C_2N Precivity Test Methods

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Contents

Background:

The analysis will be performed using C_2N's PrecivityAD2™ CLIA assay which comprises of the Precivity-Aβ, and Precivity-%p-tau217 tests and reports Aβ42, Aβ40, p-tau217, np-tau217, Aβ42/40, %p-tau217, and APS2 score. PrecivityAD2 assay has been clinically validated and results are published in Meyer et al 2024. Each of the analyte components of PrecivityAD2 test are analytically validated. Analytical validation of Precivity-Aβ has been published in Kirmess et al 2021. Analytical validation of Precivity-%p-tau217 has been published in Eastwood et al 2024. Apolipoprotein E (ApoE) proteotype is qualitatively measured in plasma samples simultaneously with Amyloid proteins using C_2N's Precivity-ApoE proteotype assay (Kirmess et.al.. 2021).

Methods and Procedures

	C_2N **Diagnostics**
Assays	PrecivityAD2™: Aβ42, Aβ40, Aβ42/40 ratio, p-tau217, np-tau217, p-tau217/np-tau217 ratio, and Amyloid Probability Score 2 (APS2) score Precivity-ApoE ™
Platform	Precivity liquid chromatography tandem mass spectrometry (LC-MS)
Performing Laboratory	C_2N Diagnostics CAP/CLIA laboratory in St. Louis, Missouri, USA

The PrecivityAD2™ test is an analytically and clinically validated blood test that aids healthcare providers in ruling in or ruling out AD in patients presenting with MCI or dementia. The PrecivityAD2™ blood test simultaneously quantifies specific plasma amyloid beta and tau peptide concentrations. The Ratios are combined into a proprietary statistical algorithm to calculate the Amyloid Probability Score 2 (APS2), a numerical value ranging from 0-100, that

determines whether a patient is Positive (has high likelihood) or Negative (has low likelihood) for the presence of brain amyloid plaques by amyloid PET scan to calculate the Aβ42/40 Ratio and p-tau217/np-tau217 (p-tau217 Ratio).

The Amyloid Beta 42 and 40 peptide concentrations are measured in plasma samples by an immunoprecipitation-mass spectrometry (IP/MS) workflow. There are two separate measurements that are performed on each sample, one that measures an Aβ42-specific peptide and one that measures an Aβ40-specific peptide. These measurements are made as part of a multiplexed LC-MS/MS assay where both peptides are measured from the same sample in the sample processing and LC-MS/MS runs as described in Kirmess et. al. 2021.

Tau217 protein is enriched through immunoprecipitation with a tau-specific antibody. Liquid chromatography-mass spectrometry is used to separate, identify, and quantify the concentrations of tau peptides (tau amino acids 212–221) phosphorylated at Thr-217 (p217) or not phosphorylated at Thr-217 (np217) in human plasma. These measurements are made as part of a multiplexed LC-MS/MS assay where both peptides are measured from the same sample in the sample processing and LC-MS/MS runs as described in Eastwood et. al. 2024

In general plasma aliquots were combined, and the final sample volumes were prepared using a Hamilton STAR® liquid handler and a Kingfisher Flex device. The samples were analyzed in 96-well plate format on Waters Acquity M-Class liquid chromatography (LC) units interfaced to Thermo Fusion Lumos Tribrid mass spectrometers (MS/MS).

Apolipoprotein E (ApoE) proteotype is qualitatively measured in plasma samples using C2N's ApoE proteotype assay (Kirmess et.a.. 2021) for each of the six APOE genotypes (APOE2/2, APOE2/3, APOE2/4, APOE3/3, APOE3/4, APOE4/4) by a combination of the presence or absence of the four targeted APOE isoform specific tryptic peptides. The presence or absence of an APOE isoform specific peptide is determined by monitoring the peak areas for each peptide. Peak areas above the previously determined Limit of Detection (LoD) are considered present, and peak areas below the LoD are considered absent.

