facility. This collection of plasma provides the lab with samples that can be used to constantly monitor the performance of the lots as new assay lots arrive in the lab. To do this, NCRAD BAL runs 31 specimens from the local collection on both the old and the new lots in the lab. If the percent coefficient of variation (%CV) is more than 10%, calibrators on the new lot are adjusted to bring the lot into alignment with the previous lot. Bias is assessed, as is the new %CV. The change is documented, and the new lot is put into use. Additionally, this same method is used to assess instrument changes or instrument-to-instrument variability for any platform for which the lab has more than one instrument. In this case, the same kit lot is used, and the variable is the instrument. If the %CV is more than 10%, an additional study may be performed and a correction applied, documented, and put into use. The use of these methods allows comparability of all studies analyzed in the lab over time. This is critical for the field as it allows studies to be combined and data to be collected over the course of studies.

3.5 | Monitoring assay performance

To further assess consistency and comparability, several types of controls are utilized within the NCRAD BAL. All assays in the NCRAD BAL have manufacturer-provided controls. These controls are monitored and allow the staff in the lab to ensure that the assay is running properly. Additionally, the NCRAD BAL uses its local collection of plasma to create reference pools. Three plasma reference pools are created for each assay. The reference pools are created to cover three points across the range of expected values in large batches and monitored on every plate or every run day of a study. Multiple reference pools are created to be able to detect the different levels (high, medium, and low) of each biomarker being run for a particular assay. The samples are monitored such that $\pm$ 2 standard deviations are considered normal performance, and plates or run days outside these boundaries are investigated to determine if the data are valid. One instance of a single reference pool being outside the 2 standard deviation boundary is considered a random occurrence; a pattern of multiple plates with the

same issue or multiple reference pools with the issue is considered a failed run. The cause of the issue is determined, and the data will be re-run when the instrument or assay has been put back into use. Data for the controls and reference pools are available to any research study for the days on which their specimens were run.

3.6 | Participation in Global Biomarker Standardization Consortium Round Robin

In addition to the internal and manufacturer controls utilized in the lab, the NCRAD BAL also participates in an external quality monitoring program, the Global Biomarker Standardizataion Consortium (GBSC) Round Robin study, in which shared samples are provided by the Neurochemistry Lab in Gothenburg, and data are returned to monitor variability between labs. This is not meant to harmonize labs but rather to study inherent variability that is seen on various platforms for the most commonly utilized biomarkers.[21] The program details on its website that in round 45 of quarterly returns, 107 labs reported Lumipulse biomarker concentrations, and 32 labs reported Quanterix Simoa biomarker concentrations.[22] The NCRAD BAL reports both Lumipulse and Quanterix concentrations for plasma as well as Lumipulse concentrations for CSF.

3.7 | Qualification studies

As new assays demonstrate clear improvements or new biomarkers show consistent clinical utility, the NCRAD BAL will consider adding new platforms or assays to the offerings of the lab. NCRAD has developed a Biomarker Advisory Committee (BAC), composed of leaders in biomarker discovery, that will meet twice yearly to discuss emerging platforms, technology, and assays. The BAC will advise NCRAD on key platforms, technologies, and assays to ensure we remain aligned with the needs of ADRD researchers.

As part of this process, qualification studies are run. The most important aspects being assessed are the measuring range and precision of the assay. The local collection of plasma is used to select five plasma samples of varying concentrations (across the expected value range, as best as possible with the local collection of plasma) to create qualification study specimens. The qualification study spans 2 days to allow both intra- and inter-day precision to be assessed by analysis of each of the five samples four times per day. In addition to precision, the qualification study typically includes an assessment of dilutional linearity and parallelism; however, this varies depending on the type of assay and the usefulness of the assessment. For example, the additional assessments are needed when assessing an assay looking at a single biomarker; however, their utility may be less in an assay looking at 150 multiplexed biomarkers. Ultimately, the goal of the qualification study is to assess the assay in such a way that the BAL is able to characterize its performance and compare that performance to validation data provided by the manufacturer or in the literature. These data are key to determining the BAL's ability to deliver high-quality, robust, and reliable results over time before putting them into use for the researchers and across the ADRCs.

3.8 | Platform comparison

As biomarkers are identified, the number of commercially available platforms and assays increases. Typically, the NCRAD BAL will adopt the first commercially available assays due to demand from the research community, assuming the assays demonstrate adequate performance in the qualification study. In time, different companies and platforms will have assays for the biomarker come to market. If the available data suggest added value or better performance, the NCRAD BAL will perform a qualification study. If a different assay has been used to provide data in the NCRAD BAL already, a platform or assay comparison study will occur in addition to the qualification study.

