August 19, 2026

C2N Diagnostics, LLC
Ilana Fogelman
Scientific Advisor for Medical Innovation
4340 Duncan Ave.
St. Louis, Missouri 63110

Re: K253240
Trade/Device Name: PrecivityAD2 Test
Regulation Number: 21 CFR 866.5840
Regulation Name: Alzheimer's disease pathology assessment test
Regulatory Class: Class II
Product Code: SET
Dated: August 18, 2026
Received: August 18, 2026

Dear Ilana Fogelman:

We have reviewed your section 510(k) premarket notification of intent to market the device referenced above and have determined the device is substantially equivalent (for the indications for use stated in the enclosure) to legally marketed predicate devices marketed in interstate commerce prior to May 28, 1976, the enactment date of the Medical Device Amendments, or to devices that have been reclassified in accordance with the provisions of the Federal Food, Drug, and Cosmetic Act (the Act) that do not require approval of a premarket approval application (PMA). You may, therefore, market the device, subject to the general controls provisions of the Act. Although this letter refers to your product as a device, please be aware that some cleared products may instead be combination products. The 510(k) Premarket Notification Database available at https://www.accessdata.fda.gov/scripts/cdrh/cfdocs/cfpmn/pmn.cfm identifies combination product submissions. The general controls provisions of the Act include requirements for annual registration, listing of devices, good manufacturing practice, labeling, and prohibitions against misbranding and adulteration. Please note: CDRH does not evaluate information related to contract liability warranties. We remind you, however, that device labeling must be truthful and not misleading.

If your device is classified (see above) into either class II (Special Controls) or class III (PMA), it may be subject to additional controls. Existing major regulations affecting your device can be found in the Code of Federal Regulations, Title 21, Parts 800 to 898. In addition, FDA may publish further announcements concerning your device in the Federal Register.

Additional information about changes that may require a new premarket notification are provided in the FDA guidance documents entitled "Deciding When to Submit a 510(k) for a Change to an Existing Device" (https://www.fda.gov/media/99812/download) and "Deciding When to Submit a 510(k) for a Software Change to an Existing Device" (https://www.fda.gov/media/99785/download).

Your device is also subject to, among other requirements, the Quality Management System Regulation (QMSR) (21 CFR Part 820), which includes, but is not limited to, ISO 13485 clause 7.3 (Design controls), ISO 13485 clause 8.3 (Nonconforming product), ISO 13485 clause 8.5.2 (Corrective action), and ISO 13485 clause 8.5.3 (Preventative action). Please note that regardless of whether a change requires premarket review, the QMSR requires device manufacturers to review and approve changes to device design and production (ISO 13485 clause 7.3 and ISO 13485 clause 7.5) and document changes and approvals in the Medical Device File (ISO 13485 clause 4.2.3).

Please be advised that FDA's issuance of a substantial equivalence determination does not mean that FDA has made a determination that your device complies with other requirements of the Act or any Federal statutes and regulations administered by other Federal agencies. You must comply with all the Act's requirements, including, but not limited to: registration and listing (21 CFR Part 807); labeling (21 CFR Part 801 and Part 809); medical device reporting (reporting of medical device-related adverse events) (21 CFR Part 803) for devices or postmarketing safety reporting (21 CFR Part 4, Subpart B) for combination products (see https://www.fda.gov/combination-products/guidance-regulatory-information/postmarketing-safety-reporting-combination-products); good manufacturing practice requirements as set forth in the Quality Management System Regulation (QMSR) (21 CFR Part 820) for devices or current good manufacturing practices (21 CFR Part 4, Subpart A) for combination products; and, if applicable, the electronic product radiation control provisions (Sections 531-542 of the Act); 21 CFR Parts 1000-1050.

All medical devices, including Class I and unclassified devices and combination product device constituent parts are required to be in compliance with the final Unique Device Identification System rule ("UDI Rule"). The UDI Rule requires, among other things, that a device bear a unique device identifier (UDI) on its label and package (21 CFR 801.20(a)) unless an exception or alternative applies (21 CFR 801.20(b)) and that the dates on the device label be formatted in accordance with 21 CFR 801.18. The UDI Rule (21 CFR 830.300(a) and 830.320(b)) also requires that certain information be submitted to the Global Unique Device Identification Database (GUDID) (21 CFR Part 830 Subpart E). For additional information on these requirements, please see the UDI System webpage at https://www.fda.gov/medical-devices/device-advice-comprehensive-regulatory-assistance/unique-device-identification-system-udi-system.

Also, please note the regulation entitled, "Misbranding by reference to premarket notification" (21 CFR 807.97). For questions regarding the reporting of adverse events under the MDR regulation (21 CFR Part 803), please go to https://www.fda.gov/medical-devices/medical-device-safety/medical-device-reporting-mdr-how-report-medical-device-problems.

For comprehensive regulatory information about medical devices and radiation-emitting products, including information about labeling regulations, please see Device Advice (https://www.fda.gov/medical-devices/device-advice-comprehensive-regulatory-assistance) and CDRH Learn (https://www.fda.gov/training-and-continuing-education/cdrh-learn). Additionally, you may contact the Division of Industry and Consumer Education (DICE) to ask a question about a specific regulatory topic. See the DICE website (https://www.fda.gov/medical-devices/device-advice-comprehensive-regulatory-

assistance/contact-us-division-industry-and-consumer-education-dice) for more information or contact DICE by email (DICE@fda.hhs.gov) or phone (1-800-638-2041 or 301-796-7100).

Sincerely,

Ying Mao -S

Ying Mao, Ph.D.
Branch Chief
Division of Immunology and
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Office of Product Evaluation and Quality
Center for Devices and Radiological Health

Enclosure

DEPARTMENT OF HEALTH AND HUMAN SERVICES
Food and Drug Administration

Indications for Use

Form Approved: OMB No. 0910-0120
Expiration Date: 07/31/2026
See PRA Statement below.

510(k) Number *(if known)*
K253240

Device Name
PrecivityAD2 test

Indications for Use *(Describe)*
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.

Type of Use *(Select one or both, as applicable)*

☒ Prescription Use (Part 21 CFR 801 Subpart D) ☐ Over-The-Counter Use (21 CFR 801 Subpart C)

CONTINUE ON A SEPARATE PAGE IF NEEDED.

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510(k) SUMMARY

I. BACKGROUND INFORMATION

510(k) Number:

K253240

Submitter:

C2N Diagnostics, LLC
4340 Duncan Avenue
St Louis, MO 63110
United States

Contact Person:

Name: Dr Ilana Fogelman
Title: Senior Advisor for Medical Innovation
C2N Diagnostics, LLC

Telephone: 202-256-2950
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Date Prepared:

August 17, 2026

Regulatory Information:

Item	Description
Proprietary Name	PrecivityAD2®
Classification Name	Alzheimer's Disease Pathology Assessment Test
Regulation Number	21 CFR 866.5840
Product Code	SET
Device Class	II
Panel	Immunology (82)
Review Panel	Immunology

II. INTENDED USE/INDICATIONS FOR USE:

1. Indications 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.

2. Special Instrument Requirements

Thermo Scientific Tribrid Mass Spectrometer

Waters M-Class ultra-high-performance liquid chromatography (UHPLC) system with Trap Valve Manager and Autosampler

3. Limitations and Warnings

For complete warnings, precautions, and limitations, refer to the product labeling.

III. DEVICE/SYSTEM CHARACTERIZATION

1. Device Description

The PrecivityAD2 test is a laboratory-developed, multianalyte assay with algorithmic analysis (MAAA) performed at a single-site laboratory. The assay measures plasma concentrations of the following analytes:

1. β-Amyloid 1-42 (Aβ42)

2. β-Amyloid 1-40 (Aβ40)
3. Phosphorylated tau protein at amino acid threonine 217 (p-tau217)
4. Non-phosphorylated tau protein at amino acid threonine 217 (np-tau217)

From the measured concentrations, two ratios are calculated:

- Aβ42/40 ratio = [Aβ42]/[Aβ40]
- %p-tau217 = ([p-tau217]/[np-tau217]) ×100

These ratios are combined in a proprietary algorithm to generate the Amyloid Probability Score 2 (APS2), which is evaluated against validated clinical cutoffs. Results are reported as Positive, Likely Positive, or Negative.

The test is performed at the C2N Diagnostics Laboratory in Missouri, USA.

2. Principle of Operation

The PrecivityAD2 test consists of two LC-MS/MS-based assays:

1. C2N-Aβ42/40 Assay
2. C2N-%p-tau217 Assay

Both 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.

2.1. C2N-Aβ42/40 Assay

The C2N-Aβ42/40 Assay quantitatively measures plasma concentrations of Aβ42 and Aβ40.

Methodology:

- Immunoprecipitation enrichment
- Lys-N protease digestion
- LC-MS/MS peptide detection
- Stable isotope internal standards
- Proprietary calibrators

The measured concentrations are used to calculate the Aβ42/40 ratio.

2.2 C2N-%p-tau217 Assay

The C2N-%p-tau217 Assay quantitatively measures plasma concentrations of p-tau217 and np-tau217.

Methodology:

- Immunoprecipitation enrichment
- Trypsin digestion
- LC-MS/MS peptide detection
- Stable isotope internal standards
- Proprietary calibrators

2.3 APS2 Algorithm

The APS2 algorithm combines the Aβ42/40 ratio and %p-tau217 into a single composite score intended to estimate the likelihood of brain amyloid pathology.

The algorithm output is reported as the APS2, a numeric value between 0 and 100.

APS2 values are categorized using validated clinical cutoffs:

Table 53.1: APS2 Clinical Cutoffs and Test Result Interpretation

APS2 Value^	Result	Result Interpretation
APS2 > 66.5	Positive	An APS2 result > 66.5 is Positive and 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	An APS2 result > 27.5 and < 66.5 is Likely Positive and likely consistent with the presence of brain amyloid pathology. The 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.
APS2 < 27.5	Negative	An APS2 result < 27.5 is Negative and not consistent with the presence of brain amyloid pathology. The result decreases the likelihood that a patient's clinical presentation is due to Alzheimer's disease. Other potential causes for the patient's cognitive symptoms should be considered.

APS2 = Amyloid Probability Score 2

^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]), 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.

The APS2 algorithm was developed using discovery cohorts and subsequently validated in pooled independent clinical cohorts (see PrecivityAD2 Cutoffs, Section V.8.).

IV. SUBSTANTIAL EQUIVALENCE INFORMATION:

1. Predicate Device Name

Lumipulse G β-Amyloid Ratio (1-42/1-40)

2. Predicate 510(k) Number

DEN200072

3. Comparison with Predicate

A comparison of the PrecivityAD2 test and the predicate device, Fujirebio Lumipulse G β-Amyloid Ratio (1-42/1-40) test (DEN200072), is provided in Table 53.2. The comparison demonstrates that the devices have the same general intended clinical purpose: assessment of brain amyloid pathology in adults being evaluated for Alzheimer's disease or other causes of cognitive decline. Although the devices differ in specimen matrix, assay technology, and biomarker composition, these differences do not raise new or different questions of safety or effectiveness. The performance of the PrecivityAD2 test is supported by analytical validation and clinical validation against accepted amyloid pathology comparators, including amyloid PET imaging and CSF biomarker testing using an FDA-authorized assay.

