510(k) SUBSTANTIAL EQUIVALENCE DETERMINATION
DECISION SUMMARY
ASSAY AND INSTRUMENT

I Background Information:

A 510(k) Number

K253240

B Applicant

C2N Diagnostics, LLC

C Proprietary and Established Names

PrecivityAD2 test

D Regulatory Information

Product Code(s)	Classification	Regulation Section	Panel
SET	Class II	21 CFR 866.5840 – Alzheimer's Disease Pathology Assessment Test	IM-Immunology

II Submission/Device Overview:

A Purpose for Submission:

New device

B Measurand:

β-Amyloid 1-42
β-Amyloid 1-40
Phosphorylated-Tau 217 (p-tau 217)
Non-phosphorylated-Tau 217 (np-tau 217)

C Type of Test:

Immunoprecipitation followed by high resolution liquid chromatography tandem mass spectrometry (LC-MS/MS).

III Intended Use/Indications for Use:

A Intended Use(s):

See Indications for Use below

B Indication(s) for Use:

The PrecivityAD2 test is an in vitro device that uses immunoprecipitation followed by liquid chromatography-tandem mass spectrometry (LC-MS/MS) to quantify β-Amyloid 1-42 (Aβ42) and β-Amyloid 1-40 (Aβ40) peptide concentrations, and phosphorylated and non-phosphorylated tau protein at amino acid threonine, position 217 (p-tau217 and np-tau217) peptide concentrations in human K2-EDTA plasma.

An algorithm combines the Aβ42/40 ratio and the p-tau217/np-tau217 ratio x 100 (%p-tau217) to generate the Amyloid Probability Score 2 (APS2) result, a numeric value ranging from 0-100.

The PrecivityAD2 test is indicated for use in adults aged 40 years and older who present with signs or symptoms of cognitive impairment undergoing evaluation for Alzheimer's disease or other forms of cognitive decline.

The PrecivityAD2 test is intended to be used in conjunction with clinical assessment to aid health care professionals experienced in the evaluation of cognitive impairment to identify patients with amyloid pathology associated with Alzheimer's disease.

The test is not intended as a screening or stand-alone diagnostic test.

C Special Conditions for Use Statement(s):

Rx- For Prescription Use Only

D Special Instrument Requirements:

Waters Acquity M-Class HPLC
Waters Acquity M-Class Trap Valve Manager
Waters Acquity M-Class Autosampler
Thermo Scientific Fusion Lumos Tribrid Mass Spectrometer

IV Device/System Characteristics:

A Device Description:

The PrecivityAD2 test is a multianalyte assay with algorithmic analysis (MAAA). The PrecivityAD2 test consists of two individual multiplexed LC-MS/MS-based assays that are run separately using different reagents and LC-MS/MS instrumentation methods but use the same patient sample and sample matrix:

- C2N-Aβ42/40 Assay measures the concentrations of Aβ42 and Aβ40 in human K2-EDTA plasma and the quantity of each peptide in pg/mL is used to calculate the Aβ42/Aβ40 ratio
- C2N-%p-tau217 Assay is processed separately from the C2N-Aβ42/40 Assay and quantifies the concentration of p-tau217 and np-tau217 in K2-EDTA plasma. The quantity of each peptide in pg/mL is used to calculate %p-tau217 ([p-tau217]/[np-tau217]) ×100).

These ratios are combined in a proprietary algorithm to generate the Amyloid Probability Score 2 (APS2), a numeric value ranging from 0 to 100, which is evaluated against clinical cutoffs, to indicate whether a subject is likely to have brain amyloid pathology.

The assays utilize immunoprecipitation enrichment, proteolytic digestion, stable isotope-labeled internal standards, liquid chromatography separation, and tandem mass spectrometry detection of absolute quantitation of target analytes. The critical reagents required to perform the assays are listed in the table below.

Critical reagents	C2N-Aβ42/40 Assay	C2N-%p-tau217 Assay
Immunoprecipitation, protein digestion, and peptide clean-up		
Monoclonal immune-capture antibody	Proprietary anti-amyloid-beta antibody HJ5.1	Proprietary anti-Tau antibody Tau1
Dynabeads and antibody coupling kit	Magnetic beads for direct coupling of antibody HJ 5.1	Magnetic beads for direct coupling of antibody Tau1
Protease	Soluble LysN protease to digest Aβ42 and Aβ40 proteins	SOLu-Trypsin protease to digest p-tau217 and np-tau217 proteins
Enolase digestion	Soluble carrier peptides	Not applicable
Internal standard (ISTD) peptides	Individual heavy. isotope-labeled ^{15}N-Aβ40 and 1^{5}N-Aβ42 full-length recombinant proteins that are spiked into every sample, calibrator, and control for normalization and quantitation of assay results	Individual chemically synthesized ^{13}C/^{15}N tau-51 peptides (amino acids 171–221)phosphorylated and not phosphorylated at amino acid threonine 217 with a single ^{3}C/^{15}N arginine residue at amino acid 221
Recombinant human serum albumin protein (rHSA)	Solubilized rHSA is used to make the final stock of ISTD peptides	Solubilized rHSA is used to make the final stock of ISTD peptides
Calibrator and quality control peptides	Individual ^{14}N-Aβ40 and ^{14}N-Aβ42 recombinant proteins solubilized in 100% formic acid	Individual chemically synthesized tau-51 peptides (amino acids 171–221) phosphorylated and not phosphorylated at amino acid threonine 217 solubilized in 10% Acetonitrile/0.1% formic acid
Charcoal stripped human plasma	Matrix used to create five levels of calibrator material	Not applicable
Human defibrinated plasma	Matrix used to create three levels of quality control material.	Matrix used to create six levels of calibrator and three levels of quality control material.

Critical reagents	C2N-Aβ42/40 Assay	C2N-%p-tau217 Assay
Solid phase extraction media plate	Hydrophilic-lipophilic balanced reversed-phase 96-well plate for peptide clean-up	Hydrophilic-lipophilic balanced reversed-phase 96-well plate for peptide clean-up
Mass Spectrometry		
High-pressure liquid chromatography (HPLC) column	nanoEase M/Z BEH C4 Trap Column: 300Å,5 µm Separation Column:300 µm X 50 mm	nanoEase M/Z HSS C18 T3, 100A, 1.8 µm, 75 µm x 150 mm

B Principle of Operation:

The patient sample is processed to quantify the Aβ42/Aβ40 ratio and %p-tau217 ratio using the LC-MS/MS assays as described below.

1. Antibody-coupled beads and internals standards (ISTDs) are mixed with patient samples, quality control (QC) samples, and calibrators in immunoprecipitation buffer in deep-well 96-well plates on the Hamilton Microlab STAR liquid handling robot and incubated at room temperature to allow antibody-protein binding.
2. The 96-well plate is manually transferred to the KingFisher Flex magnetic bead washer. The antibody-coupled magnetic beads are washed with immunoprecipitation wash buffer to remove unbound proteins.
3. The 96-well plate with precipitated magnetic beads is manually transferred to the Hamilton Microlab STAR liquid handling robot and LysN protease in LysN digestion buffer or trypsin endoproteinase in trypsin digestion buffer is added to each well. For the C2N-Aβ42/40 Assay, Lys-N endoproteinase to generate C-terminal peptides that are specific for the Aβ42 and Aβ40 isoforms of β-amyloid protein. For the C2N-%p-tau217 Assay, trypsin endoproteinase generates two peptides that contain amino acids 212 to 221 of the tau protein: one peptide that is phosphorylated on amino acid threonine 217 (sequence TPSLP(p)TPPTR); and one peptide that is not phosphorylated on amino acid threonine 217 (sequence TPSLPTPPTR).
4. The 96-well plate is manually transferred to the thermomixer and proteins are digested at 37°C.
5. The 96-well plate is manually transferred to the Hamilton Microlab STAR liquid handling robot and placed on a 96-well plate magnetic stand. Digested peptides in the supernatant are aspirated off the magnetic beads and purified on SPE plates, washed with SPE wash buffer, and eluted with SPE elution buffer into a 96-well SPE collection plate.
6. The 96-well plate is transferred to the TurboVap to lyophilize purified peptides.
7. The 96-well plate is manually transferred to the Hamilton Microlab STAR liquid handling robot and purified peptides are redissolved in peptide solubilization buffer.
8. Dissolved peptides in 96-well plates are manually transferred onto the Waters Acquity M-class Autosampler and separated and eluted chromatographically on the Waters Acquity M-class HPLC.
9. Eluted peptides are detected and quantified in the Fusion Lumos Tribrid mass spectrometer. which can distinguish peptides based on the difference in their m/z. For each run, each of the calibrators is analyzed in duplicate and the mean value for each calibrator is used to generate the calibration curves.
10. Data acquisition and quantitative analysis of the peak area ratio of the endogenous peptides to the corresponding internal standards were performed using Thermo Scientific TraceFinder General Quan software.

In the result reporting step, the Aβ42/Aβ40 ratio and %p-tau217 ratio are combined using a proprietary algorithm (PAD3 API software) to generate the patient's APS2 result which is compared against two clinical cutoffs (APS2=66.5 and APS2=27.5) to determine whether the subject is likely to have brain amyloid pathology. The APS2 result is interpreted according to the table below:

APS2^	**Result**	**Result Interpretation**
APS2 > 66.5	Positive	A positive result is consistent with the presence of brain amyloid pathology. This result increases the likelihood that a patient's clinical presentation is due to Alzheimer's disease. The APS2 result alone does not establish a diagnosis of clinical Alzheimer's disease. Clinical correlation is recommended.
27.5* < APS2 < 66.5	Likely Positive	A likely positive result is consistent with the presence of brain amyloid pathology. This result increases the likelihood that a patient's clinical presentation is due to Alzheimer's disease. The APS2 result alone does not establish a diagnosis of clinical Alzheimer's disease. Additional evaluations and/or further testing may be appropriate evaluations and/or further testing may be appropriate.
APS2 < 27.5*	Negative	A negative result is not consistent with the presence of brain amyloid pathology. This result decreases the likelihood that the patient's clinical presentation is due to Alzheimer's disease. Other potential causes for the patient's cognitive symptoms should be considered.

* Samples with initial APS2 values between 17 and 45 are re-analyzed using the existing processed specimen. A second LC-MS/MS injection is performed on the same processed specimen to remeasure p-tau217 and np-tau217 and recalculate %p-tau217; no additional blood collection is required. The processed specimen undergoing reanalysis to remeasure p-tau217 and np-tau217 will be kept refrigerated until all the reanalysis has been completed. The final APS2 result is calculated using the average of the initial and repeat %p-tau217 measurements. Aβ40 and Aβ42 are not re-analyze.

^ If APS2 cannot initially be calculated because one or more analyte results are outside the applicable Analytical Measuring Interval (AMI) (with the exception for p-tau217 below the Limit of Quantitation (LoQ); see below), the submitted specimen will not be retested and further recollection for APS2 testing is not recommended. If one or more mass spectrometry flags (i.e. ion ratio failure) are present, the submitted specimen will be retested. If the mass spectrometry flag persists upon retesting, an APS2 result cannot be determined. A new specimen may be collected and tested to address potential specimen-specific or pre-analytical factors

- If the Aβ40 result within the C2N-Aβ42/40 Assay is < 50 pg/mL or > 1,000 pg/mL, APS2 cannot be calculated.
- If the Aβ42 result within the C2N-Aβ42/40 Assay is < 5 pg/mL or > 100 pg/mL, APS2 cannot be calculated.
- If the p-tau217 result within the C2N-%p-tau217 Assay is > 50 pg/mL, APS2 cannot be calculated.
- If the p-tau217 result is < 1.3 pg/mL, then the input for p-tau217 used to calculate APS2 will be 0.65.
- If the np-tau217 result within the C2N-%p-tau217 Assay is < 6.6 pg/mL or > 400 pg/mL, APS2 cannot be calculated.

C Instrument Description Information:

1. Instrument Name:
   Waters Acquity M-Class HPLC
   Waters Acquity M-Class Trap Valve Manager
   Waters Acquity M-Class Autosampler
   Thermo Scientific Fusion Lumos Tribrid Mass Spectrometer

2. Specimen Identification:
   There are no specimen identification methods on the instruments. Specimen identification is done manually prior to sample vials being placed on the instrument.

3. Specimen Sampling and Handling:
   Specimens are manually retrieved from storage, processed using a combination of manual and robotic methods, and then placed manually on the instrument.

4. Calibration:
   Calibrators are processed the same as patient samples and a calibration curve is generated by the instrument software.

5. Quality Control:
   Three quality controls are processed the same as patient samples and tested in each run with patient samples.