The comparison study allows the NCRAD BAL to collect data to determine if it is necessary or possible to provide an equation that will allow data to be converted from the old assay's values to the new assay's values or vice versa. This is key to allowing continued comparisons of the previously analyzed samples while also moving along with the field to better performing assays. If this is not possible, studies must consider either not switching assays or re-analyzing specimens that have been analyzed on an older assay.

The comparability study utilizes specimens previously analyzed on the NCRAD BAL standard assays. The comparability study complements the qualification study in that specimens are chosen based on the availability of clinical diagnosis and PET data, which allows assessment of the ability of the new assay to predict disease status. To complete this portion of the assessment, ~100 samples, split between amyloid or tau PET positive and PET negative, are analyzed on the new assays. The data are used to generate receiver operating curves and assess the model fit for the biomarkers. Additionally, if the data are deemed comparable, typically employing *z*-scoring to standardize the data prior to comparison, the equation for the data conversion can be generated and provided to any studies requiring the information. It is most applicable if the data generating the equation are from the same study that utilizes the equation. For the NCRAD BAL, this means that it is most useful to utilize samples from the ADCFB Initiative.

Qualification and comparability studies are not just for new biomarkers or assays. These studies are employed when a company updates a kit and makes a new version of the kit commercially available. The data from the comparability study are utilized to determine, if not provided by the manufacturer, the equation for conversion of data from the previous version to the current version and vice versa. This is the same technique the BAL employs when platforms are switched. However, a new version of a kit is much more likely to retain compatibility than a switch across platforms. If a conversion equation is possible, NCRAD BAL provides it to studies.

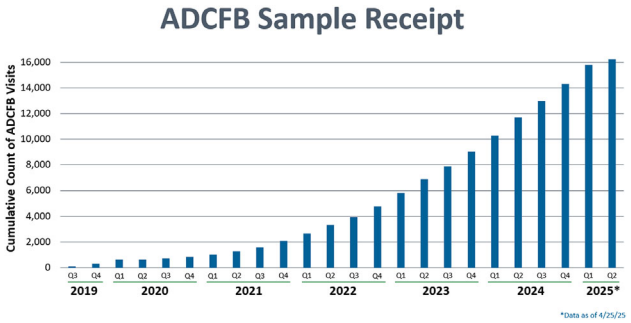

FIGURE 2 Cumulative number of participants' visit specimens received and banked at NCRAD as part of ADCFB Initiative. ADCFB, Alzheimer's Disease Centers Fluid Biomarker Initiative; NCRAD, National Centralized Repository for Alzheimer's Disease and Related Dementias

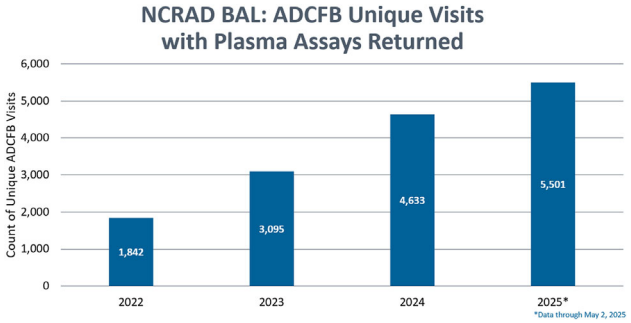

FIGURE 3 Number of unique visits with plasma biomarkers returned through the ADCFB Initiative. ADCFB, Alzheimer's Disease Centers Fluid Biomarker

4 | THE ADCFB INITIATIVE

4.1 | Overview

In 2019, NCRAD created the ADCFB Initiative, which expanded sample banking with the ADRCs to include not only DNA, but also plasma, serum, RNA, and PBMCs. The goal of the ADCFB Initiative was to rapidly generate a large set of samples to support the evaluation of promising BBBM. This initiative was expanded and supported through NIA funding at NCRAD.

A total of 71% percent of the ADRCs are now participating in the ADCFB Initiative. These ADRCs have already sent samples to NCRAD from over 8800 participants. Participation in the ADCFB Initiative requires the ADRCs to send NCRAD from each participant's visit, a buffy coat for DNA and plasma aliquots from 20 mL of blood collection. Some ADRCs also choose to provide specimens from sodium heparin tubes for PBMCs and/or CSF aliquots. To date, NCRAD has uniformly collected plasma samples from 16,000 ADRC participant visits (Figure 2).