Table 53.2: Comparison of Subject Device (PrecivityAD2 Test) with Predicate Device, Fujirebio Lumipulse G β-Amyloid Ratio (1-42/1-40) Test (DEN200072)

Characteristic	Subject Device: PrecivityAD2 Test	Predicate Device: Lumipulse G β-Amyloid Ratio (1-42/1-40) (DEN200072)	Comparison / Substantial Equivalence Rationale
Proprietary Device Name	PrecivityAD2 Test	Lumipulse G β-Amyloid Ratio (1-42/1-40)	Different device names; no impact on safety or effectiveness.
Submitter / Manufacturer	C2N Diagnostics	Fujirebio Diagnostics	Different manufacturers; no impact on substantial equivalence.
Regulatory Pathway	Traditional 510(k) submission	De Novo authorization, DEN200072	DEN200072 established the classification regulation and special controls for this device type.
Common Name	Alzheimer's Disease Pathology Assessment Test	Alzheimer's Disease Pathology Assessment Test	Same common name.
Classification Regulation	21 CFR 866.5840	21 CFR 866.5840	Same regulation.
Device Class	Class II	Class II	Same device class.

Characteristic	Subject Device: PrecivityAD2 Test	Predicate Device: Lumipulse G β-Amyloid Ratio (1-42/1-40) (DEN200072)	Comparison / Substantial Equivalence Rationale
Product Code	SET	QSE	Different product code.
Prescription Status	Rx only	Rx only	Same prescription-use limitation.
Intended Use/Indications 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	The Lumipulse ***G*** β-Amyloid Ratio (1-42/1-40) is an in vitro cerebral spinal fluid (CSF) test that combines the results of Lumipulse ***G*** β-Amyloid 1-42 and Lumipulse ***G*** β-Amyloid 1-40 assays into a ratio of β-amyloid 1-42to 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.	Different. The difference is addressed through specific analytical validation and clinical performance testing.

Characteristic	Subject Device: PrecivityAD2 Test	Predicate Device: Lumipulse G β-Amyloid Ratio (1-42/1-40) (DEN200072)	Comparison / Substantial Equivalence Rationale
	patients with amyloid pathology associated with Alzheimer's disease. The test is not intended as a screening or stand-alone diagnostic test.	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.	
Analytical Technology	High-resolution liquid chromatography–tandem mass spectrometry (LC–MS/MS) following immunoprecipitation.	Chemiluminescent enzyme immunoassay on the Lumipulse G system	Different technologies; LC-MS/MS is analytically validated for precision, linearity, analytical measuring interval, LoQ, carryover, interference, and stability.
Primary Biomarkers	Aβ42, Aβ40, p-tau217, np-tau217	Aβ42 and Aβ40	Both include Aβ42 and Aβ40. The subject device additionally includes tau biomarkers associated with Alzheimer's disease pathology. The added biomarkers do not change the intended use and are validated as part of the APS2 algorithm.
Amyloid Biomarker Ratio	Aβ42/40 ratio	Aβ42/40 ratio	Same core amyloid ratio concept.
Tau Biomarker Component	%p-tau217 = (p-tau217 / np-tau217) × 100	Not applicable	Subject device includes an additional tau-related biomarker ratio to improve classification of amyloid pathology. This difference is supported by analytical and clinical validation.

Characteristic	Subject Device: PrecivityAD2 Test	Predicate Device: Lumipulse G β-Amyloid Ratio (1-42/1-40) (DEN200072)	Comparison / Substantial Equivalence Rationale
Test Setting	Single-site, high-complexity CLIA-certified, CAP-accredited, NYS CLEP approved, ISO13485:2016 certified laboratory	Clinical laboratory using the Lumipulse G system	Different operational settings; both are laboratory-based in vitro diagnostic tests performed by trained personnel.
Test Cut-off	APS2 >66.5 = Positive; APS2 27.5-66.5 = Likely Positive; APS2 <27.5 = Negative	Two ratio cut-offs: ≤0.058 Positive; ≥0.073 Negative; 0.059-0.072 Likely Positive	Both devices use validated clinical cutoffs to categorize likelihood of amyloid pathology. The PrecivityAD2 test uses an APS2 algorithmic value based on multiple biomarker inputs, while the predicate device uses ratio-based cutoffs. The different numerical cutoffs reflect differences in output type, specimen matrix, biomarkers, assay design, and analytical methodology, and do not raise new questions of safety or effectiveness.
Sample Volume	Approximately 1.5 mL K2-EDTA plasma *Aβ42 and Aβ40:* 0.5 mL *p-tau217 and np-tau217*: 1.0 mL	Approximately 100 µL CSF *Aβ42:* 50 µL *Aβ40:* 40 µL	Different sample volume requirements are attributable to different specimen types and analytical methodologies. Both are appropriate for intended use.
Analytes	β-Amyloid 1-42 (Aβ42) and β-Amyloid 1-40 (Aβ40), phosphorylated tau (p-tau217), non-phosphorylated tau217 (np-tau217)	β-Amyloid 1-42 and β-Amyloid 1-40	Both devices measure amyloid biomarkers associated with Alzheimer's disease pathology. The subject device additionally incorporates tau biomarkers to improve classification of amyloid pathology.
Assay Antibodies	Proprietary immunoprecipitation antibodies for capture of Aβ42, Aβ40, ptau217, and np-tau217 followed by high resolution LC-MS/MS analysis	β-Amyloid 1-42*:* anti-β-amyloid 1-42 monoclonal antibody (mouse)-coated particles and biotinylated anti-β-amyloid antibody (mouse) β-Amyloid 1-40*:* anti-β-amyloid 1-40	Both devices use antibody-based analyte capture technologies. The subject device uses LC-MS/MS following immunoprecipitation enrichment, while the predicate device uses CLEIA immunoassays technology.

Characteristic	Subject Device: PrecivityAD2 Test	Predicate Device: Lumipulse G β-Amyloid Ratio (1-42/1-40) (DEN200072)	Comparison / Substantial Equivalence Rationale
		monoclonal antibody (mouse)-coated particles and alkaline-phosphatase (ALP)-labeled anti-β-amyloid antibody (mouse) conjugate	
Specimen type	Human K2-EDTA plasma	Cerebrospinal fluid (CSF)	Different specimen matrix. The difference is addressed through specimen-specific analytical validation and clinical performance testing.
Calibrators	Proprietary calibrators and stable isotope-labeled internal standards for Aβ42, Aβ40, p-tau217, and np-tau217	*β-Amyloid 1-42:* Lumipulse ***G*** β-Amyloid 1-42 Calibrator Set: 30, 129, and 2,335 pg/mL *β-Amyloid 1-40:* Lumipulse ***G*** β-Amyloid 1-40 Calibrator Set: 0, 500, and 30,000 pg/mL	Both devices use validated calibration systems traceable to defined reference materials or internal standards appropriate for their analytical technologies.
Algorithm / Calculation	Proprietary algorithm combines Aβ42/40 and %p-tau217 to generate APS2	Aβ42/40 ratio calculated from measured Aβ42 and Aβ40 concentrations	Both devices use measured analyte concentrations to generate an interpretive result but provide different outputs. Algorithmic result of subject device is validated and does not raise new questions of safety or effectiveness.
Reported Numeric Output	Amyloid Probability Score 2 (APS2), 0–100	β-Amyloid Ratio (1-42/1-40) between 0.001 to 1.000	Different numeric scales; both are used to classify amyloid pathology status.
Traceability/Standardization	Quantitation performed using Stable isotope Spike Absolute Quantitation (SISAQ™) methodology with isotopically labeled peptide internal standards	*β-Amyloid 1-42:* Standardized against three certified reference materials: ERM-DA480/IFCC, ERM-DA481/IFCC and ERM-DA482/IFCC *β-Amyloid 1-40:* Traceable to INNOTEST β-Amyloid 1-40	Both devices use validated calibration systems traceable to defined reference materials or internal standards appropriate for their analytical technologies.
Measuring Range	Aβ42: 5-100 pg/mL; Aβ40: 50-1000 pg/mL; p-tau217: 1.3 – 50.0 pg/mL; np-tau217: 6.6 - 400 pg/mL	*β-Amyloid 1-42*: 38 - 2203.5 pg/mL *β-Amyloid 1-40:*156.3 - 28450.3 pg/mL	Measuring ranges differ due to different specimen types and analytical methodologies but are appropriate for intended use and validated clinical application.

Characteristic	Subject Device: PrecivityAD2 Test	Predicate Device: Lumipulse G β-Amyloid Ratio (1-42/1-40) (DEN200072)	Comparison / Substantial Equivalence Rationale
Detection	Aβ42: 2.3 pg/mL; Aβ40: 13.7 pg/mL; p-tau217: 1.167 pg/mL; np-tau217: 3.576 pg/mL 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	*β-Amyloid 1-42:* Limit of Blank = 2.2 pg/mL Limit of Detection = 11.6 pg/mL Limit of Quantitation = 38.0 pg/mL *β-Amyloid 1-40:* Limit of Blank = 0.97 pg/mL Limit of Detection = 33.0 pg/mL Limit of Quantitation = 158.0 pg/mL	Detection limits differ due to differences in specimen matrix, analytes, assay design, and analytical technology (LC-MS/MS versus CLEIA immunoassay). The subject device demonstrates analytically validated sensitivity adequate for reliable quantitation of plasma biomarkers across the clinically relevant range. These differences do not raise new questions of safety or effectiveness because both devices demonstrated acceptable analytical performance characteristics for their intended use.
Ratio Calculation	Proprietary APS2 algorithm combines Aβ42/40 ratio and %p-tau217 into a composite score from 0-100	A web-based calculator tool calculates the ratio of *β-Amyloid 1-42 to β-Amyloid 1-40 and reports the final test results (negative, likely positive or positive). If the ratio is not calculated by the Calculator Tool, the results is manually reported as 'ratio undetermined'*	Both devices use algorithmic interpretation of biomarker concentration to classify amyloid pathology status.
Reagent Stability Calculation	Shelf-life stability of the unopened reagents is 6 months. Critical Reagents are prepared as Single Use aliquots.	Unopened: 19 months at 2-10^{0}C On-board: 15 days	Reagent stability was validated for each device according to its analytical technology and storage conditions.
Sample Stability	Long-term stability at −80°C (frozen) – 280 days for APS2 Room temperature (RT) – 3 hours for APS2	3 hours on-board 8 days at 2-8^{0}C 48 days at 23 - 27^{0}C 2 weeks at -30- -10^{0}C	Stability conditions differ due to specimen matrix and analytical methodology. Stability studies support intended clinical use for both devices.

Characteristic	Subject Device: PrecivityAD2 Test	Predicate Device: Lumipulse G β-Amyloid Ratio (1-42/1-40) (DEN200072)	Comparison / Substantial Equivalence Rationale
	Freeze–thaw (F/T) – 3 cycles for APS2 Preanalytical sample handling (Time from venipuncture to centrifugation) – Up to 120 minutes Transportation – Frozen or refrigerated (2–8°C); refrigerated for up to 48 hours	1 month at -80^{0}C Up to three freeze/thaw cycles	
Result Categories	Positive, Likely Positive, Negative	Positive, Likely Positive, Negative	Both devices provide categorical interpretation related to amyloid pathology. The subject device includes a Likely Positive category to provide additional clinically actionable information.
Positive Result Interpretation	APS2 > 66.5 is consistent with presence of brain amyloid pathology	Predicate positive result is consistent with amyloid pathology	Similar positive interpretation.
Negative Result Interpretation	APS2 < 27.5 is not consistent with presence of brain amyloid pathology	Predicate negative result is not consistent with amyloid pathology	Similar negative interpretation.