V Substantial Equivalence Information:

A Predicate Device Name(s):

Lumipulse ***G*** ß-Amyloid Ratio (1-42/1-40)

B Predicate 510(k) Number(s):

DEN200072

C Comparison with Predicate(s):

Device & Predicate Device(s):	**K253240 (Candidate Device)**	**DEN200072 (Predicate)**
Device Trade Name	The PrecivityAD2 test	Lumipulse ***G*** β-Amyloid Ratio (1-42/1-40)
General Device Characteristic Similarities		
	The PrecivityAD2 test is an in vitro device that uses immunoprecipitation followed by liquid chromatography-	The Lumipulse ***G*** β-Amyloid Ratio (1-42/1-40) is an in vitro cerebral spinal fluid (CSF) test that combines the

Device & Predicate Device(s):	K253240 (Candidate Device)	DEN200072 (Predicate)
Device Trade Name	The PrecivityAD2 test	Lumipulse ***G*** β-Amyloid Ratio (1-42/1-40)
Intended Use/ Indications for Use	tandem mass spectrometry (LC-MS/MS) to quantify β-Amyloid 1-42 (Aβ42) and β-Amyloid 1-40 (Aβ40) peptide concentrations, and phosphorylated and non-phosphorylated tau protein at amino acid threonine, position 217 (p-tau217 and np-tau217) peptide concentrations in human K2-EDTA plasma. An algorithm combines the Aβ42/40 ratio and the p-tau217/np-tau217 ratio x 100 (%p-tau217) to generate the Amyloid Probability Score 2 (APS2) result, a numeric value ranging from 0-100. The PrecivityAD2 test is indicated for use in adults aged 40 years and older who present with signs or symptoms of cognitive impairment undergoing evaluation for Alzheimer's disease or other forms of cognitive decline. The PrecivityAD2 test is intended to be used in conjunction with clinical assessment to aid health care professionals experienced in the evaluation of cognitive impairment to identify patients with amyloid pathology associated with Alzheimer's disease. The test is not intended as a screening or stand-alone diagnostic test.	results of Lumipulse ***G*** β-Amyloid 1-42 and Lumipulse ***G*** β-Amyloid 1-40 assays into a ratio of β-amyloid 1-42 to β-amyloid 1-40 concentrations using the LUMIPULSE G1200 System. The Lumipulse ***G*** β-Amyloid Ratio (1-42/1-40) is intended to be used in adult patients, aged 55 years and older, presenting with cognitive impairment who are being evaluated for Alzheimer's disease (AD) and other causes of cognitive decline. A test result ≥ 0.073 is a negative result which is consistent with a negative amyloid positron emission tomography (PET) scan result. A negative result reduces the likelihood that a patient's cognitive impairment is due to AD. A test result ≤ 0.058 is a positive result which is consistent with a positive amyloid PET scan result. A positive result does not establish a diagnosis of AD or other cognitive disorder. A test result between 0.059 and 0.072 is considered as a likely positive result as it is more likely consistent with a positive amyloid PET scan result. A likely positive result does not establish a diagnosis of AD or other cognitive disorders and has increased uncertainty in regard to amyloid PET positivity. The Lumipulse ***G*** β-Amyloid Ratio (1-42/1-40) results must be interpreted in conjunction with other patient clinical information. This test is not intended as a screening or stand-alone diagnostic test.
Assay Output	Negative, Likely Positive, Positive	Same

Device & Predicate Device(s):	**K253240 (Candidate Device)**	**DEN200072 (Predicate)**
Device Trade Name	The PrecivityAD2 test	Lumipulse ***G*** β-Amyloid Ratio (1-42/1-40)
General Device Characteristic Differences		
Assay Type and Format	Immunoprecipitation followed by high resolution liquid chromatography tandem mass spectrometry (LC MS/MS).	Chemiluminescent enzyme immunoassay (CLEIA) Two-step sandwich
Test Setting	Single-site, high-complexity CLIA-certified	Clinical laboratory
Sample Type	Human K2-EDTA plasma	Human cerebrospinal fluid (CSF)
Sample Volume	*Aβ42 and Aβ40:* 0.5 mL *p-tau217 and np-tau217*: 1.0 mL	*Aβ42:* 50 µL *Aβ40:* 40 µL
Assay Antibodies	*Aβ42 and Aβ40:* Anti-Amyloid-Beta Antibody, HJ5.1 *p-tau217 and np-tau217:* Anti-Tau antibody, Tau1	*Aβ42:* anti- Aβ42 monoclonal antibody (mouse)-coated particles and biotinylated Aβ antibody (mouse) *Aβ40:* anti-Aβ40 monoclonal antibody (mouse)-coated particles and alkaline phosphatase (ALP)-labeled anti- Aβ (mouse) conjugate
Analytes	Aβ42, Aβ40, p-tau217 and np-tau217 combined into Aβ42/Aβ40 ratio and %p-tau217 = (p-tau217 / np-tau217) × 100	Aβ42 and Aβ40 combined into Aβ42/Aβ40 ratio
Reported Numeric Output	Amyloid Probability Score 2 (APS2) between 0–100	Aβ42/Aβ40 ratio between 0.001 to 1.000.
Result Calculation and Test Result Interpretation	Proprietary APS2 algorithm combines Aβ42/40 ratio and %p-tau217 into APS2 The result is interpreted by laboratory professionals at C2N according to the device labeling	A web-based Calculator Tool calculates Aβ42/Aβ40 ratio and reports the final test result (negative, positive or likely positive relative to the two cut-offs). If the ratio is not calculated by the Calculator Tool, the result is manually reported as 'ratio undetermined'
Assay Cut-off	Two APS2 cut-offs (27.5 and 66.5)	Two ratio cut-offs (0.058 and 0.073)
Instrument	Waters Acquity M-Class HPLC Waters Acquity M-Class Trap Valve Manager Waters Acquity M-Class Autosampler Thermo Scientific Fusion Lumos Tribrid Mass Spectrometer	LUMIPULSE G l200 System

Device & Predicate Device(s):	**K253240 (Candidate Device)**	**DEN200072 (Predicate)**
Device Trade Name	The PrecivityAD2 test	Lumipulse ***G*** β-Amyloid Ratio (1-42/1-40)
Calibrators	*Aβ42 and Aβ40:* Calibrator A: 0 pg/mL Aβ42 0 pg/mL Aβ40 Calibrator C: 5 pg/mL Aβ42 50 pg/mL Aβ40 Calibrator E: 25 pg/mL Aβ42 250 pg/mL Aβ40 Calibrator F: 65 pg/mL Aβ42 650 pg/mL Aβ40 Calibrator H: 100 pg/mL Aβ42 1000 pg/mL Aβ40 *p-tau217 and np-tau217:* Calibrator A: 0 pg/mL p-tau217 0 pg/mL np-tau217 Calibrator B: 0.62 pg/mL p-tau217 5 pg/mL np-tau217 Calibrator C: 1.85 pg/mL p-tau217 15 pg/mL np-tau217 Calibrator D: 5.56 pg/mL p-tau217 44.5 pg/mL np-tau217 Calibrator E: 16.67 pg/mL p-tau217 133.3 pg/mL np-tau217 Calibrator F: 50.0 pg/mL p-tau217 400.0 pg/mL np-tau217	*Aβ42:* Lumipulse ***G*** β-Amyloid 1-42 Calibrator Set: 30, 129, and 2,335 pg/mL *Aβ40:* Lumipulse ***G*** β-Amyloid 1-40 Calibrator Set: 0, 500, and 30,000 pg/mL
Controls	*Aβ42 and Aβ40:* QC Low: 29.54 pg/mL Aβ42 377.926 pg/mL Aβ40 QC Medium: 61.566 pg/mL Aβ42 679.589 pg/mL Aβ40 QC High*:* 78.816 pg/mL Aβ42 910.224 pg/mL Aβ40 *p-tau217 and np-tau217:* QC Low: 2.5 pg/mL p-tau217 25.0 pg/mL np-tau217 QC Medium: 7.5 pg/mL p-tau217 75.0 pg/mL np-tau217 QC High: 20.0 pg/mL p-tau217 200.0 pg/mL np-tau217	*Aβ42:* Lumipulse ***G*** β-Amyloid Controls (sold separately): 4,000, 10,000, and 20,000 pg/mL *Aβ40:* Lumipulse ***G*** β-Amyloid Controls (sold separately): 274, 548, and 1,027 pg/mL

Device & Predicate Device(s):	**K253240 (Candidate Device)**	**DEN200072 (Predicate)**
Device Trade Name	The PrecivityAD2 test	Lumipulse ***G*** β-Amyloid Ratio (1-42/1-40)
Traceability/ Standardization	Aβ40, Aβ42, p-tau217, and np-tau217 are traceable to in-house reference standards	*Aβ42:* Standardized against three certified reference materials: ERM-DA480/IFCC, ERM-DA481/IFCC and ERM-DA482/IFCC *Aβ40:* Traceable to INNOTEST β-Amyloid 1-40
Measuring Range	*Aβ42:* 5–100 pg/mL *Aβ40:* 50–1000 pg/mL *p-tau217:* 1.3–50 pg/mL *np-tau217:* 6.6–400 pg/mL	*Aβ42:* 38−2203.5 pg/mL *Aβ40:*156.3−28450.3 pg/mL
Detection Capability	Limit of Blank and Limit of Detection are not applicable for the Score *Aβ42:* Limit of Quantitation = 5 pg/mL *Aβ40:* Limit of Quantitation = 50 pg/mL *p-tau217:* Limit of Quantitation = 1.3 pg/mL *np-tau217:* Limit of Quantitation = 6.6 pg/mL	*Aβ42:* Limit of Blank = 2.2 pg/mL Limit of Detection = 11.6 pg/mL Limit of Quantitation = 38.0 pg/mL *Aβ40:* Limit of Blank = 0.97 pg/mL Limit of Detection = 33.0 pg/mL Limit of Quantitation = 158.0 pg/mL
Reagent Stability	Unopened: *Aβ42 and Aβ40:* 6 months at required storage conditions *p-tau217 and np-tau217:* 6 months at required storage conditions On-board: Not applicable as the Critical Reagents are prepared as Single Use aliquots.	*Aβ42 and Aβ40*: Unopened: 19 months at 2−10°C On-board: 15 days
Sample Stability	*Aβ42, Aβ40, p-tau217 and np-tau217:* • 24 months at −80°C • Up to 3 freeze/thaw cycles • 3 hours at RT prior to processing • Refrigerated shipping conditions (2–8°C) are validated for up to 48 hours.	*Aβ42 and Aβ40:* • 3 hours on-board • 8 days at 2−8°C • 48 hours at 23−27°C • 2 weeks at -30− -10°C • 1 month at -80°C • Up to 3 freeze/thaw cycles

VI Standards/Guidance Documents Referenced:

The following Clinical and Laboratory Standards Institute (CLSI) guidelines were used:

- CLSI EP05-A3: Evaluation of Precision of Quantitative Measurement Procedures; Approved Guideline – Third Edition
- CLSI EP06, 2nd ed.: Evaluation of the Linearity of Quantitative Measurement Procedures − Second Edition
- CLSI EP07, 3rd ed.: Interference Testing in Clinical Chemistry– Third Edition
- CLSI EP17-A2: Evaluation of Detection Capability for Clinical Laboratory Measurement Procedures; Approved Guideline − Second Edition
- CLSI EP25, 2nd ed.: Evaluation of Stability of In Vitro Medical Laboratory Test Reagents
- CLSI EP28-A3c: Defining, Establishing, and Verifying Reference Intervals in the Clinical Laboratory; Approved Guideline – Third Edition
- CLSI EP37, 1st ed.: Supplemental Tables for Interference Testing in Clinical Chemistry: Approved Guideline, First Edition
- CLSI C62-A Liquid Chromatography-Mass Spectrometry Methods: Approved Guideline.
- IEC 61326-2-6 Edition 4.0: Electrical equipment for measurement, control and laboratory use - EMC requirements - Part 2-6: Particular requirements - In vitro diagnostic (IVD) medical equipment
- IEC 60601-1 Edition 3.2: Medical electrical equipment - Part 1: General requirements for basic safety and essential performance

VII Performance Characteristics (if/when applicable):

A Analytical Performance:

1. Precision/Reproducibility:

A study was conducted per the CLSI guideline EP05-A3 to evaluate the precision of the C2N-Aβ42/40 Assay, C2N-%-p-tau217 Assay, and PrecivityAD2 test.

For the C2N-Aβ42/40 Assay, a panel of six K2-EDTA plasma samples was prepared to achieve target concentrations of Aβ42 and Aβ40 that cover the measuring ranges of the individual analytes (Panel #1). For the C2N-%-p-tau217 Assay, two separate panels of six K2-EDTA plasma samples, one for np-tau217 (Panel #2) and the other for p-tau217 (Panel #3), were prepared to achieve target concentrations that cover the measuring ranges of the individual analytes. Sample panels #1−#3 were analyzed to evaluate *1)* within-laboratory precision, *2)* lot-to-lot precision, and *3)* instrument-to-instrument precision, as described below. To assess variations in the APS2 score, two panels of K2-EDTA plasma samples spiked with varying amounts of peptides corresponding to the four analytes to achieve APS2 values across the range (0–100) and within 20% of the two clinical cutoffs (Panels #4 and #5)

were tested for within-laboratory precision. The C2N Aβ42/40 and C2N %p tau217 measurements were obtained from the samples in both Panels.

i. Within-laboratory precision:

C2N-Aβ42/40 Assay

To evaluate the within-laboratory precision for the C2N-Aβ42/40 Assay, Panel #1 was analyzed over 21 non-consecutive days, with one run per day and four replicates of each sample per run for a total of 84 measurements per sample. Panels #4 and #5 were tested in five replicates per run, one run per day for 15 non-consecutive days, resulting in a total of 75 measurements per sample. Three different operators (one operator per seven days) conducted the study using one lot of reagents and one instrument system. The results for Panels #1, #4 and #5 are summarized in the tables below for each analyte:

Aβ42										
Sample	**N**	**Mean (pg/mL)**	**Within-Run**		**Between-Day**		**Between-Operator**		**Within-Laboratory**	
			SD	**%CV**	**SD**	**%CV**	**SD**	**%CV**	**SD**	**%CV**
Panel #1*										
1	84	6.56	0.52	8.0	0.36	5.6	0.20	3.1	0.67	10.2
2	84	13.57	0.68	5.0	0.53	3.9	0.13	1.0	0.87	6.4
3	84	18.36	1.12	6.1	0.75	4.1	0.40	2.2	1.40	7.6
4	84	50.24	2.69	5.4	1.51	3.0	0	0	3.09	6.2
5	84	52.46	2.67	5.1	1.60	3.1	0	0	3.11	5.9
6	84	79.89	4.14	5.2	2.66	3.3	0	0	4.92	6.2
Samples 1–5 were single native K2-EDTA plasma and Sample 6 was a single native K2-EDTA plasma spiked with Aβ42.										
Panel #4*										
1	75	31.00	1.39	4.5	1.62	5.2	0.37	1.2	2.16	7.0
2	73	31.36	2.32	7.4	1.48	4.7	1.10	3.5	2.96	9.4
3	74	39.46	2.18	5.5	1.72	4.4	0	0	2.77	7.0
4	74	43.67	3.09	7.1	1.36	3.1	1.91	4.4	3.88	8.9
5	74	44.94	1.06	2.4	1.76	3.9	1.09	2.4	2.32	5.2
6	75	68.67	4.43	6.5	1.28	1.9	2.33	3.4	5.17	7.5
Samples 1-6 were pooled K2-EDTA plasma spiked with Aβ40, Aβ42, and Tau-441 recombinant protein. Sample 2 has only 73 replicates and Samples 3-5 have 74 replicates because specimen failed to meet predefined QC acceptance criteria.										
Panel #5*										
1	75	13.33	0.67	5.1	0.35	2.6	0.23	1.7	0.79	5.9
2	75	24.48	1.44	5.9	1.29	5.3	1.42	5.8	2.40	9.8
3	75	30.84	1.01	3.3	1.47	4.8	0	0	1.78	5.8
4	75	40.24	0.98	2.4	1.95	4.9	0	0	2.18	5.4
5	75	71.76	1.81	2.5	5.05	7.0	1.60	2.2	5.60	7.8
Samples 1-5 were pooled K2-EDTA plasma spiked with Aβ40, Aβ42, and Tau-441 recombinant protein.										
*Preanalytical sample handling was less stringent for Panels #1 and #4 than for Panel #5, which was prepared under more controlled conditions. However, samples from both panels underwent pooling, aliquoting, mixing, and, when necessary, analyte spiking —steps that are not part of routine clinical sample handling.										