An incentive for the ADRCs to participate in the ADCFB Initiative is that plasma biomarkers are returned to their center to support ongoing research analyses. Plasma biomarker data for NfL, GFAP, P-tau217, Aβ 42, and Aβ 40 are returned to the ADRCs for each participant visit, with plasma sent to NCRAD. The NCRAD BAL has analyzed and returned data for over 15,000 participant visits (Figure 3). As the AD field develops new commercially available assays, NCRAD takes ad hoc or solicits input from the ADRCs and the ADRC Biomarker Steering Committee to keep current on possible changes to the list of BBBMs or platforms supported by NCRAD.

4.2 | Collaboration between ADRCs: NCRAD-NACC

The infrastructure already in existence at NCRAD is key to the movement of specimens into the NCRAD BAL (Figure 4). On average, NCRAD receives plasma from approximately 125 participant visits per week from the ADRCs as part of the ADCFB Initiative. NCRAD covers the costs for the ADRCs to batch ship samples to NCRAD. Once samples arrive at NCRAD, they are accessioned into the NCRAD Laboratory Information Management system (LIMs), where the records of each unique sample identifier are linked with participant characteristics and sample collection and processing information submitted by the ADRCs to NCRAD.

Samples are transferred weekly to the NCRAD BAL from the NCRAD Accessioning Laboratory, at which time the samples are aliquoted into the volume required for biomarker analysis, and the residual is transferred back to NCRAD. The participant data received from the ADRCs are then used to arrange the runs for the biomarker analysis by age and sex, and also group longitudinal samples from the same ADRC participant together into the same run. The arranging of runs is done by a third party within NCRAD to ensure the BAL team is not biased. Once the run order is prepared, samples are analyzed on the NCRAD BAL standard assays. Data undergoes a rigorous, standardized quality control (QC), described earlier in this review.

Once the data pass the QC process, the biomarkers are returned to the ADRCs through NACC. The data have a 90-day embargo period during which the ADRC that sent the sample has sole access to the data. After the embargo period, the data are available to the research community. Access to the data requires that a data-use agreement be signed, and a determination is made whether the requester has for-profit or not-for-profit status. This determination is required to ensure that the participant's consent is upheld and respected.

As new biomarker assays or analysis technologies become commercially available, NCRAD assesses the utility of the new assays/technologies as guided by BAC. NCRAD will also confer with its executive committee, the ADRC Biofluid Biomarker Steering Committee, and receive feedback directly from the ADRCs when deciding if the biomarker assays will be implemented for ADCFB. These groups will also advise when biomarkers can be removed because there is sufficient clinical diagnostic availability.

4.3 | Cut point development

There is a series of attributes that make the ADCFB Initiative samples ideal for the development of cut points for biomarkers. These

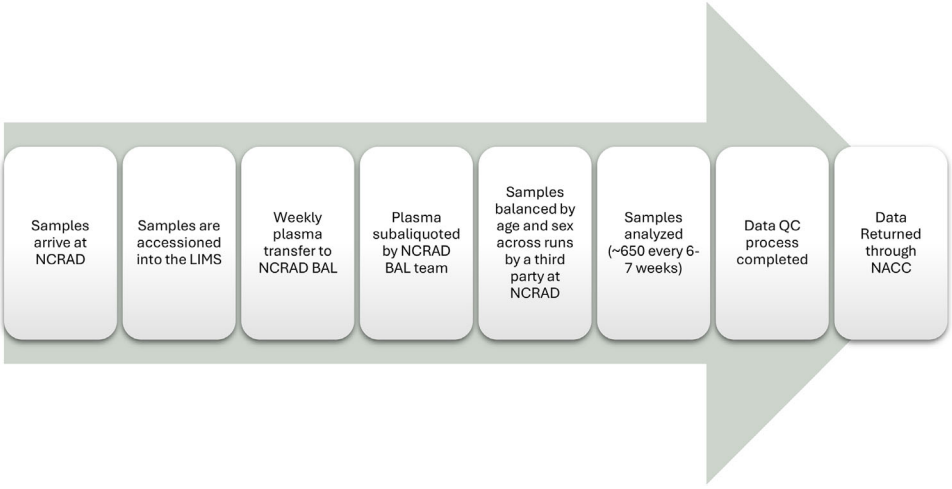

FIGURE 4 NCRAD BAL sample preparation and analysis process. BAL, Biomarker Assay Laboratory; NCRAD, National Centralized Repository for Alzheimer's Disease and Related Dementias

include the collection of these samples across the country rather than in one geographical region. In addition, participants are very well characterized, and the UDS is obtained and widely available at NACC. Furthermore, many of these participants are longitudinally assessed with biological samples and imaging, typically collected at the same visit. Finally, the plasma biomarkers are analyzed in a consistent and comparable manner through the NCRAD BAL.