4. Substantial Equivalence Discussion

The PrecivityAD2 test is substantially equivalent to the predicate device, Fujirebio Lumipulse G β-Amyloid Ratio (1-42/1-40) test (DEN200072), because:

- Both devices are intended to assess brain amyloid pathology in adults undergoing evaluation for cognitive impairment;
- Both devices use validated biomarker measurements associated with Alzheimer's disease pathology;
- Both provide clinically interpretable categorical results associated with amyloid pathology status;
- Analytical and clinical validation studies demonstrated comparable safety and effectiveness characteristics.

Technological differences, including the use of LC-MS/MS methodology and incorporation of phosphorylated and non-phosphorylated tau217 biomarkers into the APS2 algorithm, do not raise new questions of safety or effectiveness and are supported by comprehensive analytical and clinical validation studies.

V. PERFORMANCE CHARACTERISTICS

1. Analytical Performance

Analytical performance of the PrecivityAD2 test was established through comprehensive analytical validation studies evaluating precision, reproducibility, analytical sensitivity, linearity, interference, cross-reactivity, stability, and APS2 clinical interpretation robustness.

2. Precision

All precision studies were conducted based on recommendations found in CLSI guideline EP05-A3[1].

2.1. APS2 Operator-to-Operator and Within-Laboratory Precision

Precision was evaluated using 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. The C2N-Aβ42/40 and C2N-%p-tau217 measurements were obtained from the same samples. These samples were tested by three operators throughout the study. Three of the plasma samples tested were within 20% of the two clinical cutoffs within the New Sample Panel, whereas two of the plasma samples within the Original Panel were within 20% of the clinical cutoffs in the Original Panel. Each operator analyzed five replicate measurements per sample over five non-consecutive days, resulting in a total of 15 non-consecutive study days across all operators (N=75 per sample). A single reagent Lot and one instrument system were used throughout the study. Total (within-laboratory) precision was determined by combining the repeatability, between-day, and between-operator variance components. APS2 using the new sample panel demonstrated a total (within-laboratory) precision of ≤ 11.999 %CV near the two clinical cutoffs. In the original sample panel, APS2 demonstrated a total (within-laboratory) precision of ≤ 28.155 %CV near the 27.5 clinical cutoff.

Large-scale precision studies require a preanalytical workflow that differs from routine clinical specimen handling. Preparation of the validation samples included pooling, aliquoting, mixing, and, when necessary, analyte spiking. These additional activities are not part of routine clinical testing and may introduce greater preanalytical variability. All samples were prepared to reflect routine clinical conditions as closely as possible. Less stringent preparation conditions, where applicable, are noted in the corresponding table legends. The precision results should therefore be interpreted in the context of the sample preparation conditions used during each study. Adherence to the specimen collection and handling instructions described in this labeling is

important to ensure consistent assay performance. The APS2 precision results are summarized in the table below:

Table 53.3: Summary of APS2 Variability in the Precision Study

APS2										
Sample	Total Replicates	Mean APS2 Value	Within-Run		Between-Day		Between-Operator		Total (within-laboratory)	
			SD	%CV	SD	%CV	SD	%CV	SD	%CV
New Sample Panel										
1	75	4.307	0.712	16.528	1.281	29.748	0.625	14.523	1.593	37
2	75	24.533	2.84	11.577	0.774	3.154	0	0	2.944	11.999
3	75	31.613	3.247	10.27	1.294	4.092	1.189	3.761	3.692	11.677
4	75	68.493	5.058	7.385	3.06	4.467	0	0	5.912	8.631
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.										
Original Panel										
1	73	5	1.035	22.225	1.206	25.884	0.46	9.881	1.654	35.519
2	75	29	7.194	24.616	3.994	13.666	0	0	8.229	28.155
3	74	41	5.747	14.189	5.627	13.894	0	0	8.043	19.859
4	75	77	6.795	8.849	3.927	5.114	3.396	4.423	8.551	11.136
5	74	90	5.588	6.208	2.254	2.504	3.789	4.21	7.118	7.908
6	74	90	4.282	4.747	1.136	1.26	0.761	0.844	4.495	4.983
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 quality control (QC) acceptance criteria. Preanalytical sample handling was less stringent for the Original Panel than for the New Sample Panel, 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.										

2.2. Operator-to-Operator and Within-Laboratory Precision in the C2N-Aβ42/40 Assay

Precision for Aβ40 and Aβ42 was evaluated across three different sample panels. Samples within each sample panel were tested by three operators. In the New Sample Panel, each operator analyzed five samples with five replicate measurements per sample over five non-consecutive days, resulting in a total of 15 non-consecutive study days across all operators (N=75). In the Original Panel #1, each operator analyzed six samples with four replicate measurements per sample over seven non-consecutive days, resulting in a total of 21 non-consecutive study days across all operators (N=84). In the Original Panel #2, each operator analyzed six samples with five replicate measurements per sample over five non-consecutive days, resulting in a total of 15

non-consecutive study days across all operators (N=75). A single reagent Lot and one instrument system were used throughout the study for each sample panel.

Total (within-laboratory) precision was determined by combining the repeatability, between-day, and between-operator variance components. In the New Sample Panel, Aβ40 and Aβ42 demonstrated a total (within-laboratory) precision of ≤ 4.427 %CV and ≤ 9.8 %CV, respectively. In the Original Panel #1, Aβ40 and Aβ42 demonstrated a total (within-laboratory) precision of ≤ 3.512 %CV and ≤ 10.184 %CV, respectively. In the Original Panel #2, Aβ40 and Aβ42 demonstrated a total (within-laboratory) precision of ≤ 3.67 %CV and ≤ 9.445 %CV, respectively. The Aβ40 and Aβ42 precision results are summarized in the tables below:

Table 53.4: Summary of Aβ40 Variability in the Precision Study

Aβ40										
Sample	**Total Replicates**	**Mean Abeta40 Conc. (pg/mL)**	**Within-Run**		**Between-Day**		**Between-Operator**		**Within-Laboratory**	
			SD	%CV	SD	%CV	SD	%CV	SD	%CV
New Sample Panel										
1	75	126.048	4.368	3.465	2.469	1.959	2.441	1.937	5.58	4.427
2	75	228.119	3.947	1.73	5.128	2.248	2.634	1.155	6.987	3.063
3	75	360.232	9.825	2.727	6.606	1.834	5.433	1.508	13.026	3.616
4	75	454.94	9.292	2.042	10.683	2.348	4.773	1.049	14.941	3.284
5	75	672.774	11.239	1.671	15.336	2.28	6.022	0.895	19.944	2.964
Samples 1-5 were pooled K2-EDTA plasma spiked with Aβ40, Aβ42, and Tau-441 recombinant protein.										
Original Panel #1										
1	84	76.273	1.598	2.095	1.032	1.353	0	0	1.902	2.494
2	84	131.230	2.745	2.092	1.648	1.256	1.786	1.361	3.666	2.794
3	84	264.338	5.552	2.100	3.475	1.314	3.114	1.178	7.253	2.744
4	84	531.326	9.561	1.799	6.995	1.317	11.856	2.231	16.760	3.154
5	84	523.743	9.256	1.767	8.379	1.600	13.510	2.579	18.396	3.512
6	84	890.787	20.659	2.319	10.224	1.148	2.979	0.334	23.242	2.609
Samples 1–5 were single native K2-EDTA plasma and Sample 6 was a single native K2-EDTA plasma spiked with Aβ40.										
Original Panel #2										
1	75	297.33	9.4	3.16	4.429	1.49	0	0.	10.391	3.50
2	75	298.476	6.65	2.23	4.276	1.43	5.237	1.75	9.483	3.18
3	75	381.423	8.897	2.33	7.933	2.08	0	0	11.92	3.13
4	75	479.49	8.569	1.79	10.915	2.28	0	0	13.877	2.89

5	75	520.354	17.384	3.34	7.889	1.52	0.911	0.18	19.112	3.67
6	75	601.024	15.186	2.53	10.089	1.68	0	0	18.232	3.03
Samples 1-6 were pooled K2-EDTA plasma spiked with Aβ40, Aβ42, and Tau-441 recombinant protein. Preanalytical sample handling was less stringent for the Original Panels than for the New Sample Panel, 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.										

Table 53.5: Summary of Aβ42 Variability in the Precision Study

Aβ42										
Sample	**Total Replicates**	**Mean Abeta42 Conc. (pg/mL)**	**Within-Run**		**Between-Day**		**Between-Operator**		**Within-Laboratory**	
			SD	%CV	SD	%CV	SD	%CV	SD	%CV
New Sample Panel										
1	75	13.326	0.674	5.06	0.345	2.588	0.227	1.707	0.791	5.934
2	75	30.835	1.005	3.26	1.467	4.757	0	0	1.778	5.767
3	75	24.481	1.441	5.887	1.293	5.283	1.416	5.785	2.399	9.8
4	75	40.236	0.982	2.442	1.951	4.849	0	0	2.184	5.429
5	75	71.755	1.806	2.517	5.052	7.041	1.602	2.232	5.599	7.803
Samples 1-5 were pooled K2-EDTA plasma spiked with Aβ40, Aβ42, and Tau-441 recombinant protein.										
Original Panel #1										
1	84	6.556	0.522	7.960	0.364	5.558	0.202	3.075	0.668	10.184
2	84	13.571	0.682	5.023	0.528	3.893	0.131	0.969	0.872	6.428
3	84	18.362	1.117	6.082	0.747	4.071	0.401	2.184	1.402	7.638
4	84	50.238	2.692	5.358	1.510	3.006	0	0	3.086	6.144
5	84	52.463	2.665	5.080	1.597	3.045	0	0	3.107	5.923
6	84	79.885	4.137	5.178	2.663	3.334	0	0	4.920	6.159
Samples 1–5 were single native K2-EDTA plasma and Sample 6 was a single native K2-EDTA plasma spiked with Aβ42.										
Original Panel #2										
1	75	31.002	1.39	4.484	1.616	5.211	0.369	1.191	2.163	6.977
2	73	31.357	2.315	7.383	1.483	4.73	1.101	3.511	2.962	9.445
3	74	39.46	2.176	5.513	1.719	4.357	0	0	2.773	7.027
4	74	43.669	3.092	7.08	1.361	3.117	1.907	4.367	3.879	8.883
5	74	44.94	1.057	2.352	1.755	3.904	1.09	2.426	2.32	5.163
6	75	68.669	4.434	6.456	1.276	1.858	2.333	3.397	5.17	7.528

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 quality control (QC) acceptance criteria.
Preanalytical sample handling was less stringent for the Original Panels than for the New Sample Panel, 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.

2.3. Operator-to-Operator and Within-Laboratory Precision in the C2N-%p-tau217 Assay

Precision for p-tau217and np-tau217 was evaluated across three different sample panels. Samples within each sample panel were tested by three operators. In the New Sample Panel, 12 samples were analyzed for both p-tau217 and np-tau217. One sample had an np-tau217 concentration above the AMI; therefore, results were reported for 12 samples for p-tau217 and 11 samples for np-tau217. Each sample was tested in five replicate measurements per day by each operator over five non-consecutive days, resulting in 15 non-consecutive study days across all operators (N=72 - 75). In the Original Panel #1 and Original Panel #2, each operator analyzed six samples with four replicate measurements per sample over five non-consecutive days, resulting in a total of 15 non-consecutive study days across all operators (N=60). A single reagent Lot and one instrument system were used throughout the study for each sample panel.