Aβ40										
Sample	N	Mean (pg/mL)	Within-Run		Between-Day		Between-Operator		Within-Laboratory	
			SD	%CV	SD	%CV	SD	%CV	SD	%CV
Panel #1*										
1	84	76.27	1.60	2.1	1.03	1.4	0	0	1.90	2.5
2	84	131.23	2.75	2.1	1.65	1.3	1.79	1.4	3.67	2.8
3	84	264.34	5.55	2.1	3.48	1.3	3.11	1.2	7.25	2.7
4	84	531.33	9.56	1.8	7.00	1.3	11.86	2.2	16.76	3.2
5	84	523.74	9.26	1.8	8.38	1.6	13.51	2.6	18.40	3.5
6	84	890.79	20.66	2.3	10.22	1.2	2.98	0.3	23.24	2.6
Samples 1–5 were single native K2-EDTA plasma and Sample 6 was a single native K2-EDTA plasma spiked with Aβ42.										
Panel #4*										
1	75	297.33	9.4	3.2	4.43	1.5	0	0.	10.39	3.5
2	75	298.48	6.65	2.2	4.28	1.4	5.24	1.8	9.48	3.2
3	75	381.42	8.90	2.3	7.93	2.1	0	0	11.92	3.1
4	75	479.49	8.57	1.8	10.92	2.3	0	0	13.88	2.9
5	75	520.35	17.38	3.3	7.89	1.5	0.91	0.2	19.11	3.7
6	75	601.02	15.19	2.5	10.09	1.68	0	0	18.23	3.0
Samples 1-6 were pooled K2-EDTA plasma spiked with Aβ40, Aβ42, and Tau-441 recombinant protein.										
Panel #5*										
1	75	126.05	4.37	3.5	2.47	2.0	2.44	1.9	5.58	4.4
2	75	228.12	3.95	1.7	5.13	2.3	2.63	1.2	6.99	3.1
3	75	360.23	9.83	2.7	6.61	1.8	5.43	1.5	13.03	3.6
4	75	454.94	9.29	2.1	10.68	2.4	4.77	1.1	14.94	3.3
5	75	672.77	11.24	1.7	15.34	2.3	6.02	0.9	19.94	3.0
Samples 1–5 were pooled native K2-EDTA plasma spiked with Aβ40, Aβ 42, and Tau-441 recombinant protein										
*Preanalytical sample handling was less stringent for Panels ##1 and 4 than for Panel #5, which was prepared under more controlled conditions. However, samples from both panels underwent pooling, aliquoting, mixing, and, when necessary, analyte spiking —steps that are not part of routine clinical sample handling.										

C2N-%-p-tau217 Assay

To evaluate the within-laboratory precision for the C2N-%-p-tau217 Assay, Panels #2 and #3 were analyzed over 15 non-consecutive days, with one run per day and four replicates of each sample per run for a total of 60 measurements per sample. Three different operators (one operator per five days) conducted the study using one lot of reagents and one instrument system. Panels #4 and #5 were tested as described above. The results for Panels #2, #3, #4, and #5 are summarized in the tables below for each analyte:

p-tau217										
Sample	N	Mean (pg/mL)	Within-Run		Between-Day		Between-Operator		Within-Laboratory	
			SD	%CV	SD	%CV	SD	%CV	SD	%CV
Panel #2*										
1	60	2.83	0.35	12.3	0	0	0	0	0.35	12.3
2	60	8.00	0.52	6.5	0.41	5.2	0	0	0.67	8.3
3	60	13.39	1.38	10.3	0.88	6.5	0	0	1.63	12.2
4	60	23.06	2.21	9.6	2.02	8.8	0.69	3.0	3.07	13.3
5	60	31.58	2.29	7.3	1.00	3.2	0	0	2.50	7.9
6	60	39.14	3.42	8.7	2.43	6.2	0	0	4.20	10.7
Sample 1 was a pooled native K2-EDTA plasma, Samples 2–4 were pooled native K2-EDTA plasma spiked with CSF, and Samples 5 and 6 were pooled native K2-EDTA plasma spiked with Tau-441 recombinant protein.										
Panel #4*										
1	75	0.51	0.11	22.0	0.08	16.3	0.03	5.2	0.14	27.8
2	75	5.27	0.48	9.1	0.24	4.5	0.13	2.5	0.55	10.4
3	75	12.33	1.15	9.3	0.97	7.8	0.05	0.4	1.50	12.2
4	75	12.90	0.78	6.0	0.80	6.2	0.72	5.5	1.33	10.3
5	75	16.12	1.02	6.3	0.69	4.3	1.08	6.7	1.64	10.2
6	75	28.24	2.71	9.6	1.84	6.5	1.38	4.9	3.56	12.6
Samples 1-6 were pooled K2-EDTA plasma spiked with Aβ40, Aβ42, and Tau-441 recombinant protein. Sample 1 is below the lower limit of the analytical measuring interval for p-tau217.										
Panel #5*										
1	75	1.56	0.17	10.7	0.10	6.1	0	0	0.19	12.3
2	75	3.80	0.37	9.6	0.18	4.8	0	0	0.41	10.7
3	75	4.02	0.45	11.2	0.15	3.8	0.05	1.2	0.48	11.9
4	72	4.81	0.52	10.9	0	0	0	0	0.52	10.9
5	74	5.98	0.57	9.5	0.29	4.8	0	0	0.64	10.6
6	75	7.32	0.70	9.5	0.37	5.0	0	0	0.79	10.8
7	75	8.89	0.75	8.5	0.66	7.4	0.25	2.0	1.03	11.6
8	75	9.09	0.71	7.8	0.43	4.7	0	0	0.8	9.1
9	75	12.46	0.96	7.7	0.50	4.0	0.15	1.2	1.1	8.8
10	75	18.80	1.84	9.8	0.78	4.2	0	0	2.0	10.6
11	74	20.57	1.56	7.6	1.08	5.3	0	0	1.9	9.2
12	74	34.71	3.41	9.8	2.38	6.9	0.70	2.0	4.2	12.2
Samples 1, 2, 8-10 were pooled K2-EDTA plasma spiked with Aβ40, Aβ42, and Tau-441 recombinant protein. Samples 3-7, 11 and 12 were pooled native K2-EDTA plasma. Sample 4 has only 72 replicates and Samples 5, 11, and 12 have 74 replicates because specimen failed to meet predefined QC acceptance criteria.										
*Preanalytical sample handling was less stringent for Panels #2 and #4 than for Panel #5, which was prepared under more controlled conditions. However, samples from both panels underwent pooling, aliquoting, mixing, and, when necessary, analyte spiking —steps that are not part of routine clinical sample handling.										

np-tau217										
Sample	N	Mean (pg/mL)	Within-Run		Between-Day		Between-Operator		Within-Laboratory	
			SD	%CV	SD	%CV	SD	%CV	SD	%CV
Panel #3*										
1	60	14.11	1.67	11.9	0.9	6.5	0.45	3.2	1.96	13.9
2	60	49.12	4.61	9.4	4.9	9.9	2.73	5.6	7.24	14.7
3	60	69.36	6.75	9.7	3.4	5.0	4.57	6.6	8.85	12.8
4	60	106.87	11.77	11.0	8.1	7.6	4.95	4.6	15.13	14.2
5	60	191.75	18.34	9.6	13.7	7.1	16.06	8.4	27.96	14.6
6	60	334.27	30.44	9.1	28.2	8.4	7.59	2.3	42.21	12.6
Samples 1–3 were pooled native K2-EDTA plasma, Sample 4 was pooled native K2-EDTA plasma spiked with CSF, Samples 5 and 6 were pooled native K2-EDTA plasma spiked with Tau-441 recombinant protein.										
Panel #4*										
1	75	93.88	5.66	6.0	4.46	4.8	6.46	6.9	9.68	10.3
2	75	112.09	8.20	7.3	4.82	4.3	6.51	5.8	11.53	10.3
3	75	149.86	10.28	6.9	7.60	5.1	9.52	6.4	15.94	10.6
4	75	180.60	10.13	5.6	9.75	5.4	13.44	7.4	19.45	10.8
5	75	229.56	9.93	4.3	15.03	6.5	17.24	7.5	24.94	10.9
6	75	276.24	18.22	6.6	12.83	4.6	19.19	7.0	29.41	10.6
Samples 1-6 were pooled K2-EDTA plasma spiked with Aβ40, Aβ42, and Tau-441 recombinant protein.										
Panel #5*										
1	75	59.63	2.89	4.9	2.19	3.7	0	0	3.63	6.1
2	75	77.72	6.04	7.8	4.0	5.1	1.43	1.8	7.38	9.5
3	75	79.29	3.62	4.6	4.0	5.0	0	0	5.36	6.8
4	75	84.95	6.91	8.1	2.1	2.5	1.52	1.8	7.39	8.7
5	75	111.32	5.74	5.2	4.3	3.9	0	0	7.19	6.5
6	74	126.89	6.12	4.8	5.6	4.4	0	0	8.31	6.5
7	74	180.29	7.05	3.9	5.7	3.2	0	0	9.08	5.0
8	75	200.00	12.71	6.4	0	0	5.37	2.7	13.79	6.9
9	75	207.20	18.11	8.7	0	0	4.14	2.0	18.57	9.0
10	75	327.82	19.40	5.9	10.6	3.2	0	0	22.10	6.7
11	72	347.37	17.54	5.0	7.1	2.0	2.11	0.6	19.03	5.5
One sample had an np-tau217 concentration above the AMI; therefore, results were reported for 11 samples for np-tau217. Samples 2,5, 8-10 were pooled K2-EDTA plasma spiked with Aβ40, Aβ42, and Tau-441 recombinant protein. Samples 1, 3, 4, 6, 7 and 11 were pooled native K2-EDTA plasma. Sample 11 has only 72 replicates and Samples 6 and 7 have 74 replicates because specimen failed to meet predefined QC acceptance criteria.										
*Preanalytical sample handling was less stringent for Panels # 3 and #4 than for Panel #5, which was prepared under more controlled conditions. However, samples from both panels underwent pooling, aliquoting, mixing, and, when necessary, analyte spiking —steps that are not part of routine clinical sample handling.										

PrecivityAD2 APS2

To evaluate the within-laboratory precision for the PrecivityAD2 APS2, Panels #4 and #5 were tested following the study design described above, resulting in 75 replicates. The results for Panels #4 and #5 are summarized in the tables below.

APS2										
Sample	N	Mean APS2 Value	Within-Run		Between-Day		Between-Operator		Within-Laboratory	
			SD	%CV	SD	%CV	SD	%CV	SD	%CV
Panel #4*										
1	73	5	1.04	22.2	1.21	25.9	0.46	9.9	1.65	35.5
2	75	29	7.19	24.6	3.99	13.7	0	0	8.23	28.2
3	74	41	5.75	14.2	5.63	13.9	0	0	8.04	19.6
4	75	77	6.80	8.8	3.93	5.1	3.40	4.4	8.55	11.1
5	74	90	4.28	4.7	1.14	1.3	0.76	0.8	4.50	5.0
6	74	90	5.59	6.2	2.25	2.5	3.79	4.2	7.12	7.9
Samples 1-6 were pooled K2-EDTA plasma spiked with Aβ40, Aβ42, and Tau-441 recombinant protein. Sample 1 has only 73 replicates and Samples 3, 5, and 6 have 74 replicates because specimen failed to meet predefined QC acceptance criteria										
Panel #5*										
1	75	4.3	0.71	16.5	1.28	29.8	0.63	14.5	1.59	37.0
2	75	24.5	2.84	11.6	0.77	3.2	0	0	2.94	12.0
3	75	31.6	3.25	10.3	1.29	4.1	1.19	3.8	3.69	11.1
4	75	68.5	5.06	7.4	3.06	4.5	0	0	5.91	8.6
5	75	100	0	0	0	0	0	0	0	0
Samples 1-5 were pooled K2-EDTA plasma spiked with Aβ40, Aβ42, and Tau-441 recombinant protein.										
*Preanalytical sample handling was less stringent for Panel #4 than for Panel #5, which was prepared under more controlled conditions. However, samples from both panels underwent pooling, aliquoting, mixing, and, when necessary, analyte spiking —steps that are not part of routine clinical sample handling/										

ii. Lot-to-lot precision:

To evaluate between-lot precision for the C2N-Aβ42/40 Assay and C2N-%-p-tau217 Assay, Panel #1 (for the C2N-Aβ42/40 Assay) and Panels #2 and #3 (for the C2N-%-p-tau217) were analyzed over 15 non-consecutive days, with one run per day and five replicates of each sample per run, resulting in 75 measurements per sample. One operator conducted the study using three different lots of reagents (one lot per 5 days) and one instrument system. The results are summarized in the tables below for each analyte:

Sample	N	Mean (pg/mL)	Within-Run		Between-Day		Between-Lot		Within-Laboratory	
			SD	%CV	SD	%CV	SD	%CV	SD	%CV
Aβ42										
1	75	10.59	1.18	11.2	0.72	6.8	0.00	0.00	1.38	13.1
2	75	18.68	1.39	7.4	0.46	2.5	0.93	5.0	1.74	9.3
3	75	21.41	1.71	8.0	0.43	2.0	1.25	5.9	2.16	10.1
4	75	46.62	5.28	11.4	0.00	0.00	2.84	6.1	6.00	12.9
5	71	56.07	3.80	6.8	1.90	3.4	3.65	6.5	5.60	10.0
6	66	76.99	5.07	6.6	1.26	1.6	3.98	5.2	6.57	8.5
Samples 1–5 were single native K2-EDTA plasma and Sample 6 was a single native K2-EDTA plasma spiked with Aβ42. Sample 5 has only 71 replicates and Sample 6 has 66 because one and two analytical runs, respectively, failed to meet predefined QC acceptance criteria and additional aliquots (six for Sample 5 and one for Sample 6) were tested and included in the analysis.										
Aβ40										
1	75	68.45	1.61	2.4	7.32	10.7	1.85	2.7	7.78	11.3
2	75	164.60	2.62	1.6	6.48	3.9	0.00	0.0	6.99	4.3
3	75	203.35	3.84	1.9	5.58	2.8	4.04	2.0	7.89	3.9
4	75	447.16	10.40	2.3	11.80	2.6	12.52	2.8	20.10	4.5
5	71	662.29	15.53	2.4	9.30	1.4	22.85	3.5	29.15	4.4
6	66	881.48	13.09	1.5	12.52	1.4	36.75	4.17	40.97	4.7
Samples 1–5 were single native K2-EDTA plasma and Sample 6 was a single native K2-EDTA plasma spiked with Aβ40. Sample 5 has only 71 replicates and Sample 6 has 66 because one and two analytical runs, respectively, failed to meet predefined QC acceptance criteria and additional aliquots (six for Sample 5 and one for Sample 6) were tested and included in the analysis.										
p-tau217										
1	75	2.33	0.28	12.0	0.10	4.4	0.08	3.4	0.31	13.2
2	75	9.64	1.12	11.6	0.49	5.1	0.71	7.3	1.41	14.6
3	75	14.88	1.08	7.2	1.01	6.8	0.16	1.1	1.48	10.0
4	75	17.03	1.46	8.6	0.78	4.5	0.67	4.0	1.78	10.4
5	75	33.27	2.45	7.4	1.02	3.1	3.11	9.4	4.09	12.3
6	75	40.55	3.26	8.0	1.86	4.6	2.54	6.3	4.53	11.7
Samples 1 and 2 were pooled native K2-EDTA plasma, Samples 3 and 4 were single native K2-EDTA plasma spiked with CSF, and Samples 5 and 6 were pooled native K2-EDTA plasma spiked with Tau-441 recombinant protein.										
np-tau217										
1	75	24.53	2.00	8.1	0.81	3.3	2.15	8.8	3.04	12.4
2	75	49.71	3.63	7.3	0	0	3.51	7.1	5.05	10.2
3	75	75.57	4.61	6.1	1.97	2.6	4.27	5.7	6.59	8.7
4	75	155.66	8.70	5.6	4.94	3.2	6.56	4.2	11.96	7.7
5	75	207.67	13.94	6.7	9.44	4.6	15.36	7.4	22.79	11.0
6	71	349.58	18.70	5.4	0	0	25.08	7.2	31.28	9.0
Samples 1 and 2 were single native K2-EDTA plasma, Sample 3 was a pooled native K2-EDTA plasma, Samples 4 was a single native K2-EDTA plasma spiked with CSF, and Samples 5 and 6 were pooled native K2-EDTA plasma spiked with Tau-441 recombinant protein. Sample 6 had 71 replicates because four replicates with np-tau217 values above the AMI were excluded.										

iii. Instrument-to-instrument precision:

To evaluate the between-instrument precision for the C2N-Aβ42/40 Assay, Panel #1 (for the C2N-Aβ42/40 Assay) and Panels #2 and #3 (for the C2N-%-p-tau217) were analyzed following the same study design described in the lot-to-lot precision study with the exception that one instrument system was used per 5 days. The results are summarized in the tables below for each analyte:

Sample	N	Mean (pg/mL)	Within-Run		Between-Day		Between-Instrument		Within-Laboratory	
			SD	%CV	SD	%CV	SD	%CV	SD	%CV
Aβ42										
1	72	6.86	0.49	7.1	0.25	3.6	0.57	8.2	0.79	11.5
2	75	14.02	0.67	4.8	0.42	3.0	0.28	2.0	0.83	5.9
3	75	17.95	1.39	7.7	0	0	0.78	4.3	1.59	8.8
4	75	41.71	2.23	5.4	0.61	1.7	0.47	1.1	2.36	5.7
5	75	61.63	2.58	4.2	2.66	4.3	0.89	1.4	3.81	6.2
6	73	82.30	3.70	4.5	1.89	2.3	0	0	4.15	5.0
Samples 1–5 were single native K2-EDTA plasma and Sample 6 was a single native K2-EDTA plasma spiked with Aβ42. Sample 1 had 72 replicates because three replicates with Aβ42 values below the AMI were excluded. Sample 6 had 73 replicates because two replicates with Aβ42 values above the AMI were excluded.										
Aβ40										
1	75	78.52	1.42	1.8	0.72	0.9	0.76	1.0	1.77	2.3
2	75	134.34	1.88	1.4	1.28	1.0	2.99	2.2	3.76	2.8
3	75	268.51	5.06	1.9	1.94	0.7	5.27	2.0	7.56	2.8
4	75	438.86	20.04	4.6	0	0	2.61	0.6	20.21	4.6
5	75	628.03	12.88	2.1	6.65	1.1	7.34	1.2	16.25	2.6
6	75	918.17	15.80	1.7	10.53	1.2	9.17	1.0	21.08	2.3
Samples 1–5 were single native K2-EDTA plasma and Sample 6 was a single native K2-EDTA plasma spiked with Aβ40.										
p-tau217										
1	75	2.82	0.26	9.3	0.02	0.8	0.05	1.8	0.27	9.5
2	75	8.03	0.59	7.4	0.36	4.5	0.03	0.4	0.69	8.6
3	75	13.32	1.03	7.8	1.07	8.1	0	0	1.49	11.2
4	75	23.15	1.63	7.0	1.52	6.6	0	0	2.22	9.6
5	75	28.75	2.10	7.3	1.44	5.0	0	0	2.55	8.9
6	74	34.17	2.53	7.4	1.50	4.4	0.49	1.4	2.98	8.7
Sample 1 was a pooled native K2-EDTA plasma, Samples 2–4 were pooled native K2-EDTA plasma spiked with CSF, and Samples 5 and 6 were pooled native K2-EDTA plasma spiked with Tau-441 recombinant protein. Sample 6 had 74 replicates because one replicate with ISTD area out of range was excluded.										

Sample	N	Mean (pg/mL)	Within-Run		Between-Day		Between-Instrument		Within-Laboratory	
			SD	%CV	SD	%CV	SD	%CV	SD	%CV
np-tau217										
1	75	14.53	1.29	8.8	0.81	5.6	0.64	4.4	1.65	11.3
2	75	50.74	3.59	7.1	2.20	4.3	1.65	3.3	4.53	8.9
3	75	71.29	4.97	7.0	3.40	4.8	2.37	3.3	6.47	9.1
4	75	111.95	7.22	6.5	4.68	4.2	0	0	8.60	7.7
5	75	189.15	9.62	5.1	9.13	4.8	4.98	2.6	14.17	7.5
6	74	319.07	24.76	7.8	8.42	2.6	1.33	0.4	26.18	8.2
Samples 1–3 were pooled native K2-EDTA plasma, Samples 4 was a pooled native K2-EDTA plasma spiked with CSF, and Samples 5 and 6 were pooled native K2-EDTA plasma spiked with Tau-441 recombinant protein. Sample 6 had 74 replicates because one replicate with np-tau217 value above the AMI was excluded.										

iv. Precision Simulation of APS2

For a multianalyte score values, the precision performance of Score values can be different at the same Score values when the underlying combinations for (Aβ40, Aβ42, p-tau217, and np-tau217) are different. In order to evaluate the precision characteristics of the APS2 score values under different combinations of underlying variables, precision was computationally simulated from empirically obtained precision of the individual components. A simulation-based evaluation of the analytical precision of the PrecivityAD2 test was conducted based on the precision profile for each analyte (Aβ40, Aβ42, p-tau217, and np-tau217) derived from the precision study. Random measurement errors were considered normally distributed and a generator of random normally distributed numbers was used. For each analyte, the mean values of the analytes were considered as values of the corresponding analyte of 1,142 subjects of the pivotal clinical performance study. The standard deviation (SD) for each individual subject for each of four individual analytes was calculated based on the analyte specific precision profiles which was approximated by a linear regression. Each subject random measurement error was simulated by 100 Monte Carlo iterations (100 replicates of APS2 score for the sample).

For each subject, the simulated replicates of the APS2 were used to estimate: *(i)* median APS2 score*; (ii)* analog of SD, defined as (97.5th percentile- 2.5th percentile)/4; and *(iii)* analog of %CV, defined as analog of SD divided by the median APS2 score. Precision profile plot of APS2 Analog SD vs APS2 median and of APS2 analog %CV vs APS2 median were generated using results from subject-level simulations. Three ranges of APS2 values were considered and for each range, the min. SD and max. SD were calculated. The results are summarized in the table below:

	APS2 Score Interval	Number of Subjects	Min. SD	Max. SD
Combinations of 4 Analytes as in the Clinical Performance Study	0 −27.5	423	0.5	15.9
	27.5 − 66.5	198	5.4	23.2
	66.5−100	521	0	20.5

The potential clinical impact of APS2 analytical imprecision was further evaluated using a simulation-based C5–C95 approach. The results demonstrate that analytical variability around the APS2 cutoff of 27.5 may cause misclassification between the Negative and Likely Positive categories and that a re-testing zone is implemented to minimize this risk. Specimens with APS2 scores between 17 and 45 are retested to reduce the potential for patient misclassification. An additional LC-MS/MS injection for p-tau217 and np-tau217 from the same processed specimen is performed. The final APS2 value for the patient specimen within the re-testing zone is calculated using the average of the two C2N-%p-tau217 Assay measurements.

2. Linearity:

The linearity of C2N-Aβ42/40 Assay and C2N-%-p-tau217 Assay was evaluated in accordance with the CLSI guideline EP06, 2nd edition.

For the C2N-Aβ42/40 Assay, a high K2-EDTA plasma sample spiked with Aβ42, and another high K2-EDTA plasma sample spiked with Aβ40 were each mixed with a blank sample (charcoal stripped human plasma) in different proportions to create 21 sample levels for Aβ42 and 19 sample levels for Aβ40 to cover the linearity interval for each analyte. Each sample level was measured in one run with four replicates using one reagent lot of each assay and the mean of the replicates was calculated for each sample level. The Aβ42 and Aβ40 linearity studies were performed independently of each other on different days by the same operator on the same instrument system, but different lots of reagents were used for each analyte.

For the C2N-%-p-tau217 Assay, a high K2-EDTA plasma pool spiked with the synthetic p-tau217 and np-tau217 peptides and a low sample (native human plasma sample with levels of p-tau217 and np-tau217 below the expected lower limit of the linearity interval) were mixed in different proportions to create 13 sample levels covering the linearity interval for each analyte for testing. Each sample level was measured in one run with six replicates using one reagent lot of each assay and the mean of the replicates was calculated for each sample level. The -tau217 and np-tau217 linearity studies were performed by the same operator on a single instrument system.

A weighted regression analysis was performed to determine the predicted values. Percent deviation from linearity (DL) between the observed values and the best linear fit (predicted values) was calculated. The % DL was within ±10% for each sample level. The best linear regression fits and claimed analytical measuring interval (AMI) are summarized in the table below for each analyte measured by the two assays:

	Testing Range	Regression Equation	Claimed AMI
C2N-Aβ42/40 Assay			
Aβ42	4.9 – 108.7 pg/mL	y = 0.911x – 0.110	5 – 100 pg/mL
Aβ40	35.9 – 1196.5 pg/mL	y = 1.06x – 1.002	50 – 1000 pg/mL
C2N-%-p-tau217 Assay			
p-tau217	0.6 – 89.1 pg/mL	y = 1.015x + 0.137	1.3 – 50 pg/mL
np-tau217	3.4 – 540.1 pg/mL	y = 1.044x + 3.591	6.6 – 400 pg/mL

3. Analytical Specificity/Interference:

1) Interference:

The effect of potential endogenous and exogenous substances on the PrecivityAD2 APS2 was evaluated in accordance with the CLSI guidelines EP07, 3rd ed. and EP37. A panel of five pooled K2-EDTA samples with APS2 values spanning the reporting range including samples near the established clinical cutoff regions was evaluated for interference with seven endogenous substances: bilirubin, biotin, human anti-mouse antibodies (HAMA), hemoglobin, human serum albumin, triglycerides, and rheumatoid factor. A separate panel of five pooled K2-EDTA plasma samples with APS2 values cover the reporting range including samples near the established clinical cutoffs was evaluated for interference with 28 exogenous substances. Each sample was spiked with a single interferent with the volume of spiked material representing less than 5% of the final sample volume. Control samples were spiked with the solvent material in which the interferent is supplied. All control samples and samples spiked with interferents were tested with five replicates in a single run by a single operator with a single lot of reagents on a single instrument. The %Interference (%APS2 difference) was calculated by comparing measurements of the test and control samples. All the endogenous and exogenous interfering substances listed in the tables below met the acceptance criteria of within ±10% for %APS2 difference at the indicated test concentrations.

Endogenous Interferents	Test Concentration
Bilirubin, Conjugated	40 mg/dL
Bilirubin, Unconjugated	40 mg/dL
Biotin	7.02 mg/dL
HAMA*	1000 ng/mL
Hemolysate#	150 mg/dL
Albumin^	15 g/dL
Immunoglobulin A *	500 mg/dL
Immunoglobulin G*	2000 mg/dL
Immunoglobulin M*	300 mg/dL
Triglycerides^	1,500 mg/dL
Rheumatoid factor	500 IU/mL

^The spiking volume exceeded the desired 5% for triglycerides (7.3%) and 15.3% for albumin.
*HAMA, IgA, IgG, and IgM were tested in three replicates instead of five.
#Hemolysate was identified as an interfering substance during the assessment at the test concentration of 1,000 mg/dL. A dose-response study identified hemolysate concentration of 150 mg/dL to meet the acceptance criteria.

Exogenous Interferents	Test Concentration
Acetaminophen	156 mg/L
Acetylcysteine	150 mg/L
Acetylsalicylic Acid	30 mg/L
Amlodipine	0.075 mg/L
Ascorbic Acid	52.5 mg/L
Atenolol	9 mg/L
Atorvastatin	0.75 mg/dL
Citalopram	5.4 mg/L
Cyclosporine	1.8 mg/L
Denosumab	0.00015 mg/L
Donepezil	30 mg/L
Doxycycline	18 mg/L
Fluoxetine	1.4 mg/L
Heparin	3300 IU/L
Hydrochlorothiazide	1.13 mg/L
Ibuprofen	219 mg/L
Levodopa	7.5 mg/L
Levothyroxine	0.043 mg/dL
Lisinopril	0.25 mg/L
Losartan	1.2 mg/L
Memantine	0.12 mg/L
Metformin	12 mg/L
Metoprolol	1.5 mg/L
Rifampicin	48 mg/L
Risperidone	0.11 mg/L
Rivastigmine	0.17 mg/L
Simvastatin	1.68 mg/L
Theophylline	60 mg/L
Valproic Acid	318 mg/L
Warfarin	75 mg/L

To further evaluate the potential impact of endogenous and exogenous interferents on the APS2 result, a simulation analysis was performed using 25 combinations of analyte ratios for each interferent. Specifically, the mean replicates of Aβ42/Aβ40 ratio and p-tau217/np-tau217 ratio from the five interferent-treated samples and their corresponding controls were combined to generate hypothetical control and test APS2 values. For each combination, %APS2 difference was calculated. Across the evaluated interferents, %APS2 difference varied in both magnitude and direction, with both positive and negative shifts observed. Other than hemolysate, despite the observed mean %APS2 difference from control sample exceeding 10%, categorical APS2 interpretation changes were limited to transitions between adjacent interpretation categories (Negative to Likely Positive or Likely Positive to Negative, and Likely Positive to Positive or Positive to Likely Positive). No combinations resulted in categorical shifts from Negative to Positive or from Positive to Negative. These findings indicate that, except for high concentration (>150 mg/dL) hemolysate conditions, interfering substances do not alter the APS2 results that could lead to changes in medical decisions.