Although the NCRAD BAL is generating data for multiple biomarkers, the initial cut point focus has been P-tau due to the great interest in this assay and the more limited age effects on these biomarkers. To date, NCRAD has developed cut points for P-tau181 and P-tau217 for the ADCFB Initiative study. NCRAD utilizes a single cut-point strategy, understanding that the variability within the assay guides what samples fall in the "gray area" around the cut point, in which a participant may require additional assessments. The cut point was initially developed using PET and neuropathology data from the Indiana ADRC and plasma biomarker data generated by NCRAD BAL. The cut point was validated using amyloid PET data available at NACC for a subset of the ADRC participants.[23]

To aid the ADRCs in their use of the fluid biomarker data, the NCRAD BAL has developed a visualization tool (https://ncrad.iu.edu/biomarker-analysis/adcfb-data-visualization).[24] This interactive tool allows researchers utilizing ADCFB Initiative data to enter the P-tau181 or P-tau217 value into their respective tool and see where the data from the visit fall with respect to the cut point and the distribution of all other ADCFB Initiative P-tau values and clinical diagnoses (Figure 5). This newest NCRAD BAL tool will continue to be updated quarterly as newly returned data move past its embargo period. Additionally, the NCRAD BAL team is working to add additional biomarkers, particularly those without cut points, to the visualization tool, including the Aβ 42/ Aβ 40 ratio.

The cut points developed as part of the ADCFB Initiative are representative of the samples sent to NCRAD BAL by the ADRCs around the country. The AD field, including the ADRCs, is making efforts to increase enrollment and engagement from underrepresented populations. Even with these efforts, there is not yet a fluid-based biomarker cut point that is universal for all populations; therefore, the use of the NCRAD BAL P-tau cut points should be critically evaluated prior to implementation with other studies that include populations not typically represented in the ADRCs.

5 | FUTURE DIRECTIONS

The NCRAD BAL was developed as a resource for researchers to assist with high-quality analysis of study samples to drive more rapid evaluation and use of BBBMs. It is intended that this research use only data will be utilized to more rapidly identify new clinical uses and support the movement of these biomarkers from research into clinical use for medical decision-making. Once the biomarkers are FDA-cleared and are regularly used in the clinic, it is anticipated that researchers will shift to using data from the patients' medical records or obtain research study data from a CLIA-accredited lab, such as hospital pathology labs (Figure 6). This will free up capacity to allow the NCRAD BAL to bring on new biomarkers coming to the forefront of research while being able to maintain the rigorous standards to which the lab is held. The focus on research-use-only assays also ensures that NCRAD BAL can rapidly respond to new science and support research that is ongoing at the ADRCs.

Recently, the Fujirebio Lumipulse P-tau217/Aβ 42 assays received 510(k) clearance by the FDA for use in those 55 and older with clinical signs of dementia. It is anticipated that clinicians/studies utilizing these tests will shift from the NCRAD BAL to pathology labs; therefore, the NCRAD BAL has implemented a new assay platform, the Alamar ARGO HT with NULISA technology, to focus on up-and-coming biomarkers and the search for vascular and inflammation-related biomarkers, thereby supporting the ADRC research components. NCRAD BAL will not stop providing any assay until ADRCs are able to order these