Total (within-laboratory) precision was determined by combining the repeatability, between-day, and between-operator variance components. In the New Sample Panel, p-tau217 and np-tau217 demonstrated a total (within-laboratory) precision of ≤ 12.287 %CV and ≤ 9.494 %CV, respectively. In the Original Panel #1, p-tau217 and np-tau217 demonstrated a total (within-laboratory) precision of ≤ 13.323 %CV and ≤ 14.739 %CV, respectively. In the Original Panel #2, p-tau217 and np-tau217 demonstrated a total (within-laboratory) precision of ≤ 12.594 %CV and ≤ 10.862 %CV, respectively. The p-tau217 and np-tau217 precision results are summarized in the tables below:

Table 53.6: Summary of p-tau217 Variability in the Precision Study

p-tau217										
Sample	**Total Replicates**	**Mean p-tau217 Conc. (pg/mL)**	**Within-Run**		**Between-Day**		**Between-Operator**		**Within-Laboratory**	
			SD	%CV	SD	%CV	SD	%CV	SD	%CV
New Sample Panel										
1	75	1.558	0.166	10.662	0.095	6.108	0	0	0.191	12.287
2	75	3.804	0.366	9.612	0.182	4.794	0	0	0.409	10.741
3	75	4.023	0.45	11.174	0.151	3.751	0.05	1.247	0.477	11.852
4	72	4.807	0.524	10.908	0	0	0	0	0.524	10.908
5	74	5.983	0.567	9.486	0.285	4.768	0	0	0.635	10.616

6	75	7.32	0.698	9.536	0.367	5.011	0	0	0.789	10.772
7	75	8.889	0.752	8.455	0.659	7.416	0.254	2.862	1.032	11.605
8	75	9.085	0.711	7.822	0.425	4.681	0	0	0.828	9.116
9	75	12.456	0.96	7.708	0.502	4.029	0.15	1.204	1.094	8.781
10	75	18.803	1.843	9.803	0.78	4.15	0	0	2.002	10.645
11	74	20.571	1.557	7.571	1.082	5.262	0	0	1.897	9.22
12	74	34.706	3.412	9.832	2.38	6.857	0.702	2.024	4.219	12.156
Samples 1, 2, and 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 quality control (QC) acceptance criteria.										
Original Panel #1										
1	60	2.827	0.348	12.317	0	0	0	0	0.348	12.317
2	60	7.997	0.523	6.544	0.412	5.147	0	0	0.666	8.326
3	60	13.388	1.380	10.311	0.875	6.535	0	0	1.634	12.207
4	60	23.062	2.210	9.581	2.020	8.758	0.691	2.997	3.072	13.323
5	60	31.580	2.292	7.256	0.999	3.163	0	0	2.500	7.916
6	60	39.137	3.422	8.743	2.429	6.206	0	0	4.196	10.721
Sample 1 was a single native K2-EDTA plasma and 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.										
Original Panel #2										
1	75	0.512*	0.113	21.994	0.083	16.252	0.027	5.179	0.143	27.834
2	75	5.269	0.477	9.060	0.238	4.514	0.13	2.458	0.549	10.416
3	75	12.331	1.148	9.309	0.965	7.825	0.05	0.405	1.5	12.168
4	75	12.904	0.777	6.022	0.803	6.224	0.715	5.544	1.327	10.283
5	75	16.121	1.021	6.332	0.692	4.294	1.084	6.724	1.642	10.185
6	75	28.243	2.712	9.601	1.844	6.531	1.377	4.876	3.557	12.594
Samples 1-6 were pooled K2-EDTA plasma spiked with Aβ40, Aβ42, and Tau-441 recombinant protein. *Sample 1 is below LLMI for p-tau217 Preanalytical sample handling was less stringent for the Original Panels than for the New Sample Panel, 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.										

Table 53.7: Summary of np-tau217 Variability in the Precision Study

np-tau217										
Sample	Total Replicates	Mean np-tau217 Conc. (pg/mL)	Within-Run	Between-Day	Between-Operator	Within-Laboratory				
			SD	%CV	SD	%CV	SD	%CV	SD	%CV
New Sample Panel										
1	75	59.633	2.892	4.85	2.194	3.679	0	0	3.63	6.088
2	75	77.724	6.044	7.776	3.986	5.128	1.427	1.836	7.379	9.494
3	75	79.286	3.616	4.561	3.959	4.994	0	0	5.362	6.763
4	75	84.952	6.912	8.136	2.132	2.509	1.52	1.789	7.391	8.7
5	75	111.319	5.735	5.152	4.34	3.899	0	0	7.192	6.461
6	74	126.886	6.119	4.823	5.62	4.429	0	0	8.308	6.548
7	74	180.287	7.049	3.91	5.718	3.172	0	0	9.076	5.034
8	75	200.000	12.705	6.352	0	0	5.365	2.682	13.791	6.896
9	75	207.196	18.106	8.739	0	0	4.136	1.996	18.573	8.964
10	75	327.819	19.396	5.917	10.591	3.231	0	0	22.099	6.741
11	72	347.373	17.541	5.049	7.063	2.033	2.108	0.607	19.026	5.477
Samples 2, 5, and 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 6-7 have 74 replicates because specimen failed to meet predefined quality control (QC) acceptance criteria.										
Original Panel #1										
1	60	14.108	1.674	11.866	0.916	6.495	0.452	3.203	1.961	13.901
2	60	49.119	4.612	9.389	4.870	9.914	2.727	5.551	7.240	14.739
3	60	69.364	6.748	9.728	3.436	4.954	4.574	6.594	8.846	12.754
4	60	106.873	11.771	11.014	8.111	7.590	4.945	4.627	15.126	14.154
5	60	191.747	18.343	9.566	13.669	7.129	16.058	8.374	27.949	14.576
6	60	334.273	30.444	9.107	28.240	8.448	7.594	2.272	42.213	12.628
Samples 1-3 were pooled native K2-EDTA plasma, Sample 4 was 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.										
Original Panel #2										
1	75	93.876	5.659	6.028	4.459	4.750	6.464	6.886	9.68	10.311
2	75	112.091	8.203	7.318	4.823	4.303	6.506	5.804	11.527	10.283
3	75	149.857	10.281	6.861	7.596	5.069	9.517	6.351	15.937	10.635
4	75	180.6	10.132	5.610	9.747	5.397	13.436	7.440	19.447	10.768

5	75	229.562	9.928	4.325	15.027	6.546	17.244	7.511	24.935	10.862
6	75	276.242	18.222	6.596	12.832	4.645	19.193	6.948	29.412	10.647

Samples 1-6 were pooled K2-EDTA plasma spiked with Aβ40, Aβ42, and Tau-441 recombinant protein.
Preanalytical sample handling was less stringent for the Original Panels than for the New Sample Panel, 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.

2.4 Lot-to-Lot Precision

2.4.1 Lot-to-Lot Precision in the C2N-Aβ42/40 Assay

Precision was evaluated using six human K2-EDTA plasma samples tested by one operator using three separate reagent Lots. The operator analyzed five replicate measurements per sample per reagent Lot over five days, resulting in a total of 15 non-consecutive study days across all Lots (N=75). A single instrument system was used throughout the study. The Aβ40 and Aβ42 precision results are summarized in the tables below:

Table 53.8: Summary Results for Aβ40 in the Lot-to-Lot Precision Study

Aβ40										
Sample	**Total Replicates**	**Mean Abeta40 Conc. (pg/mL)**	**Within-Run**		**Between-Day**		**Between-Operator**		**Within-Laboratory**	
			SD	%CV	SD	%CV	SD	%CV	SD	%CV
1	75	68.454	1.614	2.357	7.315	10.687	1.854	2.708	7.717	11.274
2	75	164.596	2.624	1.594	6.480	3.937	0.000	0.000	6.992	4.248
3	75	203.345	3.838	1.887	5.584	2.746	4.041	1.987	7.889	3.880
4	75	447.156	10.395	2.325	11.798	2.639	12.518	2.799	20.099	4.495
5	71	662.288	15.531	2.345	9.304	1.405	22.847	3.450	29.151	4.402
6	66	881.479	13.093	1.485	12.520	1.420	36.745	4.169	40.968	4.648

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 quality control (QC) acceptance criteria and additional aliquots (six for Sample 5 and one for Sample 6) were tested and included in the analysis.

Table 53.9: Summary Results for Aβ42 in the Lot-to-Lot Precision Study

Aβ42										
Sample	**Total Replicates**	**Mean Abeta42 Conc. (pg/mL)**	**Within-Run**		**Between-Day**		**Between-Operator**		**Within-Laboratory**	
			SD	%CV	SD	%CV	SD	%CV	SD	%CV
1	75	10.588	1.183	11.169	0.716	6.767	0.000	0.000	1.383	13.059
2	75	18.674	1.390	7.444	0.464	2.486	0.928	4.969	1.735	9.289
3	75	21.411	1.708	7.978	0.427	1.994	1.253	5.850	2.161	10.092

4	75	46.616	5.279	11.325	0.000	0.000	2.842	6.096	5.996	12.862
5	71	56.068	3.796	6.771	1.897	3.384	3.648	6.507	5.597	9.982
6	66	76.990	5.074	6.590	1.258	1.633	3.981	5.171	6.571	8.535
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 quality control (QC) acceptance criteria and additional aliquots (six for Sample 5 and one for Sample 6) were tested and included in the analysis.										

2.4.2 Lot-to-Lot Precision in the C2N-%p-tau217 Assay

Precision was evaluated using six human K2-EDTA plasma samples tested by one operator using three separate reagent Lots. The operator analyzed five replicate measurements per sample per reagent Lot over five days, resulting in a total of 15 non-consecutive study days across all Lots (N=75). A single instrument system was used throughout the study. The p-tau217 and np-tau217 precision results are summarized in the tables below:

Table 53.10: Summary Results for p-tau217 in the Lot-to-Lot Precision Study

p-tau217										
Sample	**Total Replicates**	**Mean p-tau217 Conc. (pg/mL)**	**Within-Run**		**Between-Day**		**Between-Operator**		**Within-Laboratory**	
			SD	%CV	SD	%CV	SD	%CV	SD	%CV
1	75	2.333	0.279	11.973	0.102	4.375	0.080	3.439	0.308	13.203
2	75	9.641	1.117	11.585	0.494	5.120	0.706	7.327	1.411	14.633
3	75	14.879	1.075	7.228	1.009	6.779	0.162	1.089	1.483	9.969
4	75	17.034	1.460	8.571	0.759	4.456	0.673	3.949	1.778	10.436
5	75	33.272	2.454	7.376	1.017	3.056	3.110	9.346	4.090	12.292
6	75	40.550	3.259	8.036	1.860	4.588	2.538	6.259	4.530	11.172
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.										