APS2 values were calculated as reported in Meyer et al (2025)

Data Description

Data provided over the course of this study will include Aβ42, Aβ40, p-tau217, np-tau217 concentrations in plasma, APOE proteotype, Aβ42/40 and p-tau217/np-tau217 ratios, as well as the Amyloid Probability Score 2 (APS2), which is determined by entering the Aβ and tau217 ratios into an algorithm. APS2 determines the likelihood that a participant will be amyloid positive or negative and is provided on a 0-100 scale. A score of 0-47 corresponds to an amyloid negative PET (positron emission tomography) scan and a score of 48-100 corresponds to an amyloid positive PET scan.

This data import includes sample data from the first and second batch analysis of ADNI4 in-clinic participant plasma samples, with a total number of 676 samples included. Additional sample metric information is contained in Table 1 below.

Condition	Batch 1	Batch 2
Date first sample received	24Sep2024	14Mar2025
Date last sample reported for this period	21Nov2024	24Jun2025
Number of Samples Shipped to C_2N	232	444
Samples with Missing Abeta Values Due to Contaminant	5	3
Samples with Missing pTau Values Due to Contaminant	2	0
Samples with Missing pTau Values Due to QNS	1	0
Samples with Missing pTau Values Due to ALQ	1	0
Samples with Missing APOE Values	0	1
Samples with All Reportable Results	223	440

There were a total of 9 runs for each of the analytes reported; AB42, AB40, p-tau217, np-tau217, and ApoE. All QCs and Calibrators met the acceptance criteria, and all runs passed. However, during the analysis of the second batch of samples, one run of AB42, AB40 from date 23Apr2025 had one failed QC, the remaining QCs and calibrators all met the acceptance criteria. On further examination this run was considered pass as documented in NTF-3 and all runs are considered passing.

User Guidance

1. For ADNI Researchers, when you download C_2N plasma biomarker data from LONI, PLEASE:

   Cite and describe C_2N analytical methods in manuscripts, abstracts, grant applications, and any publicly accessible documents using any of these relevant articles.

   Eastwood, Stephanie M., Meyer, Matther R., Kirmess, Kristopher M., Wente-Roth, Traci L., Irvin, F., Holubasch, Mary S., Verghese, Phillip B., West, Tim, Braunstein, Joel B., Yarasheski, Kevin E., Contois, John H. "PrecivityAD2™ Blood Test: Analytical Validation of an LC-MS/MS Assay for Quantifying Plasma Phospho-tau217 and Non-Phospho-tau217 Peptide Concentrations that are used with Plasma Amyloid-β42/40 in a Multianalyte Assay with Algorithmic Analysis for Detecting Brain Amyloid Pathology" Diagnostics (Basel) 10 August 2024 14(16):1739 https://doi.org/10.3390/diagnostics14161739

   Kirmess, K. M., Meyer, M. R., Holubasch, M. S., Knapik, S. S., Hu, Y., Jackson, E. N., Harpstrite, S. E., Verghese, P. B., West, T., Fogelman, I., Braunstein, J. B., Yarasheski, K. E., & Contois, J. H. (2021). The PrecivityADTM test: Accurate and reliable LC-MS/MS assays for quantifying plasma amyloid beta 40 and 42 and apolipoprotein E proteotype for the assessment of brain amyloidosis. Clinica Chimica Acta, 519, 267–275. https://doi.org/10.1016/j.cca.2021.05.011

Meyer, M. R., Kirmess, K. M., Eastwood, S., Wente-Roth, T. L., Irvin, F., Holubasch, M. S., Venkatesh, V., Fogelman, I., Monane, M., Hanna, L., Rabinovici, G. D., Siegel, B. A., Whitmer, R. A., Apgar, C., Bateman, R. J., Holtzman, D. M., Irizarry, M., Verbel, D., Sachdev, P., … West, T. (2024). Clinical validation of the PrecivityAD2 blood test: A mass spectrometry-based test with algorithm combining %p-tau217 and Aβ42/40 ratio to identify presence of brain amyloid. Alzheimer's & Dementia, 20(5), 3179–3192. https://doi.org/10.1002/alz.13764