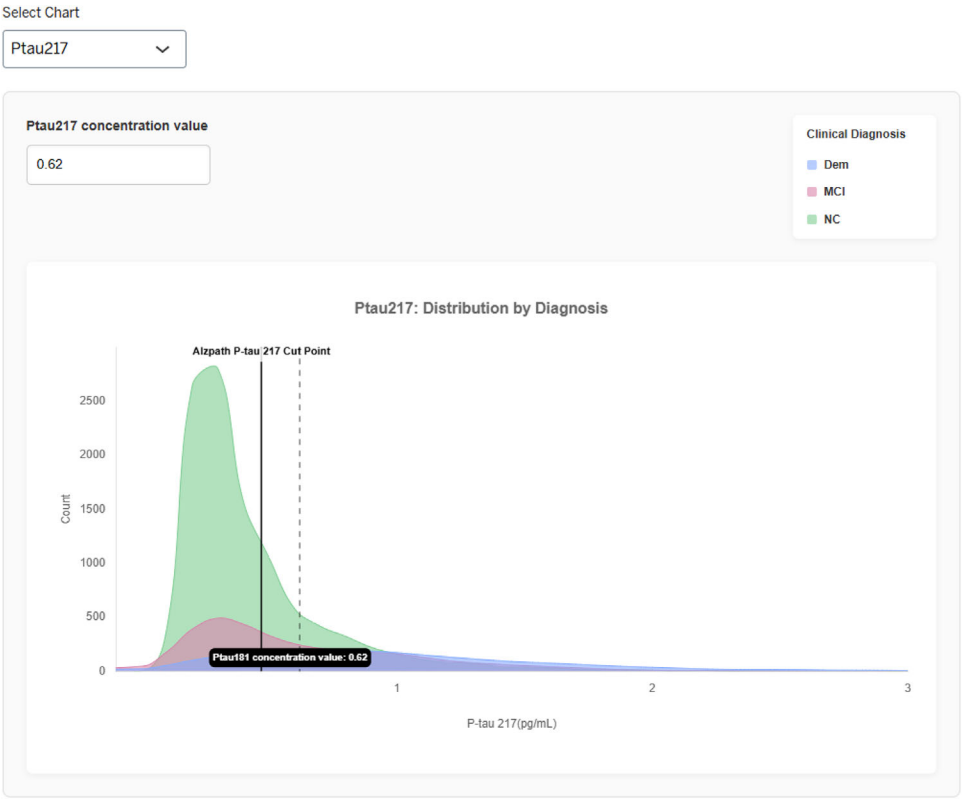

FIGURE 5 NCRAD BAL ADCFB data visualization tool. ADCFB, Alzheimer's Disease Centers Fluid Biomarker Initiative; BAL, Biomarker Assay Laboratory; NCRAD, National Centralized Repository for Alzheimer's Disease and Related Dementias

assays for prodromal and healthy controls who do not have AD; therefore, there will be a period of time where the BAL continues to offer FDA approved assays to ensure important ADRC research is able to continue uninterrupted.

The Alamar ARGO HT, in combination with the NULISAseq assay panels, holds great potential for studies interested in biomarkers of not only AD but also other neurodegenerative disorders and co-pathologies of AD. The NULISAseq assays are much larger multiplexes than are available on the Quanterix Simoa platform, and work assessing multiple biomarkers as signatures for disease states is ongoing. A limitation at this time is that not all biomarkers generate data for absolute quantitation.[25]

Most research assays use non-standardized external calibrator material, resulting in reporting of units relative to an external calibrator (RU) and often use the concentration of the calibrator in place of the RU (e.g., pg/mL). In the case of NULISAseq panels and most multiplex panels, it is inefficient, difficult to control, and costly to prepare external non-standardized calibrator materials, so assays are not calibrated and, thus, only report relative units in relation to or relative to an internal measure within the assay. This relationship across the measuring range is usually established during assay/panel creation and monitored/checked as part of manufacturing. However, this assay is now considered to report results in RU, relative to an internal non-analyte-related reference. Using any approach still results in an assay that can be used to define and establish a cut point and monitor longitudinal changes.[26] The actual performance across manufacturing lots and over time, as source materials change, will determine if robust and reliable assay performance is achievable.

The NULISAseq assays are different from the standard Quanterix and Fujirebio assays in that, rather than directly generating results on the instrument, the large multiplexes like the NULISA Inflammation AQ Panel and the CNS Panel generate a pooled library, which then must undergo next generation sequencing (NGS) on an independent platform.[27] The Alamar ARGO HT and NULISAseq technology is desirable because of the number of multiplexed biomarkers available (upwards of 120 on the previously mentioned panels) as well as the 25 µL assay volume. However, with the complexity of the multiplexed assays, comparability between current assay platforms (Quanterix, Fujirebio) and lot-to-lot or instrument-instrument variability may be complex questions. Therefore, having the Alamar platform and assays in the NCRAD BAL, given the lab's experience with assessing and monitoring different assay platforms as well as the standardized procedures and quality control, will be of great value to the ADRCs as evaluations of new biomarkers occur.