Table 53.11: Summary Results for np-tau217 in the Lot-to-Lot Precision Study

np-tau217										
Sample	**Total Replicates**	**Mean np-tau217 Conc. (pg/mL)**	**Within-Run**		**Between-Day**		**Between-Operator**		**Within-Laboratory**	
			SD	%CV	SD	%CV	SD	%CV	SD	%CV
1	75	24.525	1.995	8.136	0.810	3.304	2.151	8.773	3.044	12.412
2	75	49.708	3.625	7.292	0	0	3.510	7.060	5.045	10.150
3	75	75.565	4.606	6.096	1.972	2.609	4.274	5.656	6.586	8.716
4	75	155.659	8.704	5.592	4.940	3.174	6.556	4.212	11.964	7.686

5	75	207.669	13.939	6.712	9.440	4.546	15.355	7.394	22.786	10.972
6	71	349.584	18.702	5.350	0	0	25.076	7.173	31.282	8.948
Samples 1 and 2 were single native K2-EDTA plasma, Sample 3 was a pooled native K2-EDTA plasma, Sample 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.										

2.5 Instrument-to-Instrument Precision

2.5.1 Instrument-to-Instrument Precision for the C2N-Aβ42/40 Assay

Instrument-to-instrument precision was evaluated using six human K2-EDTA plasma samples tested on three instrument systems. One operator analyzed five replicate measurements per sample on each instrument over five days, resulting in a total of 15 non-consecutive study days (N=75). Total (within-laboratory) precision was estimated by combining the repeatability, between-day, and between-instrument variance components. The Aβ40 and Aβ42 precision results are summarized in the tables below:

Table 53.12: Summary Results for Aβ40 in the Instrument-to-Instrument Precision Study

Aβ40										
Sample	**Total Replicates**	**Mean Abeta40 Conc. (pg/mL)**	**Within-Run**		**Between-Day**		**Between-Instrument**		**Within-Laboratory**	
			SD	%CV	SD	%CV	SD	%CV	SD	%CV
1	75	78.519	1.419	1.807	0.722	0.92	0.761	0.969	1.765	2.248
2	75	134.34	1.883	1.402	1.283	0.955	2.989	2.225	3.759	2.798
3	75	268.513	5.058	1.884	1.939	0.722	5.269	1.962	7.557	2.814
4	75	438.862	20.043	4.567	0	0	2.613	0.595	20.213	4.606
5	75	628.032	12.883	2.051	6.648	1.059	7.338	1.168	16.249	2.587
6	75	918.169	15.797	1.721	10.526	1.146	9.166	0.998	21.08	2.296
Samples 1–5 were single native K2-EDTA plasma and Sample 6 was a single native K2-EDTA plasma spiked with Aβ40.										

Table 53.13: Summary Results for Aβ42 in the Instrument-to-Instrument Precision Study

Aβ42										
Sample	**Total Replicates**	**Mean Abeta42 Conc. (pg/mL)**	**Within-Run**		**Between-Day**		**Between-Instrument**		**Within-Laboratory**	
			SD	%CV	SD	%CV	SD	%CV	SD	%CV
1	72	6.863	0.486	7.075	0.25	3.642	0.565	8.228	0.786	11.446
2	75	14.023	0.667	4.754	0.415	2.962	0.276	1.967	0.833	5.937
3	75	17.949	1.385	7.714	0	0	0.775	4.319	1.587	8.841

4	75	41.708	2.232	5.352	0.608	1.457	0.474	1.136	2.361	5.662
5	75	61.634	2.582	4.189	2.66	4.316	0.885	1.436	3.811	6.183
6	73	82.298	3.697	4.492	1.885	2.29	0	0	4.15	5.042
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.										

2.5.2 Instrument-to-Instrument Precision in the C2N-%p-tau217 Assay

Instrument-to-instrument precision was evaluated using six human K2-EDTA plasma samples tested on three instrument systems. One operator analyzed five replicate measurements per sample on each instrument over five days, resulting in a total of 15 non-consecutive study days (N=75). A single reagent Lot was used throughout the study. The p-tau217 and np-tau217 precision results are summarized in the tables below:

Table 53.14: Summary Results for p-tau217 in the Instrument-to-Instrument Precision Study

p-tau217										
Sample	**Total Replicates**	**Mean p-tau217 Conc. (pg/mL)**	**Within-Run**		**Between-Day**		**Between-Instrument**		**Within-Laboratory**	
			SD	%CV	SD	%CV	SD	%CV	SD	%CV
1	75	2.818	0.263	9.328	0.022	0.771	0.051	1.794	0.269	9.530
2	75	8.027	0.591	7.361	0.363	4.516	0.029	0.355	0.694	8.643
3	75	13.323	1.032	7.745	1.072	8.046	0	0	1.488	11.168
4	75	23.146	1.625	7.020	1.516	6.551	0	0	2.222	9.602
5	75	28.750	2.103	7.316	1.436	4.995	0	0	2.547	8.859
6	74	34.171	2.526	7.391	1.497	4.381	0.492	1.440	2.977	8.712
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.										

Table 53.15: Summary Results for np-tau217 in the Instrument-to-Instrument precision Study

np-tau217										
Sample	**Total Replicates**	**Mean np-tau217 Conc. (pg/mL)**	**Within-Run**		**Between-Day**		**Between-Instrument**		**Within-Laboratory**	
			SD	%CV	SD	%CV	SD	%CV	SD	%CV
1	75	14.529	1.285	8.843	0.811	5.583	0.636	4.379	1.647	11.338
2	75	50.741	3.591	7.077	2.203	4.341	1.651	3.254	4.525	8.917
3	75	71.287	4.972	6.975	3.402	4.772	2.367	3.320	6.473	9.080

4	75	111.945	7.217	6.447	4.678	4.179	0	0	8.600	7.683
5	75	189.153	9.617	5.084	9.130	4.827	4.983	2.635	14.166	7.489
6	74	319.066	24.758	7.759	8.419	2.639	1.331	0.417	26.184	8.206
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.										

2.6 APS2 Precision Simulation

For 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 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 result for the sample).

For each subject, the simulated replicates of the APS2 were used to estimate: *(i)* median APS2 value*; (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 value. 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:

Table 53.16: APS2 Standard Deviation Range by Value Interval

	APS2 Value 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 retesting zone is implemented to minimize this risk of 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 a patient specimen within the retesting zone is calculated using the average of the two C2N-%p-tau217 Assay measurements.

3. Linearity

Linearity studies were conducted to evaluate the relationship between analyte concentration and measured assay response across the validated analytical measuring ranges for Aβ42, Aβ40, p-tau217, and np-tau217. Linearity was assessed according to CLSI EP06, 2nd Edition[2]. The linearity interval for each analyte was validated as the linearity interval within which the acceptable deviation from linearity is ±10%.

The AMI for Aβ40 and Aβ42 in the C2N-Aβ42/40 Assay are 50–1000 pg/mL and 5–100 pg/mL, respectively.

The AMI for p-tau217 and np-tau217 in the C2N-%p-tau217 Assay are 1.3–50 pg/mL and 6.6–400 pg/mL, respectively.

4. Analytical Specificity/Interference

Analytical specificity studies were conducted based on the recommendations of CLSI EP07, 3rd edition[3]. The study evaluated potential endogenous interferents, potential exogenous interferents, and simulated interference conditions directly on the APS2 result (Table 53.17. and Table 53.18).

A total of five human plasma K2-EDTA samples covering the APS2 range were tested. All samples were paired such that both the Aβ42/40 ratio and %p tau217 (p-tau217/np-tau217 × 100) interference testing were evaluated from the same sample. Each individual sample used for measurement of Aβ40, Aβ42, p-tau217, and np-tau217 was spiked with a single interferent, with the volume of spiked material comprising less than 5% of the total sample volume for all but two interferents tested (triglycerides [7.3%] and albumin [15.3%]). Control samples were spiked with the corresponding solvent material in which the interferent was supplied. Each test sample and control had five technical replicates for which the APS2 was averaged.

Except for hemolysate, no significant interference (acceptance criterion of ±10% relative difference in APS2) was observed for evaluated substances at the indicated test concentrations listed in the Tables 53.17 and 53.18.

Table 53.17: Interference Testing – Endogenous Interference

Endogenous Interferent	Concentration Tested
Triglycerides	1,500 mg/dL
Protein (albumin)	15 g/dL

Bilirubin, Conjugated	40 mg/dL
Bilirubin, Unconjugated	40 mg/dL
IgA	500 mg/dL
IgG	2,000 mg/dL
IgM	300 mg/dL
Rheumatoid Factor	500 IU/mL
HAMA	1,000 ng/mL
Hemolysate*	150 mg/dL
Biotin	7.02 mg/dL

HAMA = human anti-mouse antibody;
**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*

Table 53.18: Interference Testing – Exogenous Interference

Exogenous Interferent	Concentration Tested
Acetaminophen	15.6 mg/dL
Acetylcysteine	15.0 mg/dL
Acetylsalicylic Acid	3.0 mg/dL
Amlodipine	0.0075 mg/dL
Ascorbic Acid	5.25 mg/dL
Atenolol	0.9 mg/dL
Atorvastatin	0.075 mg/dL
Citalopram	0.543 mg/dL
Cyclosporine	0.18 mg/dL
Denosumab	0.0000147 mg/dL
Donepezil	3.0 mg/dL
Doxycycline	1.80 mg/dL
Fluoxetine	0.142 mg/dL
Heparin	330 U/dL
Hydrochlorothiazide	0.113 mg/dL

Ibuprofen	21.9 mg/dL
Levodopa	0.75 mg/dL
Levothyroxine	0.0429 mg/dL
Lisinopril	0.0246 mg/dL
Losartan	0.116 mg/dL
Memantine	0.0117 mg/dL
Metformin	1.2 mg/dL
Metoprolol	0.15 mg/dL
Rifampicin	4.8 mg/dL
Risperidone	0.0114 mg/dL
Rivastigmine	0.0167 mg/dL
Simvastatin	0.168 mg/dL
Theophylline	6.0 mg/dL
Valproic Acid	31.8 mg/dL
Warfarin	7.5 mg/dL

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. For each combination, %APS2 difference was calculated. The simulation analysis demonstrates that the PrecivityAD2 test is robust to the interferences evaluated under the tested conditions. Those that did not meet the predefined acceptance criterion of ±10% APS2 difference were samples with APS2 values of less than 10 and had no clinical impact on APS2 interpretation. Across the evaluated endogenous and exogenous interferents, observed 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).

The results support that APS2 maintains clinical accuracy under the intended use conditions of the PrecivityAD2 test, and no clinically significant interference has been demonstrated.

5. Cross-reactivity

Cross-reactivity studies were performed to evaluate the potential impact of elevated internal standards (ISTDs) and structurally related amyloid beta peptides on biomarker quantitation and APS2. Four samples were spiked with np-tau217 ISTD or p-tau217 ISTD at three-fold the normal control concentration. Samples spiked with three-fold np-tau217 ISTD were evaluated

for p-tau217, while samples spiked with three-fold p-tau217 ISTD were evaluated for np-tau217. The analyte corresponding to the elevated ISTD was not evaluated because the increased ISTD concentration was expected to directly impact its quantitation. Samples spiked with both tau ISTDs were evaluated for Aβ40 and Aβ42, and APS2 was generated using the previously collected tau cross-reactivity data. Two additional samples were spiked with Aβ40 ISTD or Aβ42 ISTD at three-fold the normal control concentration. Samples spiked with Aβ40 ISTD were evaluated for Aβ42, and samples spiked with Aβ42 ISTD were evaluated for Aβ40. As with the tau ISTDs, the analyte corresponding to the elevated ISTD was not evaluated due to the expected impact on quantitation. Samples spiked with Aβ40 and Aβ42 ISTDs were evaluated for np-tau217 and p-tau217, and APS2 was generated and compared to matched controls. Three additional samples were spiked individually with Aβ1-37, Aβ1-38, or Aβ1-43 at two-fold their normal endogenous concentrations and evaluated for their impact on biomarker quantitation and APS2. For all conditions, the total spike volume was less than 5% of the final sample volume, and matched control samples were spiked with an equivalent volume of surrogate matrix to maintain equivalent sample matrices. Five technical replicates were prepared and analyzed for each control and cross-reactant condition. For the cross-reactivity studies, none of the cross-reactants tested showed interference with the APS2 result.