2) *Cross-reactivity:*

The performance of the PrecivityAD2 test in the presence of potential cross-reactive pTau and β-amyloid species and the heavy isotope-labeled internal standards (ISTDs) is based on the effect of these potential cross-reactants on the performance of the two component assays, the C2N-Aβ42/40 Assay and C2N-%-p-tau217 Assay.

For cross-reactivity testing with the C2N-Aβ42/40 Assay, a panel of six K2-EDTA plasma samples with varying concentrations of Aβ42 (28.7 pg/mL ¬ 88.4 pg/mL) and Aβ40 (311.5 pg/mL¬893.9 pg/mL) was tested. A separate panel of six K2-EDTA plasma samples with varying concentrations of p-tau217 (1.84 pg/mL ¬ 16.4 pg/mL) and np-tau217 (16.5 pg/mL¬72.6 pg/mL) was tested with the C2N-%-p-tau217 Assay.

Each sample was spiked with a single potential cross-reactant listed in the table below and the volume of spiked material was less than 5% of the total volume of the sample. Matched control samples were spiked with an equivalent volume of 2% recombinant human serum albumin (rHSA) to maintain equivalent sample matrices between control and test conditions. Except for the Aβ40, Aβ42, and ISTDs, no additional cross-reactants of the tau proteins were evaluated. A Basic Local Alignment Search Tool (BLAST) search revealed that the highest sequence identity between Tau-441 (longest human tau isoform) and human non-tau proteins was observed for microtubule associated protein 2 (MAP2; 53.9% sequence identity) and microtubule associated protein 4 (MAP4; 63.1% sequence identity). Both MAP sequences were aligned with Tau-441 and the epitope for the Tau1 antibody that is used for immunoprecipitation of tau protein in the PrecivityAD2 test. The alignment showed that the epitope is not present in the MAP2 and MAP4 proteins and that there are no cross-reacting proteins in human plasma that could affect the performance of the PrecivityAD2 test.

The number of replicates, operator, lots, and instruments was the same as for endogenous and exogenous interferent testing. The % cross-reactivity was calculated by comparing measurements of the test and control samples using the equation below.

$$\text{Cross-Reactivity:} = \frac{\left[\text{Mean Concentration } (\frac{\text{pg}}{\text{mL}})_{(\text{Test})} - \text{Mean Concentration } (\frac{\text{pg}}{\text{mL}})_{(\text{Control})}\right]}{\text{Mean Concentration } (\frac{\text{pg}}{\text{mL}})_{(\text{Control})}} \text{ x } 100$$

The highest observed % cross-reactivity values are summarized in the table below for each of the four analytes.

Cross-Reactant	Test Concentration (pg/mL)	Highest Observed Cross-Reactivity (%) across Test Samples[#]
Aβ42		
Aβ40 3X ISTD	600*	-17.33
Aβ $_{1-37}$	235 ^	5.75
Aβ $_{1-38}$	695^	3.16
Aβ $_{1-43}$	23 ^	6.82

Cross-Reactant	Test Concentration (pg/mL)	Highest Observed Cross-Reactivity (%) across Test Samples[#]
Aβ40		
Aβ42 3X ISTD	90*	1.65
Aβ $_{1-37}$	235 ^	6.91
Aβ $_{1-38}$	695^	6.08
Aβ $_{1-43}$	23 ^	8.56
p-tau217		
p-tau 3X ISTD	30*	16.98
np-tau217		
np-tau 3X ISTD	497*	4.94

*Test concentration represents three-fold excess relative to the concentrations routinely added during standard assay processing conditions.

^ Test concentration represents four-fold or greater elevations above the expected endogenous concentrations observed in patient plasma samples based on C2N internal data generated from 46 commercially available K2EDTA plasma samples.

[#] Samples in each six-member panel span the analytical measuring range of the respective analytes.

To assess the impact of individual analytes having higher levels of %cross-reactivity on the final APS2 result, the quantitative outputs of the C2N-Aβ42/40 Assay and C2N-%p-tau217 Assay for the same samples were used to calculate the APS2. The results are summarized in the table below.

APS2		
Cross-Reactant	**Test Concentration (pg/mL)**	**Highest Observed Cross-Reactivity (%)**
Aβ $_{1-37}$	235	7.91
Aβ $_{1-38}$	695	7.95
Aβ $_{1-43}$	23	9.41
Aβ40 3X ISTD	600	1.64
Aβ42 3 X ISTD	90	9.46
np-tau217 3X ISTD	497	9.47
p-tau217 3X ISTD	30	1.92

The %APS2 differences for the samples spiked with ISTDs or cross-reactive β-amyloid species met the acceptance criteria. Although measurable analytical cross-reactivity was observed for Aβ40 3X ISTD and p-Tau 3X ISTD at these intentionally elevated concentrations, no clinically meaningful changes in APS2 interpretation (%Difference <10% across samples) were identified, and no samples demonstrated category shifts that would impact clinical interpretation of APS2 results.

4. Detection Limit:

The detection capabilities of the C2N-Aβ42/40 Assay and C2N-%-p-tau217 Assay were evaluated by Limit of Quantitation (LoQ) studies in accordance with the CLSI guideline EP17-A2. The studies evaluated three lots of C2N-Aβ42/40 Assay and C2N-%-p-tau217

Assay reagents on three different instrument systems with a different operator performing the testing for each lot of reagents. In total, there are nine lot/instrument combinations that were used for testing. The calculations were carried out separately for each lot. The requirement for the LoQ was %CV≤20% and linearity deviation of within ±10%. The LoQ was set to the highest value provided by the different lots.

For the C2N-Aβ42/40 Assay, a set of four pooled native K2-EDTA plasma samples diluted in charcoal-stripped plasma were tested for both Aβ42 and Aβ40. Each sample was tested in three replicates on three different days. Each lot of reagents yielded a total of 108 replicates across all samples (n = 4), replicates (n = 3), days (n = 3), and instruments (n = 3). The LoQ for Aβ42 was determined for each lot as 1.565 pg/mL, 1.465 pg/mL, and 2.328 pg/mL. The claimed LoQ for Aβ42 was 5.0 pg/mL at the lower limit of the analytical measuring interval. The LoQ for Aβ40 was determined for each lot as 12.438 pg/mL, 12.740 pg/mL, and 13.664 pg/mL. The claimed LoQ for Aβ40 was 50 pg/mL at the lower limit of the analytical measuring interval.

For the C2N-%-p-tau217 Assay, two sets of five K2-EDTA samples were tested, one for p-tau217 and the other for np-tau217. Each sample was tested in three replicates on three different days. Each lot of reagents yielded a total of 108 replicates across all samples (n = 4), replicates (n = 3), days (n = 3), and instruments (n = 3). The LoQ for p-tau217 was determined for each lot as 0.868 pg/mL, 1.167 pg/mL, and 0.868 pg/mL. The claimed LoQ for p-tau217 was 1.3 pg/mL at the lower limit of the analytical measuring interval. The LoQ for np-tau217 was determined for each lot as 2.101 pg/mL, 3.576 pg/mL, and 3.302 pg/mL The claimed LoQ for np-tau217 was 6.6 pg/mL at the lower limit of the analytical measuring interval.

5. Traceability, Stability, Expected Values (Controls, Calibrators, or Methods):

1) Traceability

Commercially available certified reference standards for the four measurands, Aβ40, Aβ42, p-tau217, and np-tau217 in plasma, and their heavy isotope-labeled internal standards, are not available. Because of this, all reference standards and heavy isotope-labeled internal standards for the four measurands (recombinant or synthetic) are quantified by amino acid analysis (AAA). The AAA method used is traceable to the United States Pharmacopeia (USP) harmonized standard and used a NIST-certified bovine serum albumin (BSA) reference standard to ensure quantitative accuracy.

The calibrators for use with the PrecivityAD2 test were prepared gravimetrically under internal quality system and are traceable to in-house reference calibrators (i.e. Master Calibrator Lot). The quality control samples in the C2N-Aβ42/40 assay and C2N-%p-tau217 assay share the same level of traceability as the calibrators.

2) Stability

Stability for the PrecivityAD2 test is based on reagent stability for the C2N-Aβ42/40 and C2N-%-p-tau217 Assays, and the PrecivityAD2 APS2. The PrecivityAD2 test only uses the assay reagents which are stored under the claimed storage conditions and within the

claimed expiration date for each assay. The stability of the C2N-Aβ42/40 Assay and C2N-%-p-tau217 Assay-was evaluated based on the following studies:

a. Reagent shelf-life:

To evaluate reagent shelf-life stability, three different lots of final critical reagents for the C2N-Aβ42/40 Assay and C2N-%-p-tau217 Assay were prepared. Each lot consists of the eight critical reagents that are used to perform each assay, and each of the eight critical reagents have been prepared with different lots of raw materials where possible. Hence, the study is designed to establish the shelf-life of all eight of the critical reagents used to perform the C2N-Aβ42/40 and C2N-%-p-tau217 Assays.

C2N-Aβ42/40 Assay
A panel of seven K2-EDTA plasma samples with varying Aβ42 (range 0.144 pg/mL–7.495 pg/mL) and Aβ40 (range 42.5 pg/mL–762.2 pg/mL) concentrations (Panel #1) were tested in five replicates on Day 0 and then in two replicates on two subsequent days, one between Day 207-Day 217 and another between Days 369-430. All samples were aliquoted and stored at −80°C prior to the day of testing. Multiple operators and instrument systems were used across the different days of the study. For each lot, the assay results obtained at different time points were compared to the assay results at baseline (0 month). Results support a shelf-life for all critical reagents of at least 180 days. The shelf-life of critical reagents used in the C2N-Aβ42/40 Assay is six (6) months.

C2N-%-p-tau217 Assay
A panel of seven K2-EDTA plasma samples with varying p-tau217 (range 0.144 pg/mL–7.495pg/mL) and np-tau217 (range 42.5 pg/mL–762.2 pg/mL) concentrations (Panel #2) were tested in five replicates on Day 0 and then in two replicates on two subsequent days, one between Days 188, Days 212 and another on Day 450, All samples were aliquoted and stored at −80°C prior to the day of testing. Multiple operators and instrument systems were used across the different days of the study. For each lot, the assay results obtained at different time points were compared to the assay results at baseline (0 month). Results support a shelf-life for all critical reagents of at least 180 days. The shelf-life of critical reagents used in the C2N-%-p-tau217 Assay is six (6) months.

PrecivityAD2 APS2
Reagent stability based on APS2 values from sample combinations from Panels #1 and Panel #2 was evaluated for each lot. For each analyte, sample, and time point, the mean of the replicate measurements was first calculated. For each samples, the Aβ42/Aβ40 ratio was then calculated. For each lot, all possible combinations of Aβ and p-tau samples were generated, resulting in a total of 15 combinations (three Aβ42/40 samples × five p-tau217 /np-Tau samples) for Lot 1 or 18 combinations (three Aβ42/40 samples x six p-tau217 /np-Tau samples) for Lot 2 and Lot 3. The quantitative outputs of the C2N-Aβ42/40 assay and C2N-%p-tau217 assay were used to calculate APS2 for each sample combination. After six months of storage, seven of the 15 sample combinations for lot 1 exceeded the pre-specified mean APS2 %difference, nine of the 18 sample combinations for lot 2, and 10 of the 18 sample combinations for lot 3. To evaluate the effect of the observed stability trends on APS2

values and resulting classification categories, a clinical impact analysis was conducted using samples from the pivotal clinical validation study to evaluate whether potential reagent-related drift in APS2 would meaningfully affect patient classification. Results show no categorical shifts from Negative to Positive or from Positive to Negative were observed for the three lots of critical reagent for the C2N-Aβ42/40 Assay and C2N-%-p-tau217 Assay stored at −80°C for up to six (6) months.

b. Sample stability:

i. Sample storage stability at room temperature (RT)

A panel of five K2-EDTA plasma samples with APS2 values spanning the reporting range including samples near the established clinical cutoff regions were freshly collected aliquoted, frozen, and shipped on dry ice per C2N's pre-analytical processing instructions. All samples were initially stored at -80℃. Each sample was thawed and left at room temperature for 0.5, 1, 2, 3, and 4 hours prior to testing. 0.5 hours was tested as the baseline condition (t = 0.5) with six replicates per sample. Three replicates were tested for other time points (t = 1, 2, 3, 4 hours). Each set of aliquots from each sample were tested in a single run, by a single operator, with a single Lot of reagents on a single instrument. The quantitative outputs of the C2N-Aβ42/40 assay and C2N-%p-tau217 assay were used to calculate APS2 for each replicate. The range of %APS2 differences for all samples across the timepoints is -1.16% –9.26%. The data supports that samples tested are stable at room temperature for up to three hours.

ii. Sample freeze/thaw (F/T) stability

A panel of five K_2-EDTA plasma samples with APS2 values spanning the reporting range including samples near the established clinical cutoff regions were freshly collected aliquoted, frozen, and shipped on dry ice per C2N's pre-analytical processing instructions. All aliquots were stored immediately at -80℃ upon receipt. Aliquots of each sample were subjected to two, three, and four F/T cycles with each cycle involving thawing according to the standard operating procedure for the assay, refreezing, 24-hour storage at −80℃, and thawing prior to testing. F/T cycle 1 is the baseline sample and is defined as a sample that was not subjected to any additional F/T cycles and was only thawed once prior to testing. F/T cycle 1 aliquots were tested with six replicates per sample and F/T cycle 2, 3, and 4 aliquots were tested with three replicates for each sample. Each set of F/T cycle aliquots from each sample were tested in a single run, by a single operator, with a single Lot of reagents on a single instrument. The quantitative outputs of the C2N-Aβ42/40 assay and C2N-%p-tau217 assay were used to calculate APS2 for each replicate. The range of %APS2 differences for all samples across F/T cycles is 0 % − 8.48%. The data supports that samples tested are stable for up to three F/T cycles.