As the field makes strides with new assay development and new biomarker identification, the NCRAD BAL will be assessing the use of these assays and biomarkers in the hopes of aiding researchers in the identification of co-pathologies. For example, currently, the field is working to move the α-synuclein seed amplification assay into plasma. As this work progresses and assays become commercially available, it would be very important that the NCRAD BAL bring on the assay

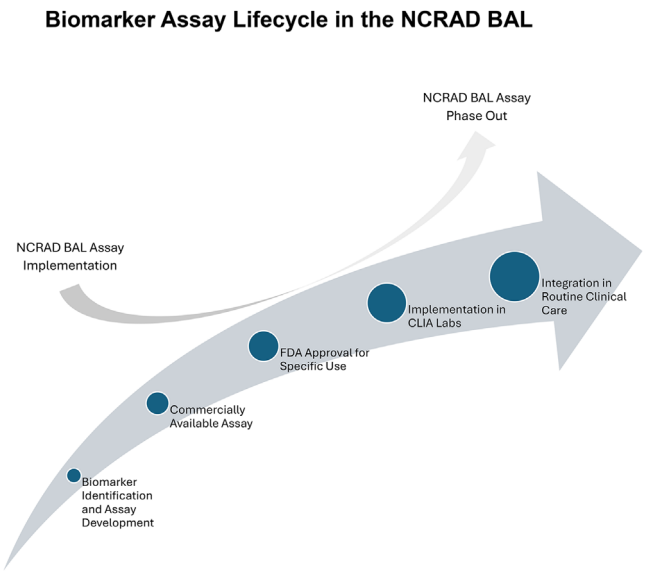

FIGURE 6 As biomarkers currently used in research-use-only assays transition into assays used routinely by clinicians, the NCRAD BAL will transition into providing new research-use-only assays for newly identified, commercially available biomarkers. Of particular interest are biomarkers of AD co-pathologies. BAL, Biomarker Assay Laboratory; NCRAD, National Centralized Repository for Alzheimer's Disease and Related Dementias

to support the ADRCs and assist the field in the rapid assessment of possible clinical uses of such a test.

Additionally, new biomarkers for vascular dementia and frontotemporal lobar degeneration (FTLD) are being researched. The Alamar platform and assays for inflammatory markers may be a great step towards identifying key plasma biomarkers or combinations of biomarkers for early diagnosis and treatment; however, the field moves quickly, and new platforms may come to the forefront. A centralized lab with the resources, standardized procedures, and quality control monitoring systems to ensure robust and reliable data will be essential as new biomarkers and new assay platforms become commercially available.

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CONFLICT OF INTEREST STATEMENT

J.L.D. is an inventor on patents or patent applications assigned to Eli Lilly and Company relating to the assays, methods, reagents, and/or compositions of matter for P-tau assays and Aβ targeting therapeutics. J.L.D. has/is served/serving as a consultant or on advisory boards for Eisai, Abbvie, Genotix Biotechnologies Inc, Gates Ventures, Gate Neurosciences, Dolby Family Ventures, Karuna Therapeutics, Alzheimer's Disease Drug Discovery Foundation, AlzPath Inc., Cognito Therapeutics, Inc., Eli Lilly and Company, Prevail Therapeutics, Neurogen Biomarking, Spear Bio, Rush University, University of Kentucky, Tymora Analytical Operations, MindImmune Therapeutics, Inc, Early is Good, and Quanterix. J.L.D. has received research support from ADx Neurosciences, Fujirebio, Roche Diagnostics and Eli Lilly and Company in the past two years. J.L.D. has received speaker fees from Eli Lilly and Company and LabCorp. J.L.D. is a founder and advisor for Monument Biosciences and Dage Scientific LLC. J.L.D. has stock or stock options in Eli Lilly and Company, Genotix Biotechnologies, MindImmune Therapeutics Inc., AlzPath Inc., Neurogen Biomarking, and Monument Biosciences. K.A.R. has received in-kind kits for assessment from Fujirebio and Alamar Biosciences. Both Guest Editor Andrew Saykin, PsyD, and Editor-in-Chief Donna Wilcock, PhD, hold faculty appointments at the same institution as the authors. Additionally, NCRAD banks, analyzes, and distributes some of Dr. Saykin's research samples. NCRAD has also distributed samples for Dr. Wilcock's research. No other conflicts of interest are reported by T.F. Author disclosures are available in the Supporting Information.

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SUPPORTING INFORMATION

Additional supporting information can be found online in the Supporting Information section at the end of this article.

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