2. Refer to this Data Dictionary when interpreting C_2N values and results:

Item	Explanation	Notes
QNS	Quantity (sample volume) not sufficient for sample analysis	minimum 1.5 mL frozen EDTA plasma
LOD	Value is below the limit of quantitation for indicated analyte	
ALQ	Value is above the limit of quantitation for indicated analyte	
Sample_ID	Sample Identifier	
AB40	C_2N Abeta 40 concentration	pg/mL
AB42	C_2N Abeta 42 concentration	pg/mL
RATIO_AB4240	C_2N Abeta 42/40 ratio	If AB40 or AB42 is LOD or ALQ, no RATIO can be reported
PTAU217	C_2N Phosphorylated tau217 concentration	pg/mL
NPTAU217	C_2N Non phosphorylated tau217 concentration	pg/mL
RATIO_PTAU217	C_2N % phosphorylated tau217	If PTAU217 or NPTAU217 is LOD or ALQ, no RATIO can be reported
APS2	Amyloid Probability Score 2 (APS2) result for PrecivityAD2™	0 - 100; >47.5 = high likelihood brain amyloid positive; or <47.5 = high likelihood brain amyloid negative
NA	Not Available	
IP	Interference/Contaminant present	No value can be reported
Hemolyzed	Excessive hemolysis in sample	No value can be reported
Icteric	Excessive bilirubin	No value can be reported
Lipemic	Excessive lipids	No value can be reported

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The National Institutes of Health: NIH SBIR R44 AG059489; BrightFocus Foundation: CA2016636; Alzheimer's Drug Discovery Foundation: GC-201711-2013978, and a charitable contribution from GHR Foundation.

Version Information

Is this an update to a previous existing data set? If yes, then please note what has changed between this version, and the previous version dated [xx-yy-zzz]: Yes, this update contains additional information regarding the second batch of samples.

This is the second version of the dataset from C_2N. Any future dataset uploads will include this dataset along with any additional new data. The name of the file for upload is : C2N_PRECIVITYAD2_PLASMA.csv

References

1. Kirmess, Kristopher M., Meyer, Matthew R., Holubasch, Mary S., Knapik, Stephanie S., Hu, Yan, Jackson, Erin N., Harpstrite, Scott E., Verghese, Phillip B., West, Tim, Fogelman, Ilana, Braunstein, Joel B., Yarasheski, Kevin E., Contois, John H. "The PrecivityAD™ test: Accurate and reliable LC-MS/MS assays for quantifying plasma amyloid beta 40 and 42 and apolipoprotein E proteotype for the assessment of brain amyloidosis" *Clinica Chimica Acta 519 (2021) 267-275* https://doi.org/10.1016/j.cca.2021.05.011

2. Meyer, Matthew R., Kirmess, Kristopher M., Eastwood, Stephanie, Wente-Roth, Traci L., Irvin, Faith, Holubasch, Mary S., Venkatesh, Venky, Fogelman, Ilana, Monane, Mark, Hanna, Lucy, Rabinovici, Gil D., Siegel, Barry A., Whitmer, Rachel A., Apgar, Charles, Bateman, Randall J., Holtzman, David M., Irizarry, Michael, Verbel, David, Sachdev, Pallavi, Ito, Satoshi, Contois, John, Yarasheski, Kevin E., Braunstein, Joel B., Verghese, Phillip B., West, Tim "Clinical validation of the PrecivityAD2 blood test: A mass spectrometry-based test with algorithm combining %p-tau217 and Aβ42/40 ratio to idenfity presence of brain amyloid" *Alzheimer's & Dementia The Journal of the Alzheimer's Association* 16 March 2024 ***https://doi.org/10.1002/alz.13764***

3. Eastwood, Stephanie M., Meyer, Matther R., Kirmess, Kristopher M., Wente-Roth, Traci L., Irvin, F., Holubasch, Mary S., Verghese, Phillip B., West, Tim, Braunstein, Joel B., Yarasheski, Kevin E., Contois, John H. "PrecivityAD2™ Blood Test: Analytical Validation of an LC-MS/MS Assay for Quantifying Plasma Phospho-tau217 and Non-Phospho-tau217 Peptide Concentrations that are used with Plasma Amyloid-β42/40 in a Multianalyte Assay with Algorithmic Analysis for Detecting Brain Amyloid Pathology" *Diagnostics (Basel) 10 August 2024 14(16):1739* ***https://doi.org/10.3390/diagnostics14161739***

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