6. Assay Cut-Off:

The APS2 algorithm was developed using data from 583 subjects with an average age of 72.6 years. The study subjects consisted of 51.3% (299/583) males and 48.7% females (284/583).

Of the 583 evaluable samples, 302 (51.8%) were PET positive and 281 (48.2%) were PET negative. The following APS2 clinical cutoffs were selected: 66.5 (upper PrecivityAD2 cutoff) and 27.5 (lower PrecivityAD2 cutoff).

7. Accuracy (Instrument):

To evaluate the analytical accuracy of the C2N-Aβ42/40 Assay, 11 different individual native plasma samples were spiked with different amounts of the same Aβ42 (8 −70 pg/mL) and Aβ40 (80 −700 pg/mL) recombinant proteins that are used to make the calibrators and controls for the C2N-Aβ42/40 Assay. To evaluate the analytical accuracy of the C2N-%p-tau217 Assay, ten different individual native plasma sample pools were spiked with different amounts of the same p-Tau217 (1.83 − 44.43 pg/mL) and np-Tau217 (15.39 −3 50.69 pg/mL) recombinant proteins that are used to make the calibrators and controls for the C2N-%p-tau217 Assay. Each unspiked sample and spiked sample was tested with seven replicates in a single run by a single operator with a single lot of reagents on a single instrument. The linear regression fits are summarized in the table below for each analyte measured by the two assays:

C2N-Aβ42/40 Assay	
Aβ42	y = 1.03 x – 0.64
Aβ40	y = 0.95 x – 10.2
C2N-%-p-tau217 Assay	
p-tau217	y = 1.05 x – 2.34
np-tau217	y = 0.99 x – 0.03

8. Carry-Over:

A low-concentration sample with each analyte near their respective lower limit of the measuring interval (LLMI) and a high concentration sample with each analyte at approximately 5 times the upper limit of the measuring interval were tested. The study comprised five runs performed over five days, with each run structured as follows: 10 initial replicates of the low concentration sample, followed by 10 cycles of alternating injections (high and low samples), totaling 30 injections per run. The initial 10 replicates were designated as the protected low concentration sample, whereas the subsequent 10 low concentration replicates in the alternating cycles were labeled as the unprotected low concentration sample. Based on the mean differences and estimates of 90%CIs, none of these four analytes (Aβ40, Aβ42, p-tau217, and np-tau217) exhibit interference between values of protected and unprotected low samples.

B. Comparison Studies:

1. Method Comparison with Predicate Device:

Not applicable.

2. Matrix Comparison:

Not applicable.

C. Clinical Studies:

1. Clinical Performance

A clinical validation study was conducted to evaluate the performance of the PrecivityAD2 Test as an aid in the assessment of whether a patient presenting with cognitive impairment and being evaluated for Alzheimer's disease (AD) and other causes of cognitive decline would test positive or negative for amyloid plaques at the time of testing, as measured by amyloid PET imaging using an FDA-approved tracer or with an FDA authorized cerebrospinal fluid (CSF) biomarker test for the assessment of brain amyloid pathology status. The study included 1142 subjects from three independent clinical cohorts (cohorts 1-3). The distribution of the amyloid PET scan and CSF biomarker test results in the individual clinical cohorts is summarized in the table below. Of the 1142 study participants included in the final data set, 209 (18.3%) had an amyloid PET imaging scan and 933 (81.7%) had a CSF biomarker test result.

Clinical cohort	Total subjects in Pooled Validation Set	Amyloid PET Visual Read	CSF Biomarker Test
1	209	209	92*
2	889	0	889
3	44	0	44
Total	1142	209	933**

*Of the 209 individuals, 92 had both amyloid PET imaging and CSF biomarker test results.
**CSF biomarker test result was not used to assess brain amyloid pathology status in clinical cohort 1. Subjects in this cohort evaluated with the CSF biomarker test were not included in the total number of subjects evaluated with the CSF biomarker test.

Amyloid PET images were read and scored as positive or negative by two independent readers. If the readers disagreed, a third reader acted as adjudicator and the score of the third reader was taken as the final result.

Subjects represented the clinical spectrum of Alzheimer's disease, including individuals with subjective cognitive decline (SCD; N=242), mild cognitive impairment (MCI; N=540), and dementia (N=360). The demographic and clinical characteristics of the study subjects according to diagnostic groups and amyloid status determined by amyloid PET scan or CSF test results are presented in the table below.

		Clinical Presentation			PET Visual Read		CSF Test		Amyloid Pathology		Total
		SCD	MCI	Dementia	Positive	Negative	Positive	Negative	Present	Absent	
N		242	540	360	96	113	506	427	602	540	1142
Sex	Male	121 (50%)	295 (54.6%)	183 (50.8%)	45 (46.9%)	75 (66.4%)	233 (46%)	246 (57.6%)	278 (46.2%)	321 (59.4%)	599 (52.5%)
	Female	121 (50%)	245 (45.4%)	177 (49.2%)	51 (53.1%)	38 (33.6%)	273 (54%)	181 (42.4%)	324 (53.8%)	219 (40.6%)	543 (47.5%)
Age	40–49	3 (1.2%)	10 (1.9%)	1 (0.3%)	0 (0%)	2 (1.8%)	2 (0.4%)	10 (2.3%)	2 (0.3%)	12 (2.2%)	14 (1.2%)
	50–59	34 (14%)	41 (7.6%)	7 (1.9%)	5 (5.2%)	8 (7.1%)	13 (2.6%)	56 (13.1%)	18 (3%)	64 (11.9%)	82 (7.2%)
	60–69	77 (31.8%)	109 (20.2%)	38 (10.6%)	26 (27.1%)	30 (26.5%)	55 (10.9%)	113 (26.5%)	81 (13.5%)	143 (26.5%)	224 (19.6%)
	70–79	92 (38%)	253 (46.9%)	178 (49.4%)	41 (42.7%)	52 (46%)	262 (51.8%)	168 (39.3%)	303 (50.3%)	220 (40.7%)	523 (45.8%)
	≥80	36 (14.9%)	127 (23.5%)	136 (37.8%)	24 (25%)	21 (18.6%)	174 (34.4%)	80 (18.7%)	198 (32.9%)	101 (18.7%)	299 (26.2%)
	Mean	69.4	72.8	76.7	73.3	71.8	76.4	70.1	75.9	70.4	73.3
Race	White	0 (0%)	32 (5.9%)	9 (2.5%)	0 (0%)	0 (0%)	34 (6.7%)	7 (1.6%)	34 (5.6%)	7 (1.3%)	41 (3.6%)
	Black or African American	0 (0%)	2 (0.4%)	0 (0%)	0 (0%)	0 (0%)	2 (0.4%)	0 (0%)	2 (0.3%)	0 (0%)	2 (0.2%)
	Others*	0 (0%)	1 (0.2%)	0 (0%)	0 (0%)	0 (0%)	1 (0.2%)	0 (0%)	1 (0.2%)	0 (0%)	1 (0.1%)
	Missing	242 (100%)	505 (93.5%)	351 (97.5%)	96 (100%)	113 (100%)	469 (92.7%)	420 (98.4%)	565 (93.9%)	533 (98.7%)	1098 (96.1%)
MMSE	<18	3 (1.2%)	21 (3.9%)	64 (17.8%)	5 (5.2%)	0 (0%)	60 (11.9%)	23 (5.4%)	65 (10.8%)	23 (4.3%)	88 (7.7%)
	18–23	9 (3.7%)	77 (14.3%)	155 (43.1%)	17 (17.7%)	7 (6.2%)	148 (29.2%)	69 (16.2%)	165 (27.4%)	76 (14.1%)	241 (21.1%)
	24–30	210 (86.8%)	411 (76.1%)	116 (32.2%)	70 (72.9%)	97 (85.8%)	268 (53%)	302 (70.7%)	338 (56.1%)	399 (73.9%)	737 (64.5%)
	Missing	20 (8.3%)	31 (5.7%)	25 (6.9%)	4 (4.2%)	9 (8%)	30 (5.9%)	33 (7.7%)	34 (5.6%)	42 (7.8%)	76 (6.7%)
Apolipoprotein E (ApoE) Status	ε2/ε2	1 (0.4%)	5 (0.9%)	2 (0.6%)	0 (0%)	1 (0.9%)	3 (0.6%)	4 (0.9%)	3 (0.5%)	5 (0.9%)	8 (0.7%)
	ε2/ε3	22 (9.1%)	42 (7.8%)	16 (4.4%)	2 (2.1%)	18 (15.9%)	14 (2.8%)	46 (10.8%)	16 (2.7%)	64 (11.9%)	80 (7%)
	ε2/ε4	6 (2.5%)	14 (2.6%)	11 (3.1%)	6 (6.2%)	3 (2.7%)	16 (3.2%)	6 (1.4%)	22 (3.7%)	9 (1.7%)	31 (2.7%)
	ε3/ε3	124 (51.2%)	241 (44.6%)	149 (41.4%)	22 (22.9%)	69 (61.1%)	160 (31.6%)	263 (61.6%)	182 (30.2%)	332 (61.5%)	514 (45%)
	ε3/ε4	77 (31.8%)	194 (35.9%)	143 (39.7%)	51 (53.1%)	17 (15%)	245 (48.4%)	101 (23.7%)	296 (49.2%)	118 (21.9%)	414 (36.3%)
	ε4/ε4	12 (5%)	40 (7.4%)	38 (10.6%)	15 (15.6%)	5 (4.4%)	65 (12.8%)	5 (1.2%)	80 (13.3%)	10 (1.9%)	90 (7.9%)
	Missing	0 (0%)	4 (0.7%)	1 (0.3%)	0 (0%)	0 (0%)	3 (0.6%)	2 (0.5%)	3 (0.5%)	2 (0.4%)	5 (0.4%)

		Clinical Presentation			PET Visual Read		CSF Test		Amyloid Pathology		Total
		SCD	MCI	Dementia	Positive	Negative	Positive	Negative	Present	Absent	
Years of Education	High School diploma or less than 13	98 (40.5%)	295 (54.6%)	244 (67.8%)	50 (52.1%)	58 (51.3%)	286 (56.5%)	243 (56.9%)	336 (55.8%)	301 (55.7%)	637 (55.8%)
	Bachelor degree or more than 13	140 (57.9%)	233 (43.1%)	109 (30.3%)	46 (47.9%)	54 (47.8%)	206 (40.7%)	176 (41.2%)	252 (41.9%)	230 (42.6%)	482 (42.2%)
	Missing	4 (1.7%)	12 (2.2%)	7 (1.9%)	0 (0%)	1 (0.9%)	14 (2.8%)	8 (1.9%)	14 (2.3%)	9 (1.7%)	23 (2%)
Brain Amyloid Pathology	Present	58 (24%)	291 (53.9%)	253 (70.3%)	96 (100%)	0 (0%)	506 (100%)	0 (0%)	602 (100%)	0 (0%)	602 (52.7%)
	Absent	184 (76%)	249 (46.1%)	107 (29.7%)	0 (0%)	113 (100%)	0 (0%)	427 (100%)	0 (0%)	540 (100%)	540 (47.3%)
*Asian or America Indian or Alaska Native or more than one.											

The prevalence of amyloid positivity among patients with mild dementia was 70.3% (253/360) compared to 53.9% (291/540) and 24.0% (58/242) among those with MCI and SCD, respectively. A breakdown of the PET scan and CSF test results by clinical status is provided in the table below.

	Diagnostic Groups			Total
	SCD	MCI	Dementia	
Amyloid PET Scan	86	94	29	209
CSF Test	156	446	331	933
Total	242	540	360	1142
Prevalence of Amyloid Pathology	24.0% (58/242)	53.9% (291/540)	70.3% (253/360)	52.7% (602/1142)

Of the 209 subjects with an adjudicated amyloid PET visual read, 96 (45.9%) had a Positive scan result and 113 (54.1%) had a Negative scan result, Of the 933 subjects with a CSF test result, 506 (54.2%) had a Positive test result and 427 (45.8%) had a Negative test result. The percentages of positive PET scan and CSF test results were 8.4% (96/1142) and 44.3% (506/1142), respectively, in the study population. Overall, 52.7% (602/1142) of the evaluable subjects had a positive amyloid status.

The study population included participants with comorbidities frequently encountered in clinical practice such as coronary heart disease (n=96), atrial fibrillation (n=78), chronic heart failure (n=27), obesity (n=184), hypertension (n=416), depression (n=258), metabolic conditions (n=228), dyslipidemia (n=230), autoimmune/inflammatory (n=105), chronic kidney disease (n=194), diabetes (n=142), depression (n=186), history of stroke or TIA (n=97), and history of cancer (n=145).

As the clinical specimens tested to establish the clinical validity of the PrecivityAD2 test were stored frozen at -80°C for an extended duration, a long-term specimen stability study was conducted to demonstrate the integrity of the archived clinical specimens. The data supports that clinical specimens stored frozen at -80◦C are stable for up to two years. All archived specimens from clinical cohorts 1-3 included in the pivotal clinical performance study were stored no longer than two years after collection.

Results:

a. Clinical Performance

To estimate the clinical performance measures of the PrecivityAD2 test, the test result using the APS2 cut-offs of 27.5 and 66.5 was compared to the adjudicated amyloid PET scan or CSF biomarker test result for each patient. The adjudicated amyloid PET scan positive or CSF biomarker result positive is considered as "Amyloid positive". The data are summarized in the table below.

The performance estimates of the PrecivityAD2 test are described by PET scan and CSF biomarker predictive value (indicated as Predictive Value, PV, percent of subjects with amyloid positive results) and frequency of positive test results from the tested samples (indicated as Frequency of Results, FR) for positive, intermediate, and negative test results. The agreement rates with visual read amyloid PET or CSF biomarker test classification and predictive values for the PrecivityAD2 test at the pre-specified APS2 cut-offs of 27.5 and 66.5 and likelihood ratios are summarized in the table below.

		Amyloid PET Scan^ or CSF Test#		
		Positive	**Negative**	**Total**
PrecivityAD2 test	**Positive** (APS2>66.5)	413	10	423
	Likely Positive (27.5<APS2< 66.5)	153	45	198
	Negative (APS2<27.5)	36	485	521
	Total	**602**	**540**	**1142**

^Amyloid PET scan result based on visual read.
#CSF biomarker test result based on Lumipulse ***G*** β-Amyloid Ratio (1-42/1-40) (DEN200072).