The maximum observed APS2 cross-reactivity was 9.74%, which remained within the predefined acceptance criterion of ±10%. The table below shows the highest observed cross-reactivity on APS2 for all cross-reactants tested.

Table 53.19: Cross-Reactants Tested as Part of the PrecivityAD2 Test and Their Highest Observed Cross-Reactivity

Cross-Reactant	Test Concentration (pg/mL)	Highest Observed Cross-Reactivity (%)
Aβ1-37	235	7.91
Aβ1-38	695	7.95
Aβ1-43	23	9.41
Aβ40 ISTD	600	1.64
Aβ42 ISTD	90	9.46
np-tau217 ISTD	497	9.47
p-tau217 ISTD	30	1.92

Aβ = β-Amyloid; Aβ40 = β-Amyloid 1-40; Aβ42 = β-Amyloid 1-42; ISTD = internal standard; np-tau217 = non-phosphorylated tau protein at amino acid threonine, position 217; p-tau217 = phosphorylated tau protein at amino acid threonine, position 217.

6. Assay Reportable Range

The reportable range within the C2N-Aβ42/40 Assay and C2N-%p-tau217 Assay will be identical to AMI as no dilutional linearity experiments were performed to extend analyte concentrations beyond the AMI. The reportable range for the four analytes is shown below in Table 53.20.

Table 53.20: Reportable Range

Analyte	Reportable 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

Aβ40 = β-Amyloid 1-40; Aβ42 = β-Amyloid 1-42; np-tau217 = non-phosphorylated tau protein at amino acid threonine, position 217; p-tau217 = phosphorylated tau protein at amino acid threonine, position 217.

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

7.1 Traceability

The PrecivityAD2 test uses internally controlled LC-MS/MS methodologies incorporating stable isotope-labeled internal standards, calibrators, and QC materials to ensure analytical traceability and quantitative consistency. Critical assay components, calibrators, and QC materials are monitored with every analytical run using predefined acceptance criteria. This control strategy supports consistent assay performance and enables identification of meaningful analytical drift prior to affecting patient results. The calibrators for Aβ40, Aβ42, p-tau217, and np-tau217 are traceable to in-house reference standards. This traceability framework ensures accurate and reproducible calibration across different Lots of calibrators within the C2N-Aβ42/40 Assay and C2N-%p-tau217 Assay. The quality control samples in the C2N-Aβ42/40 Assay and C2N-%p-tau217 Assay share the same level of traceability as the calibrators, however, they are generated from different Lots of source material. This ensures the accurate quantitation of these samples in parallel with patient samples.

7.2 Specimen and Reagent Stability

Stability studies were conducted in accordance with CLSI EP25, 2nd edition[4] to evaluate both specimen and reagent performance.

7.2.1 Reagent Stability

Three independent Lots of critical reagents were prepared at C2N for the C2N-Aβ42/40 and %p-tau217 Assays, using different raw-material Lots. Reagent stability was evaluated using samples spanning low, medium, and high concentrations of Aβ40, Aβ42, p-tau217, and np-tau217. Samples were tested on Day 0 and at subsequent time points using multiple operators and instrument systems. Reagent stability was assessed using APS2 values calculated from combinations of Aβ40, Aβ42, p-tau217, and np-tau217 sample results.

All three reagent Lots demonstrated stability for up to six months under the recommended storage conditions specified for each reagent. Long-term stability studies are ongoing.

7.2.2 Specimen Stability and Transportation

Specimen stability was evaluated using native plasma samples tested at baseline and subsequent time points under the recommended storage conditions. Baseline samples were tested in six replicates, and samples at subsequent time points were tested in three replicates using the same operator, reagent Lot, and instrument.

The study assessed the effects of pre-analytical processing, transport conditions, and real-world sample handling on APS2 results. Test conditions included delayed centrifugation, different centrifugation parameters, frozen and refrigerated shipping, and simulated summer and winter transport profiles. Conditions, measurements, and storage duration details are presented in table 53.21 below.

Table 53.21: Sample Stability Under Validated Storage and Handling Conditions

Storage Condition	Timepoints of Measurements/F/T Cycles	Claimed Storage Duration or F/T Cycle
Frozen stability at -80ºC	0, 63, 90, 182, 280, 385 days	280 days for APS2
Room Temperature (RT)	0.5, 1, 2, 3, 4 hours	3 hours for APS2
Freeze-thaw (F/T)	1, 2, 3, 4 cycles	3 cycles for APS2
Preanalytical Sample Handling	Time from venipuncture to centrifugation: <20 min, 60 min, 120 min, 140 min	Up to 120 minutes
Transportation	Frozen and refrigerated (2-8 ºC)	Frozen or refrigerated (2-8 ºC); Refrigerated up to 48 hours. Samples received more than 48 hours after collection will be rejected for PrecivityAD2 testing

7.2.3 Detection Limit

The limit of quantitation (LoQ) for all analytes within the C2N-Aβ42/40 Assay and C2N-%-p-tau217 Assay were established in accordance with CLSI EP17-A2[5].

7.2.3.1 Aβ42 and Aβ40 Limits of Quantification

Four human K2-EDTA plasma samples near the expected lower limit of quantification were evaluated for both Aβ42 and Aβ40 using the same sample set for each analyte. Each sample was tested in triplicate over three days using three reagent Lots and three instrument systems. A different operator performed testing with each reagent Lot. All nine reagent Lot/instrument combinations were evaluated. Each reagent Lot generated a total of 108 measurements across four samples. The LoQ for Aβ42 was determined to be 2.3 pg/mL and 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 to be 13.7 pg/mL and the claimed LoQ for Aβ40 was 50 pg/mL at the lower limit of the analytical measuring interval.

7.2.3.2 p-tau217 and np-tau217 Limits of Quantification

Four human K2-EDTA plasma samples near the expected lower limit of quantification were evaluated for both p-tau217 and np-tau217 using the same sample set for each analyte. Each sample was tested in triplicate over three days using three reagent Lots and three instrument systems. A different operator performed testing with each reagent Lot. All nine reagent Lot/instrument combinations were evaluated. Each reagent generated a total of 108 measurements across four samples. The LoQ for p-tau217 was determined to be 1.167 pg/mL and 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 to be 3.576 pg/mL and the claimed LoQ for np-tau217 was 6.6 pg/mL at the lower limit of the analytical measuring interval.

7.3 Carryover

All analytes were evaluated for LC-MS carryover using a low concentration sample near the lower limit of the measuring interval (LLMI) and a high concentration sample at approximately 5× the upper limit of the measuring interval (ULMI). The study consisted of five runs over five days. Each run included 10 initial replicates of the low concentration sample (protected), followed by 10 alternating high and low concentration injections, yielding 10 additional low concentration replicates (unprotected) and 30 total injections per run. No significant carryover was observed for any of the four analytes.

8. PrecivityAD2 Cutoffs

The APS2 algorithm was developed using a Discovery Cohort of 583 subjects with cognitive impairment. The model was trained on amyloid PET as the clinical comparator, with the Aβ42/40 ratio and %p-tau217 serving as the input variables. To arrive at the final cutoffs of the APS2 algorithm, performance (Positive Predictive Value [PPV], Negative Predictive Value [NPV; 100%-Predictive Value]) was evaluated in the Discovery Cohort against visual read or quantitative interpretation of the amyloid PET scan. The two cutoffs of 27.5 and 66.5 were determined based on achieving comparable performance to the Fujirebio Lumipulse G β-Amyloid Ratio (1-42/1-40) device (K200072) predicate comparator against amyloid visual read PET.

Table 53.22: APS2 Cutoffs and PrecivityAD2 test Result Interpretation

APS2 Value	Result	Result Interpretation
APS2 > 66.5	Positive	An APS2 value > 66.5 is Positive and 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	An APS2 value > 27.5 and < 66.5 is Likely Positive and likely consistent with the presence of brain amyloid pathology. The 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.
APS2 < 27.5	Negative	An APS2 value < 27.5 is Negative and not consistent with the presence of brain amyloid pathology. The result decreases the likelihood that a patient's clinical presentation is due to Alzheimer's disease. Other potential causes for the patient's cognitive symptoms should be considered.

9. Comparison Studies

9.1. Method Comparison with Predicate Device

Not applicable

9.2. Matrix Comparison

Not applicable. Human K2-EDTA plasma is the only claimed sample matrix.

10. Clinical Performance

10.1. Clinical Study Description

10.1.1 Source of Samples

A clinical validation study was conducted to evaluate the performance of the PrecivityAD2 test in the detection of brain amyloid pathology in subjects aged 40 years and older presenting with signs or symptoms of cognitive impairment, as compared to amyloid PET visual read using an FDA-approved tracer or the FDA-authorized Fujirebio Lumipulse G β-Amyloid Ratio (1-42/1-40) CSF test. The study included 1,142 subjects from three cohorts (the Pooled Validation Cohort). Among the study subjects, 21.2% had a clinical presentation of subjective cognitive

decline (SCD; n=242), 47.3% had mild cognitive impairment (MCI; n=540), and 31.5% had dementia (n=360).

10.2. Demographic and Clinical Characteristics

The demographic and clinical characteristics of the study subjects according to diagnostic groups and amyloid status determined by amyloid PET scan or CSF ratio test results are presented in Table 53.23 below.

Table 53.23: Demographic and Clinical Characteristics.