		Amyloid PET Scan^ or CSF Test[#]		Predictive Value % [n/N] (95% CI*)	Frequency of Results % [n/N]	Likelihood Ratio (95%CI**)
		Positive	Total			
PrecivityAD2 Test Result	**Positive** (APS2>66.5)	413	423	97.6% [413/423] (95.7%; 98.7%)	37.0% [423/1142]	37.05 (20.0; 68.62)
	Likely Positive (27.5<APS2<66.5)	153	198	77.3% [153/198] (70.9%; 82.6%)	17.3% [198/1142]	3.05 (2.23; 4.16)
	Negative (APS2<27.5)	36	521	6.9% [36/521] (5.0%; 9.4%)	45.6% [521/1142]	0.07 (0.05; 0.09)
	Total	**602**	**1142**	**Prevalence = 52.7% [602/1142]**		

^Amyloid PET scan result based on visual read.
[#]CSF biomarker test result based on Lumipulse ***G*** β-Amyloid Ratio (1-42/1-40) (DEN200072).
*95%CIs are calculated based on 95%CIs of corresponding likelihood ratios.
**95%CIs are calculated using an asymptotic method for ratios of two independent binomial proportions.

Of the 1142 evaluable subjects, 602 had amyloid PET or CSF biomarker positive results and 540 had amyloid PET or CSF biomarker negative results.

- The prevalence of amyloid positivity was 52.7% (602/1142) overall in the study population.
- Of the 602 subjects with an amyloid PET or CSF biomarker result, 413 subjects had a positive PrecivityAD2 test result (positive percent agreement was 68.6% (413/602) with 95% CI: (64.8%, 72.2%). Of the 540 with an amyloid PET or CSF biomarker negative result, 485 subjects had a negative PrecivityAD2 test result (negative percent agreement was 83.6% (485/580) with 95% CI: (80.4%, 86.4%).
- There were 423 out of 1142 (37.0%) subjects with positive PrecivityAD2 test results. Among them, 413 subjects had amyloid PET or CSF biomarker positive results (PV for positive test results was 97.6% (413/423) with 95%CI: (95.7%, 98.7%)). The positive PrecivityAD2 test results indicate an increase of the likelihood of an amyloid PET or CSF biomarker positive result (PV for subjects with positive PrecivityAD2 test results (97.6.5%) is statistically higher than the prevalence of 52.7%: the lower bound of 95% CI for the PV of 95.7% > prevalence of 52.7%).
- There were 521 out of 1142 (45.6%) subjects with negative PrecivityAD2 test results. Among them, 36 subjects had amyloid PET or CSF biomarker positive results (PV for negative test results was 6.9% (36/521) with 95% CI: (5.0%, 9.4%) and NPV=93.1% (485/521) with 95% CI: (90.6%, 95.0%)). The negative PrecivityAD2 test results indicate a low likelihood of an amyloid PET or CSF biomarker positive result (PV for subjects with negative PrecivityAD2 test results (6.9%) is statistically lower than the prevalence of 52.7%: the upper bound of 95% CI for the PV of 9.4% < prevalence of 52.7%).
- There were 198 out 1142 (17.3%) subjects with likely positive PrecivityAD2 test results. Of the 198 subjects with a likely positive PrecivityAD2 test result, 153 had

amyloid PET or CSF biomarker positive results. The PV for likely positive PrecivityAD2 test results was 77.3% (153/198) with 95% CI: (70.9%, 82.6%).

The data of clinical performance study supports that positive PrecivityAD2 test results are consistent with amyloid PET scan or CSF biomarker test positive results and PrecivityAD2 test negative results are consistent with amyloid PET scan or CSF biomarker test negative results.

b. Sub-group Analysis

i. Amyloid PET scan or CSF biomarker test used for determining amyloid status.

The performance measures of the PrecivityAD2 test stratified by the methods used for determining brain amyloid status are summarized in the table below.

Clinical Comparator	**PrecivityAD2 Test Result**	**Amyloid Status**		**Predictive Value % [n/N] (95% CI*)**	**Frequency of Results % [n/N]**	**Likelihood Ratio (95% CI**)**
		Positive	**Total**			
PET^	**Positive** (APS2>66.5)	67	74	90.5% (67/74) (81.7%; 95.3%)	35.4% (74/209)	11.26 (5.43; 23.37)
	Likely Positive (27.5<APS2<66.5)	23	40	57.5% (23/40) (42.2%; 71.5%)	19.1% (40/209)	1.6 (0.91; 2.80)
	Negative (APS2<27.5)	6	95	6.3% (6/95) (2.9%; 13.1%)	45.5% (95/209)	0.08 (0.04; 0.173)
	Total	**96**	**209**	**Prevalence = 45.9% [96/209]**		
CSF Test#	**Positive** (APS2>66.5)	346	349	99.1% [346/349] (97.5%; 99.7%)	37.4% [349/933]	97.33 (31.47; 301.04)
	Likely Positive (27.5<APS2<66.5)	130	158	82.3% [130/158] (75.6%; 87.4%)	16.9% [158/933]	3.92 (2.66; 5.77)
	Negative (APS2< 27.5)	30	426	7.0% [30/426] (5.0%; 9.9%)	45.7% [426/933]	0.064 (0.05; 0.09)
	Total	**506**	**933&**	**Prevalence = 54.2% [506/933]**		

^Amyloid PET scan result based on visual read.
CSF biomarker test result based on Lumipulse ***G*** β-Amyloid Ratio (1-42/1-40) (DEN200072).
& Of the 933 subjects who had CSF testing, 92 subjects also had amyloid PET scan. For these 92 subjects, amyloid PET scan was used to determine brain amyloid pathology status.
*95%CIs are calculated based on 95%CIs of corresponding likelihood ratios.
** 95%CIs are calculated using an asymptotic method for ratios of two independent binomial proportions.

ii. Diagnostic group

The performance measures of the PrecivityAD2 test stratified by diagnostic groups are summarized in the table below.

Diagnosis	PrecivityAD2 Test Result	Amyloid PET Scan^ or CSF Test[#]		Predictive Value % [n/N] (95% CI*)	Frequency of Results % [n/N]	Likelihood Ratio (95% CI**)
		Positive (n)	N			
Dementia	**Positive** (APS2>66.5)	198	201	98.5% [198/201] (95.7%; 99.5%)	55.8% [201/360]	27.96 (9.13; 85.33)
	Likely Positive (27.5<APS2<66.5)	47	60	78.3% [47/60] (66.4%; 86.9%)	16.7% [60/360]	1.53 (0.86; 2.70)
	Negative (APS2<27.5)	8	99	8.1% [8/99] (4.2%; 15.1%)	27.5% [99/360]	0.038 (0.019; 0.074)
	Toal	**253**	**360**	**Prevalence = 70.3% [253/360]**		
MCI	**Positive** (APS2>66.5)	187	191	97.9% [187/191] (94.7%; 99.2%)	35.4% [191/540]	40.18 (15.07; 106.14)
	Likely Positive (27.5<APS2<66.5)	83	104	79.8% [83/104] (71.1%; 86.4%)	19.3% [104/540]	3.39 (2.16; 5.29)
	Negative (APS2<27.5)	21	245	8.6% [21/245] (5.7%; 12.7%)	45.4% [245/540]	0.08 (0.053; 0.121)
	Total	**291**	**540**	**Prevalence = 53.9% [291/540]**		
SCD	**Positive** (APS2>66.5)	28	31	90.3% [28/31] (75.1%; 96.7%)	12.8% [31/242]	30.18 (9.34; 93.84)
	Likely Positive (27.5<APS2<66.5)	23	34	67.6% [23/34] (50.8%; 80.9%)	14.0% [34/242]	6.61 (3.44; 12.77)
	Negative (APS2<27.5)	7	177	4.0% [7/177] (1.9%; 7.9%)	73.1% [177/242]	0.131 (0.065; 0.262)
	Total	**58**	**242**	**Prevalence = 24.0% [58/242]**		

^Amyloid PET scan result based on visual read.

[#] CSF biomarker test result based on Lumipulse ***G*** β-Amyloid Ratio (1-42/1-40) (DEN200072).

*95%CIs are calculated based on 95%CIs of corresponding likelihood ratios.

**95%CIs are calculated using an asymptotic method for ratios of two independent binomial proportions.

iii. Sex

The performance measures of PrecivityAD2 test stratified by sex are summarized in the table below.

Sex	PrecivityAD2 Test Result	Amyloid PET Scan^ or CSF Test#		Predictive Value % [n/N] (95% CI*)	Frequency of Results % [n/N]	Likelihood Ratio (95% CI**)
		Positive (n)	N			
Male	**Positive** (APS2>66.5)	175	181	96.7% [175/181] (93.0%; 98.5%)	30.2% [181/599]	33.10 (15.16; 74.78)
	Likely Positive (27.5<APS2<66.5)	86	113	76.1% [86/113] (67.5%; 83.0%)	18.9% [113/599]	3.67 (2.46; 5.49)
	Negative (APS2<27.5)	17	305	5.6% [17/305] (3.5%; 8.7%)	50.9% [305/599]	0.068 (0.043; 0.108)
	Total	**278**	**599**	**Prevalence = 46.4% [278/599]**		
Female	**Positive** (APS2>66.5)	238	242	98.3% [238/242] (95.8%; 99.4%)	44.6% [242/543]	40.83 (15.19; 106.43)
	Likely Positive (27.5<APS2<66.5)	67	85	78.8% [67/85] (69.0%; 86.2%)	15.7% [85/543]	2.52 (1.53; 4.11)
	Negative (APS2<27.5)	19	216	8.8% [19/216] (5.7%; 13.3%)	39.8% [216/543]	0.066 (0.042; 0.101)
	Total	**324**	**543**	**Prevalence = 59.7% [324/543]**		

^Amyloid PET scan result based on visual read.
#CSF biomarker test result based on Lumipulse ***G*** β-Amyloid Ratio (1-42/1-40) (DEN200072).
*95%CIs are calculated based on 95%CIs of corresponding likelihood ratios.
**95%CIs are calculated using an asymptotic method for ratios of two independent binomial proportions.

iv. Age

The performance measures of the PrecivityAD2 test were also stratified by age and summarized in the table below.

Age (years)	PrecivityAD2 Test Result	Amyloid PET Scan^ or CSF #		Predictive Value % [n/N] (95% CI*)	Frequency of Results % [n/N]	Likelihood Ratio (95% CI**)
		Positive (n)	N			
40-49	**Positive** (APS2>66.5)	2	2	100.0% [2/2] (34.2%, 100.0%)	14.3% [2/14]	Inf (NaN; Inf)
	Likely Positive (27.5<APS2<66.5)	0	1	0.0% [0/1] (0.0%; 79.3%)	7.1% [1/14]	0 (0; NaN)
	Negative (APS2<27.5)	0	11	0.0% [0/11] (0.0%; 25.9%)	78.6% [11/14]	0 (0; NaN)
	Total	**2**	**14**	**Prevalence = 14.3% [2/14]**		
50-59	**Positive** (APS2>66.5)	14	14	100.0% [14/14] (78.5%; 100.0%)	17.1% [14/82]	Inf (NaN; Inf)
	Likely Positive (27.5 <APS2<66.5)	3	6	50.0% [3/6] (18.8%; 81.2%)	7.3% [6/82]	3.55 (0.78; 16.14)
	Negative (APS2<27.5)	1	62	1.6% [1/62] (0.3%; 8.6%)	75.6% [62/82]	0.059 (0.009; 0.392)
	Total	**18**	**82**	**Prevalence = 22.0% [18/82]**		
60-69	**Positive** (APS2>66.5)	56	57	98.2% [56/57] (90.7%; 99.7%)	25.4% [57/224]	98.7 (13.95; 700.8)
	Likely Positive (27.5<APS2<66.5)	21	26	80.8% [21/26] (62.1%; 91.5%)	11.6% [26/224]	7.4 (2.91; 18.91)
	Negative (APS2<27.5)	4	141	2.8% [4/141] (1.1%; 7.1%)	62.9% [141/224]	0.051 (0.02; 0,134)
	Total	**81**	**224**	**Prevalence = 36.2% [81/224]**		
70-79	**Positive** (APS2>66.5)	197	203	97.0% [197/203] (93.7%; 98.6%)	40.5% (592/1462)	24.07 (10.78; 52.71)
	Likely Positive (27.5<APS2<66.5)	85	107	79.4% [85/107] (70.8%; 86.0%)	20.5% [107/523]	2.81 (1.82; 4.34)
	Negative (APS2<27.5)	21	213	9.9% [21/213] (6.5%; 14.6%)	40.7% [213/523]	0.079 (0.052; 0.12)
	Total	**303**	**523**	**Prevalence = 57.9% [303/523]**		

Age (years)	PrecivityAD2 Test Result	Amyloid PET Scan^ or CSF #		Predictive Value % [n/N] (95% CI*)	Frequency of Results % [n/N]	Likelihood Ratio (95% CI**)
		Positive (n)	N			
≥80	**Positive** (APS2>66.5)	144	147	98.0% [144/147] (94.2%; 99.3%)	49.2% [147/299]	24.23 (8.0;74.89)
	Likely Positive (27.5<APS2<66.5)	44	58	75.9% [44/58] (63.5%; 85.0%)	19.4% [58/299]	1.60 (0.923; 2.78)
	Negative (APS2<27.5)	10	94	10.6% [10/94] (5.9%; 18.5%)	31.4% [94/299]	0.061 (0,033; 0.112)
	Total	**198**	**299**	**Prevalence = 66.2% [198/299]**		

^Amyloid PET scan result based on visual read.
#CSF biomarker test result based on Lumipulse ***G*** β-Amyloid Ratio (1-42/1-40) (DEN200072).
*95%CIs are calculated based on 95%CIs of corresponding likelihood ratios.
**95%CIs are calculated using an asymptotic method for ratios of two independent binomial proportions.

v. Race

No stratified analysis of the PrecivityAD2 test performance by race was performed due to a large proportion of the subjects in the Pooled Validation Cohort with missing race categorization. The PrecivityAD2 labeling under 'Limitations' states that "*The performance of the PrecivityAD2® test across racial subgroups has not been fully established because of the relatively small number of subjects who report racial categories other than white and the large proportion of subjects in the Pooled Validation Cohort with missing racial categorization. Results for these subgroups should be interpreted with caution*."

vi. Comorbidities

The predictive values (PV) of the PrecivityAD2 test in subjects with 12 different documented comorbidities as well as in subjects with one, two, or three or more of these comorbidities are summarized in the table below.