		Clinical Presentation			Brain Amyloid Pathology		Total = 1142 n (%)
		SCD = 242 n (%)	MCI = 540 n (%)	Dementia = 360 n (%)	Present = 602 n (%)	Absent = 540 n (%)	
Sex	Male	121 (50%)	295 (54.6%)	183 (50.8%)	278 (46.2%)	321 (59.4%)	599 (52.5%)
	Female	121 (50%)	245 (45.4%)	177 (49.2%)	324 (53.8%)	219 (40.6%)	543 (47.5%)
Age (years)	40-49	3 (1.2%)	10 (1.9%)	1 (0.3%)	2 (0.3%)	12 (2.2%)	14 (1.2%)
	50–59	34 (14%)	41 (7.6%)	7 (1.9%)	18 (3%)	64 (11.9%)	82 (7.2%)
	60–69	77 (31.8%)	109 (20.2%)	38 (10.6%)	81 (13.5%)	143 (26.5%)	224 (19.6%)
	70–79	92 (38%)	253 (46.9%)	178 (49.4%)	303 (50.3%)	220 (40.7%)	523 (45.8%)
	≥80	36 (14.9%)	127 (23.5%)	136 (37.8%)	198 (32.9%)	101 (18.7%)	299 (26.2%)
	Mean	69.4	72.8	76.7	75.9	70.4	73.3
	Median	70	75	78	77	72	75
	Min.	43	40	49	48	40	40
	Max.	89	90	91	91	90	91
Race	White	0 (0%)	32 (5.9%)	9 (2.5%)	34 (5.6%)	7 (1.3%)	41 (3.6%)
	Black or African American	0 (0%)	2 (0.4%)	0 (0%)	2 (0.3%)	0 (0%)	2 (0.2%)
	Asian	0 (0%)	1 (0.2%)	0 (0%)	1 (0.2%)	0 (0%)	1 (0.1%)

	or America Indian or Alaska Native or More than one						
	Unknown	0 (0%)	0 (0%)	0 (0%)	0 (0%)	0 (0%)	0 (0%)
	Not reported	0 (0%)	0 (0%)	0 (0%)	0 (0%)	0 (0%)	0 (0%)
	Missing	242 (100%)	505 (93.5%)	351 (97.5%)	565 (93.9%)	533 (98.7%)	1098 (96.1%)
MMSE	**<18**	3 (1.2%)	21 (3.9%)	64 (17.8%)	65 (10.8%)	23 (4.3%)	88 (7.7%)
	18–23	9 (3.7%)	77 (14.3%)	155 (43.1%)	165 (27.4%)	76 (14.1%)	241 (21.1%)
	24–30	210 (86.8%)	411 (76.1%)	116 (32.2%)	338 (56.1%)	399 (73.9%)	737 (64.5%)
	Missing	20 (8.3%)	31 (5.7%)	25 (6.9%)	34 (5.6%)	42 (7.8%)	76 (6.7%)
Apolipo protein E (ApoE) Status	**ε2/ε2**	1 (0.4%)	5 (0.9%)	2 (0.6%)	3 (0.5%)	5 (0.9%)	8 (0.7%)
	ε2/ε3	22 (9.1%)	42 (7.8%)	16 (4.4%)	16 (2.7%)	64 (11.9%)	80 (7%)
	ε2/ε4	6 (2.5%)	14 (2.6%)	11 (3.1%)	22 (3.7%)	9 (1.7%)	31 (2.7%)
	ε3/ε3	124 (51.2%)	241 (44.6%)	149 (41.4%)	182 (30.2%)	332 (61.5%)	514 (45%)
	ε3/ε4	77 (31.8%)	194 (35.9%)	143 (39.7%)	296 (49.2%)	118 (21.9%)	414 (36.3%)
	ε4/ε4	12 (5%)	40 (7.4%)	38 (10.6%)	80 (13.3%)	10 (1.9%)	90 (7.9%)
	Missing	0 (0%)	4 (0.7%)	1 (0.3%)	3 (0.5%)	2 (0.4%)	5 (0.4%)
Years of Education	**<13**	98 (40.5%)	295 (54.6%)	244 (67.8%)	336 (55.8%)	301 (55.7%)	637 (55.8%)
	≥13	140 (57.9%)	233 (43.1%)	109 (30.3%)	252 (41.9%)	230 (42.6%)	482 (42.2%)
	Missing	4 (1.7%)	12 (2.2%)	7 (1.9%)	14 (2.3%)	9 (1.7%)	23 (2%)
Brain Amyloid Pathology	**Present**	58 (24%)	291 (53.9%)	253 (70.3%)	602 (100%)	0 (0%)	602 (52.7%)
	Absent	184 (76%)	249 (46.1%)	107 (29.7%)	0 (0%)	540 (100%)	540 (47.3%)

Note: Brain amyloid pathology status was determined by Amyloid PET imaging (visual read) or Cerebrospinal Fluid Testing with Fujirebio Lumipulse G β-Amyloid Ratio (1-42/1-40 (FDA Authorization DEN200072)
MCI = mild cognitive impairment; MMSE = Mini-Mental State Examination; SCD = subjective cognitive decline

10.3. Primary Analyses

10.3.1. PrecivityAD2 Test Performance Against Amyloid Pathology Reference Standards

The performance of the PrecivityAD2 test described by the predictive value (PV), likelihood ratio, and frequency of test results, is summarized in Table 53.24. The positive predictive value (PPV) for Positive and Likely Positive results corresponds to the PV while the negative predictive value (NPV) for Negative results can be calculated as 100%-PV. At the calculated amyloid prevalence of 52.7%, the PPV for APS2 Positive subjects is 97.6% and the NPV for APS2 Negative subjects is 93.1%. The likelihood ratio for APS2 Positive subjects is 37.05 and 0.07 for APS2 Negative subjects.

Table 53.24: Clinical Performance of the PrecivityAD2Test in the Pooled Validation Cohort at the Calculated Amyloid Prevalence of 52.7%

PrecivityAD2 Test	Brain Amyloid Pathology			Predictive Value [n/N] (95% CI)	Frequency [n/N]	Likelihood Ratio
	Present	Absent	Total			
Positive (APS2>66.5)	413	10	423	97.6% [413/423] (95.7%; 98.7%)	37.0% [423/1142]	37.05 (20; 68.62)
Likely Positive (27.5<APS2<66.5)	153	45	198	77.3% [153/198] (70.9%; 82.6%)	17.3% [198/1142]	3.05 (2.23; 4.16)
Negative (APS2<27.5)	36	485	521	6.9% [36/521] (5.0%; 9.4%)	45.6% [521/1142]	0.07 (0.05; 0.09)
Total	602	540	1,142	Prevalence = 52.7% [602/1142]		

APS2 = Amyloid Probability Score 2

10.3.2. Clinical Comparator (Amyloid PET Visual Read, CSF Testing)

The performance of the PrecivityAD2 test across clinical comparators is shown in tables 53.25 and 53.26. The performance of the PrecivityAD2 test is similar across clinical comparators, providing evidence of the poolability of the data from the two clinical comparators into the Pooled Validation Cohort.

Table 53.25: Clinical Performance of the PrecivityAD2 Test Using Amyloid PET Visual Read as the Clinical Comparator

PrecivityAD2 Test	PET Visual Read			Predictive Value [n/N] (95% CI)	Frequency [n/N]	Likelihood Ratio
	Positive	Negative	Total			
Positive (APS2 > 66.5)	67	7	74	90.5% [67/74] (81.7%; 95.3%)	35.4% [74/209]	11.27 (5.43; 23.37)
Likely Positive (27.5 < APS2 < 66.5)	23	17	40	57.5% [23/40] (42.2%; 71.5%)	19.1% [40/209]	1.59 (0.91; 2.8)
Negative (APS2 < 27.5)	6	89	95	6.3% [6/95] (2.9%; 13.1%)	45.5% [95/209]	0.08 (0.04; 0.17)
Total	96	113	209	Prevalence = 45.9% [96/209]		

APS2 = Amyloid Probability Score 2

Table 53.26: Clinical Performance of the PrecivityAD2 Test Using CSF Lumipulse as the Clinical Comparator

PrecivityAD2 Test	Lumipulse G β-Amyloid Ratio (1-42/1-40)			Predictive Value [n/N] (95% CI)	Frequency [n/N]	Likelihood Ratio
	Positive	Negative	Total			
Positive (APS2 > 66.5)	346	3	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	28	158	82.3% [130/158] (75.6%; 87.4%)	16.9% [158/933]	3.92 (2.66; 5.77)
Negative (APS2 < 27.5)	30	396	426	7.0% [30/426] (5.0%; 9.9%)	45.7% [426/933]	0.06 (0.05; 0.09)
Total	506	427	933	Prevalence = 54.2% [506/933]		

APS2 = Amyloid Probability Score 2

10.4. Analyses Across Subgroups

10.4.1. Clinical Disease Stages

Table 53.27 describes the performance of the PrecivityAD2 test in the clinical presentation subgroups of SCD, MCI, and dementia.

The observed amyloid positivity prevalence differs between the disease stage categories of SCD (24.0%), MCI (53.9%) and dementia (70.3%), consistent with previously published clinical and population-based data. Despite the lower underlying amyloid prevalence, the PrecivityAD2 test

performance in the SCD category is similar to that of the Pooled Validation Cohort and the other diagnostic subgroups with more advanced disease.

Table 53.27: PrecivityAD2 Test Performance Across Clinical Disease Stages

PrecivityAD2 Test	Disease Stage			
	SCD (n = 242)	**MCI (n = 540)**	**Dementia (n = 360)**	**All (N = 1,142)**
Positive Results PV [n/N] (95% CI)	90.3% [28/31] (75.1%; 96.7%)	97.9% [187/191] (94.7%; 99.2%)	98.5% [198/201] (95.7%; 99.5%)	97.6% [413/423] (95.7%; 98.7%)
Likely Positive Results PV [n/N] (95% CI)	67.6% [23/34] (50.8%; 80.9%)	79.8% [83/104] (71.1%; 86.4%)	78.3% [47/60] (66.4%; 86.9%)	77.3% [153/198] (70.9%; 82.6%)
Negative Results PV [n/N] (95% CI)	4.0% [7/177] (1.9%; 7.9%)	8.6% [21/245] (5.7%; 12.7%)	8.1% [8/99] (4.2%; 15.1%)	6.9% [36/521] (5.0%; 9.4%)
Prevalence [n/N]	24.0% [58/242]	53.9% [291/540]	70.3% [253/360]	52.7% [602/1142]
Frequency of Likely Positive Results [n/N]	14.0% [34/242]	19.3% [104/540]	16.7% [60/360]	17.3% [198/1142]

Note: Positive = APS2 > 66.5; Likely Positive = 27.5 < APS2 < 66.5; Negative = APS2 < 27.5
MCI = mild cognitive impairment; MMSE = Mini-Mental State Examination; PV = predictive value; SCD = subjective cognitive decline

10.4.2 Age

Table 53.28 describes the performance of the PrecivityAD2 test across age groups. The predictive values in the Pooled Validation Cohort are comparable across all age groups, with the PPV for APS2 Positive subjects varying between 97.0% and 100%. For APS2 Negative subjects, NPV ranged between 89.4% and 100% and was similar across the age spectrum, decreasing with age as the observed prevalence of brain amyloid pathology expectedly increased.