PrecivityAD2 Test	Comorbidities				
	Coronary Heart Disease (CHD)	**Atrial Fibrillation**	**Chronic Heart Failure**	**Obesity**	**Hypertension**
Positive PV [n/N] (95% CI)	92.1% [35/38] (79.2%; 97.3%)	100.0% [33/33] (89.6%; 100.0%)	100.0% [10/10] (72.2%;100.0%)	96.9% [31/32] (84.3%; 99.4%)	96.7% [147/152] (92.5%; 98.6%)
Likely Positive PV [n/N] (95% CI)	93.3% [14/15] (70.2%; 98.8%)	66.7% [12/18] (43.7%; 83.7%)	42.9% [3/7] (15.8%; 75.0%)	77.3% [17/22] (56.6%; 89.9%)	75.0% [54/72] (63.9%; 83.6%)

PrecivityAD2 Test	Comorbidities				
	Coronary Heart Disease (CHD)	**Atrial Fibrillation**	**Chronic Heart Failure**	**Obesity**	**Hypertension**
Negative PV [n/N] (95% CI)	0.0% [0/43] (0.0%; 8.2%)	0.0% [0/27] (0.0%; 12.5%)	0.0% [0/10] (0.0%; 27.8%)	8.5% [11/130] (4.8%; 14.5%)	5.7% [11/192] (3.2%; 10.0%)
Prevalence [n/N]	51.0% [49/96]	57.7% [45/78]	48.1% [13/27]	32.1% [59/184]	51.0% [212/416]
Frequency of Intermediate [n/N]	15.6% [15/96]	23.1% [18/78]	25.9% [7/27]	12.0% [22/184]	17.3% [72/416]

PrecivityAD2 Test	Comorbidities				
	Dyslipidemia	**Autoimmune/ Inflammatory Disease**	**Chronic Kidney Disease**	**Diabetes**	**History of Stroke or TIA**
Positive PV [n/N] (95% CI)	95.2% [79/83] (88.3%; 98.1%)	91.8% [45/49] (80.8%; 96.8%)	100.0% [97/97] (96.2%;100.0%)	100.0% [43/43] (91.8%; 100.0%)	97.0% [32/33] (84.7%; 99.5%)
Likely Positive PV [n/N] (95% CI)	72.1% [31/43] (57.3%; 83.3%)	75.0% [15/20] (53.1%; 88.8%)	73.2% [30/41] (58.1%; 84.3%)	73.5% [25/34] (56.9%; 85.4%)	50.0% [10/20] (29.9%; 70.1%)
Negative PV [n/N] (95% CI)	4.8% [5/104] (2.1%; 10.8%)	5.6% [2/36] (1.5%; 18.1%)	1.8% [1/56] (0.3%; 9.4%)	6.2% [4/65] (2.4%; 14.8%)	2.3% [1/44] (0.4%; 11.8%)
Prevalence [n/N]	50.0% [115/230]	59.0% [62/105]	66.0% [128/194]	32.1% [59/184]	44.3% [43/97]
Frequency of Intermediate [n/N]	18.7% [43/230]	19.0% [20/105]	21.1% [41/194]	50.7% [72/142]	20.6% [20/97]

PrecivityAD2 Test	Comorbidities		Number of comorbidities		
	Depression	**History of Cancer**	**One**	**Two**	**Three or More**
Positive PV [n/N] (95% CI)	98.2% [54/55] (90.4%; 99.7%)	95.5% [64/67] (87.6%; 98.5%)	98.3% [118/120] (94.1%; 99.5%)	98.5% [67/68] (92.1%; 99.7%)	96.3% [104/108] (90.9%; 98.6%)
Likely Positive PV [n/N] (95% CI)	57.1% [16/28] (39.1%; 73.5%)	64.0% [16/25] (44.5%; 79.8%)	80.0% [44/55] (67.6%; 88.4%)	77.8% [21/27] (59.2%; 89.4%)	68.9% [42/61] (56.4%; 79.1%)
Negative PV [n/N] (95% CI)	1.9% [2/103] (0.5%; 6.8%)	5.7% [3/53] (1.9%; 15.4%)	8.3% [11/132] (4.7%; 14.3%)	3.8% [3/80] (1.3%; 10.5%)	4.6% [7/151] (2.3%; 9.3%)

PrecivityAD2 Test	Comorbidities		Number of comorbidities		
	Depression	History of Cancer	One	Two	Three or More
Prevalence [n/N]	38.7% [72/186]	57.2% [83/145]	56.4% [173/307]	52.0% [91/175]	47.8% [153/320]
Frequency of Intermediate [n/N]	15.1% [28/186]	17.2% [25/145]	17.9% [55/307]	15.4% [27/175]	19.1% [61/320]

2. Clinical Cut-Off

   Refer to Assay Cut-off.

3. Other Clinical Supportive Data (When 1. and 2. Are Not Applicable):

   Not applicable

D Expected Values/Reference Range:

1. Reference Interval

   A reference interval of the PrecivityAD2 test was determined in cognitively normal subjects in accordance with the CLSI guideline EP28-A3c. The subjects were cognitively normal individuals with no reported memory concerns and no functional decline and completed the Mini Mental State Examination (MMSE) and Normal Delayed Recall Performance on Rey Auditory Verbal Learning Test (RAVLT). A total of 464 cognitive normal subjects (187 males and 277 females aged 40 to 92 years old) from the three study cohorts were tested and stratified by age groups (40–59, 60–69, and 70–85 years), sex, race (White, African American, and Other). The results of the cognitively normal subjects tested with the PrecivityAD2 test are summarized in the table below for all subjects (N=464).

	All Cognitively Normal Subjects								
	All	Age			Sex		Race		
		40–59	60–69	70–85	Male	Female	White	African American	Other
N	464	93	188	183	187	277	402	46	16
Aβ42									
Mean (SD)	48.60 (10.4)	47.44 (12.8)	47.54 (8.7)	50.27 (10.4)	46.89 (10.0)	49.75 (10.5)	48.56 (10.4)	49.77 (10.6)	46.21 (8.9)
Median	47.53	44.37	46.86	49.10	45.87	48.39	47.60	49.69	44.68
Range (Min. – Max.)	14.17–90.51	20.97–85.73	14.17–83.93	27.60–90.51	14.17–83.93	31.36–90.51	14.17–90.51	33.36–83.93	36.51–68.07
2.5th Percentile, 97.5th Percentile	32.93, 76.66	29.04, 82.12	34.08, 66.50	35.18, 77.40	29.42, 69.03	34.93, 79.04	32.58, 76.78	35.96, 75.85	N/A*, N/A*

	All Cognitively Normal Subjects								
	All	Age			Sex		Race		
		40–59	60–69	70–85	Male	Female	White	African American	Other
Aβ40									
Mean (SD)	482.65 (100.2)	425.31 (80.8)	470.24 (84.5)	524.53 (106.3)	471.84 (99.9)	489.94 (99.9)	486.06 (101.9)	466.13 (92.4)	444.31 (68.8)
Median	470.40	418.00	464.72	509.22	458.87	476.46	472.84	444.28	430.27
Range (Min. – Max.)	93.01–954.05	236.32–905.69	93.01–954.05	265.29–951.06	93.01–795.35	316.13–954.1	93.0–786.05	306.69–786.10	365.24–630.87
2.5th Percentile, 97.5th Percentile	337.22, 761.71	314.84, 559.76	347.60, 633.78	370.40, 785.21	321.59, 734.29	353.39, 756.91	337.04, 769.83	340.94, 701.79	N/A*, N/A*
Aβ42/Aβ40 Ratio									
Mean (SD)	0.101 (0.01)	0.111 (0.02)	0.102 (0.011)	0.096 (0.010)	0.100 (0.013)	0.102 (0.014)	0.101 (0.014)	0.107 (0.011)	0.104 (0.012)
Median	0.101	0.108	0.102	0.096	0.100	0.101	0.100	0.106	0.102
Range (Min. – Max.)	0.075–0.190	0.075–0.190	0.078–0.152	0.076–0.128	0.075–0.190	0.076–0.175	0.075–0.190	0.081–0.136	0.089–0.140
2.5th Percentile, 97.5th Percentile	0.081, 0.130	0.083, 0.167	0.082, 0.120	0.081, 0.114	0.082, 0.120	0.081, 0.137	0.081, 0.127	0.086, 0.131	N/A* N/A*
p-tau217									
Mean (SD)	10.99 (19.4)	44.63 (14.8	3.31 (10.9)	1.80 (1.7)	12.77 (20.7)	9.79 (18.4)	10.28 (19.1)	15.32 (20.3)	16.56 (24.2)
Median	1.35	41.61	0.65	0.65	1.53	0.65	1.34	1.67	1.1
Range (Min. – Max.)	0.65–86.34	15.35–86.34	0.65–85.71	0.65–7.70	0.65–81.44	0.65–86.34	0.65–86.34	0.65–63.72	0.65–60.33
2.5th Percentile, 97.5th Percentile	0.65, 64.35	19.45, 73.16	0.65, 43.44	0.65, 6.62	0.65, 68.41	0.65, 61.43	0.65, 67.4	0.65, 60.24	N/A* N/A*
np-tau217									
Mean (SD)	37.98 (26.4)	0.81 (0.5)	44.47 (21.0)	50.22 (20.4)	35.51 (24.8)	39.66 (27.3)	38.85 (25.6)	30.28 (27.7)	38.49 (38.3)
Median	40.13	0.68	42.67	45.02	39.29	40.65	40.44	33.27	41.57
Range (Min. – Max.)	0.32–201.72	0.32–3.59	0.47–164.21	14.53–201.72	0.33–116.21	0.32–201.72	0.32–201.72	0.34–116.21	0.40–149.40
2.5th Percentile, 97.5th Percentile	0.47, 89.84	0.35, 1.91	0.85, 90.29	26.43, 95.86	0.47, 84.02	0.50, 95.94	0.48, 87.34	0.50, 68.27	N/A* N/A*
%p-tau217									
Mean (SD)	2.81 (2.4)	1.89 (0.9)	2.51 (2.0)	3.59 (3.0)	2.87 (2.5)	2.78 (2.3)	2.89 (2.5)	2.37 (1.7)	2.13 (1.8)
Median	1.84	1.61	1.75	2.15	1.83	1.91	1.84	1.81	1.55
Range (Min. – Max.)	0.44–17.48	0.60–5.75	0.44–12.96	0.68–17.48	0.77–17.48	0.44–14.33	0.60–17.48	0.95–7.52	0.44 8.01
2.5th Percentile, 97.5th Percentile	0.94, 10.26	0.78, 4.03	0.92, 8.10	1.05, 12.34	0.93, 10.01	0.95, 10.26	0.96, 10.5	1.02, 7.40	N/A* N/A*

	All Cognitively Normal Subjects								
	All	Age			Sex		Race		
		40–59	60–69	70–85	Male	Female	White	African American	Other
APS2									
Mean (SD)	20.7 (23.6)	9.3 (8.4)	17.6 (20.1)	29.6 (28.6)	21.5 (24.2)	20.1 (23.3)	21.7 (24.3)	13.6 (17.0.)	14.3 (18.7)
Median	11	6	10	16	11	10.0	10	8	8.5
Range (Min. – Max.)	0–100	0-48	1–99	3–100	0–100	0–99	0–100	2–84	2–80
2.5th Percentile, 97.5th Percentile	2, 94	0, 29.7	3.7, 81	4, 98	3, 90.7	2, 92.2	2, 94.0	2.1, 64.5	N/A* N/A*
PrecivityAD2 test (%, n)									
Positive (APS2>66.5)	8.84% (41)	0% (0)	5.32% (10)	16.94% (31)	10.16% (19)	7.94% (22)	9.70% (39)	2.17% (1)	6.25% (1)
Likely Positive (27.5<APS2<66.5)	11.64% (54)	4.30% (4)	10.64% (20)	16.39% (30)	10.70% (20)	12,27% (34)	12.44% (50)	8.70% (4)	0% (0)
Negative (APS2<27.5)	79.53% (369)	95.70% (89)	84.04% (158)	66.67% (122)	79.14% (148)	79.78% (221)	77.86% (313)	89.13% (41)	93.75% (15)

*Results listed as not applicable (N/A) are due to an insufficient number of samples to properly calculate the value.

The established 95% reference interval is 2 to 94 for APS2. In this cognitively normal cohort, APS2 values were predominantly low, with the majority (80%) of the participants falling below the Negative threshold (APS2<27.5), indicating that the PrecivityAD2 test identifies individuals at low likelihood of Alzheimer's-related pathology within the intended-use population. Approximately 9% and 12% of participants were classified as Positive (APS2>66.5) and Likely Positive (27.5<APS2<66.5), respectively. Across the age groups, APS2 Positive classification demonstrated an expected increase with advancing age, from 0% at ages between 40–59 to 5.3% and 17.0% at ages between 60–69 years and between 70–85 years, respectively. No meaningful differences in the 95% reference interval and APS2 classification were observed between males and females. The 95% reference intervals were 2 to 94 in Whites and 2.1 to 64.5 in African Americans and the median is also higher in Whites compared to African Americans.

2. **Expected Values**

The expected values for Alzheimer's disease (AD)-related neurological conditions and other diseases prevalent in the intended-use population were investigated. A total of 47 evaluable human K_2-EDTA plasma samples with clinical and diagnostic information were collected from participants 40 years and older who presented with a diagnosis of non-AD dementia and other neurological conditions. The test results are summarized in the tables below:

Disease	APS2				PrecivityAD2 test (%, n)		
	N	Mean (SD)	Median	2.5th Percentile, 97.5th Percentile	Positive (APS2 > 66.5)	Likely Positive (27.5<APS2 < 66.5)	Negative (APS2 < 27.5)
Parkinson's	40	32.2 (26)	23	7, 97	12.5% (5)	35.0% (14)	52.5% (21)
Lewy Body Dementia	1	28 (NA)	28	N/A*, N/A*	0% (0)	100.0% (1)	0% (0)
Behavioral Frontotem-poral Dementia	3	45 (22)	22	N/A*, N/A*	33.3% (1)	0% (0)	66.7% (2)
Frontotem-poral Dementia	3	12.7 (13)	13	N/A*, N/A*	0% (0)	0% (0)	100.0% (1)
*Results listed as not applicable (N/A) are due to an insufficient number of samples to properly calculate the value							

E Other Supportive Instrument Performance Characteristics Data:

None.

VIII Proposed Labeling:

The labeling supports finding of substantial equivalence for this device.

IX Conclusion:

The submitted information in this premarket notification is complete.