Table 53.28: Clinical Performance of the PrecivityAD2 Test in the Pooled Validation Cohort: Subgroup Analysis – Age

Age	PrecivityAD2 Test	Brain Amyloid Pathology			Predictive Value [n/N] (95% CI)	Frequency [n/N]
		Present	Absent	Total		
40-49	Positive (APS2 > 66.5)	2	0	2	100.0% [2/2] (34.2%; 100.0%)	14.3% [2/14]
	Likely Positive (27.5 < APS2 < 66.5)	0	1	1	0.0% [0/1] (0.0%; 79.3%)	7.1% [1/14]
	Negative (APS2 < 27.5)	0	11	11	0.0% [0/11] (0.0%; 25.9%)	78.6% [11/14]
	Total	2	12	14	Prevalence = 14.3% [2/14]	
50-59	Positive (APS2 > 66.5)	14	0	14	100.0% [14/14] (78.5%; 100.0%)	17.1% [14/82]
	Likely Positive (27.5 < APS2 < 66.5)	3	3	6	50.0% [3/6] (18.8%; 81.2%)	7.3% [6/82]
	Negative (APS2 < 27.5)	1	61	62	1.6% [1/62] (0.3%; 8.6%)	75.6% [62/82]
	Total	18	64	82	Prevalence = 22.0% [18/82]	
60-69	Positive (APS2 > 66.5)	56	1	57	98.2% [56/57] (90.7%; 99.7%)	25.4% [57/224]
	Likely Positive (27.5 < APS2 < 66.5)	21	5	26	80.8% [21/26] (62.1%; 91.5%)	11.6% [26/224]
	Negative (APS2 < 27.5)	4	137	141	2.8% [4/141] (1.1%; 7.1%)	62.9% [141/224]
	Total	81	143	224	Prevalence = 36.2% [81/224]	
70-79	Positive (APS2 > 66.5)	197	6	203	97.0% [197/203] (93.7%; 98.6%)	38.8% [203/523]
	Likely Positive (27.5 < APS2 < 66.5)	85	22	107	79.4% [85/107] (70.8%; 86.0%)	20.5% [107/523]
	Negative (APS2 < 27.5)	21	192	213	9.9% [21/213] (6.5%; 14.6%)	40.7% [213/523]
	Total	303	220	523	Prevalence = 57.9% [303/523]	
≥80	Positive (APS2 > 66.5)	144	3	147	98.0% [144/147] (94.2%; 99.3%)	49.2% [147/299]

	Likely Positive (27.5 < APS2 < 66.5)	44	14	58	75.9% [44/58] (63.5%; 85.0%)	19.4% [58/299]
	Negative (APS2 < 27.5)	10	84	94	10.6% [10/94] (5.9%; 18.5%)	31.4% [94/299]
	Total	198	101	299	Prevalence = 66.2% [198/299]	

APS2 = Amyloid Probability Score 2

10.4.3. Sex

Table 53.29 describes the performance of the PrecivityAD2 test in both females and males. The prevalence of brain amyloid pathology is higher in females (59.7%) than in males (46.4%). This leads to a slightly higher PPV for APS2 Positive subjects in females (98.3%) than in males (96.7%). The NPV for APS2 negative subjects is also slightly lower in females (91.2%) than in males (94.4%), and this difference is expected to diminish if prevalence between females and males is adjusted to be equivalent. Taken together, the performance of the PrecivityAD2 test is similar in female and male subjects.

Table 53.29: Clinical Performance of the PrecivityAD2 Test in the Pooled Validation Cohort: Subgroup Analysis – Sex

Sex	PrecivityAD2 Test	Brain Amyloid Pathology			Predictive Value [n/N] (95% CI)	Frequency [n/N]
		Present	Absent	Total		
Female	Positive (APS2 > 66.5)	238	4	242	98.3% [238/242] (95.8%; 99.4%)	44.6% [242/543]
	Likely Positive (27.5 < APS2 < 66.5)	67	18	85	78.8% [67/85] (69.0%; 86.2%)	15.7% [85/543]
	Negative (APS2 < 27.5)	19	197	216	8.8% [19/216] (5.7%; 13.3%)	39.8% [216/543]
	Total	324	219	543	Prevalence = 59.7% [324/543]	
Male	Positive (APS2 > 66.5)	175	6	181	96.7% [175/181] (93.0%; 98.5%)	30.2% [181/599]
	Likely Positive (27.5 < APS2 < 66.5)	86	27	113	76.1% [86/113] (67.5%; 83.0%)	18.9% [113/599]
	Negative (APS2 < 27.5)	17	288	305	5.6% [17/305] (3.5%; 8.7%)	50.9% [305/599]
	Total	278	321	599	Prevalence = 46.4% [278/599]	

APS2 = Amyloid Probability Score 2

10.4.4. Race

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.

10.4.5. Comorbidities

The performance of the PrecivityAD2 test in 12 different documented comorbidities, as well as in subjects with one, two, or three or more of these comorbidities, was evaluated. Not all studies reported all 12 different comorbidities. Thus, it was not possible to evaluate the clinical performance of the PrecivityAD2 test in subjects with no comorbidities, since it is not clear that the absence of a reported comorbidity for an individual is evidence of the absence of that specific comorbidity. The data depicted in Tables 53.30 and 53.31 below demonstrate that the clinical performance of the PrecivityAD2 test is similar in all subjects regardless of the presence of any single comorbidity or multiple number of comorbidities.

Table 53.30: Clinical Performance of the PrecivityAD2 Test by Comorbidity Category (Alphabetized)

PrecivityAD2 Test	Comorbidity		
	Atrial Fibrillation	**Autoimmune/ Inflammatory Disease**	**Chronic Heart Failure**
Positive Result PV [n/N] (95% CI)	100.0% [33/33] (89.6%; 100.0%)	91.8% [45/49] (80.8%; 96.8%)	100.0% [10/10] (72.2%; 100.0%)
Likely Positive Result PV [n/N] (95% CI)	66.7% [12/18] (43.7%; 83.7%)	75.0% [15/20] (53.1%; 88.8%)	42.9% [3/7] (15.8%; 75.0%)
Negative Result PV [n/N] (95% CI)	0.0% [0/27] (0.0%; 12.5%)	5.6% [2/36] (1.5%; 18.1%)	0.0% [0/10] (0.0%; 27.8%)
Prevalence [n/N]	57.7% [45/78]	59.0% [62/105]	48.1% [13/27]
Frequency of Likely Positive [n/N]	23.1% [18/78]	19.0% [20/105]	25.9% [7/27]
PrecivityAD2 Test	**Comorbidity**		
	Chronic Kidney Disease	**Coronary Heart Disease (CHD)**	**Depression**
Positive Result PV [n/N] (95% CI)	100.0% [97/97] (96.2%; 100.0%)	92.1% [35/38] (79.2%; 97.3%)	98.2% [54/55] (90.4%; 99.7%)

Likely Positive Result PV [n/N] (95% CI)	73.2% [30/41] (58.1%; 84.3%)	93.3% [14/15] (70.2%; 98.8%)	57.1% [16/28] (39.1%; 73.5%)
Negative Result PV [n/N] (95% CI)	1.8% [1/56] (0.3%; 9.4%)	0.0% [0/43] (0.0%; 8.2%)	1.9% [2/103] (0.5%; 6.8%)
Prevalence [n/N]	66.0% [128/194]	51.0% [49/96]	38.7% [72/186]
Frequency of Likely Positive [n/N]	21.1% [41/194]	15.6% [15/96]	15.1% [28/186]
PrecivityAD2 Test	**Comorbidity**		
	Diabetes	**Dyslipidemia**	**History of Cancer**
Positive Result PV [n/N] (95% CI)	100.0% [43/43] (91.8%; 100.0%)	95.2% [79/83] (88.3%; 98.1%)	95.5% [64/67] (87.6%; 98.5%)
Likely Positive Result PV [n/N] (95% CI)	73.5% [25/34] (56.9%; 85.4%)	72.1% [31/43] (57.3%; 83.3%)	64.0% [16/25] (44.5%; 79.8%)
Negative Result PV [n/N] (95% CI)	6.2% [4/65] (2.4%; 14.8%)	4.8% [5/104] (2.1%; 10.8%)	5.7% [3/53] (1.9%; 15.4%)
Prevalence [n/N]	50.7% [72/142]	50.0% [115/230]	57.2% [83/145]
Frequency of Likely Positive [n/N]	23.9% [34/142]	18.7% [43/230]	17.2% [25/145]
PrecivityAD2 Test	**Comorbidity**		
	History of Stroke or TIA	**Hypertension**	**Obesity**
Positive Result PV [n/N] (95% CI)	97.0% [32/33] (84.7%; 99.5%)	96.7% [147/152] (92.5%; 98.6%)	96.9% [31/32] (84.3%; 99.4%)
Likely Positive Result PV [n/N] (95% CI)	50.0% [10/20] (29.9%; 70.1%)	75.0% [54/72] (63.9%; 83.6%)	77.3% [17/22] (56.6%; 89.9%)
Negative Result PV [n/N] (95% CI)	2.3% [1/44] (0.4%; 11.8%)	5.7% [11/192] (3.2%; 10.0%)	8.5% [11/130] (4.8%; 14.5%)
Prevalence [n/N]	44.3% [43/97]	51.0% [212/416]	32.1% [59/184]
Frequency of Likely Positive [n/N]	20.6% [20/97]	17.3% [72/416]	12.0% [22/184]

Note: Positive = APS2 > 66.5; Likely Positive = 27.5 < APS2 < 66.5; Negative = APS2 < 27.5
APS2 = Amyloid Probability Score 2; PV = predictive value; TIA = transient ischemic attack

Table 53.31: Clinical Performance of the PrecivityAD2 Test by Number of Documented Comorbidities

PrecivityAD2 Test	Number of Comorbidities		
	One	Two	Three or More
Positive Result PV [n/N] (95% CI)	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 Result PV [n/N] (95% CI)	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 Result PV [n/N] (95% CI)	8.3% [11/132] (4.7%; 14.3%)	3.8% [3/80] (1.3%; 10.5%)	4.6% [7/151] (2.3%; 9.3%)
Prevalence [n/N]	56.4% [173/307]	52.0% [91/175]	47.8% [153/320]
Frequency of Likely Positive [n/N]	17.9% [55/307]	15.4% [27/175]	19.1% [61/320]

Note: Positive = APS2 > 66.5; Likely Positive = 27.5 < APS2 < 66.5; Negative = APS2 < 27.5
APS2 = Amyloid Probability Score 2; PV = predictive value

11. Clinical Cut-Off

Refer to Section V.8.: PrecivityAD2 Cutoffs

12. Reference Range and Expected Values

12.1. Reference Range Study

The reference range for the PrecivityAD2 test in cognitively normal individuals was evaluated in accordance with CLSI EP28-A3c[6]. The analysis was based on data from cognitively normal individuals who participated in three separate studies: the Bio-Hermes Cohort[7] (N=363); the PPAS008 study (N=74); and BioIVT[8] Normal Control study (N=27). In total, 470 cognitively normal individuals were enrolled across the three studies, of whom 464 had reportable APS2 results and were included in the analysis.

Table 53.32: Distribution of APS2 in All Subjects in Bio-Hermes, PPAS008 and BioIVT Healthy Control Cohorts

	All	Age			Gender		Race		
		40-59	60-69	70-85	Male	Female	White	African American	Other
n	464	93	188	183	187	277	402	46	16

	APS2								
Mean	20.67	9.28	17.63	29.57	21.52	20.09	21.72	13.63	14.31
SD	23.64	8.4	20.06	28.59	24.22	23.27	24.32	16.95	18.68
Median	11	6	10	16	11	10	11	8	8.5
Minimum	0	0	1	3	0	0	0	2	2
Maximum	100	48	99	100	100	99	100	84	80
2.5th Percentile	2	0	3.67	4	3	2	2	2.12	N/A*
97.5th Percentile	92	29.70	81	98	90.70	92.20	93.95	64.50	N/A*
	$A\beta 40$								
Mean	482.65	425.31	470.24	524.53	471.84	489.94	486.06	466.13	444.31
SD	100.18	80.81	84.51	106.25	99.9	99.89	101.86	92.43	63.83
Median	470.40	418	464.72	509.22	458.87	476.46	472.84	444.28	430.27
Minimum	93.01	236.32	93.01	265.29	93.01	316.13	93.01	306.69	365.24
Maximum	954.05	905.69	954.05	951.06	795.35	954.05	954.05	786.10	630.87
2.5th Percentile	337.22	314.84	347.6	370.4	321.59	353.39	337.04	340.94	N/A*
97.5th Percentile	761.71	559.76	633.78	785.21	734.29	756.91	769.83	701.79	N/A*
	$A\beta 42$								
Mean	48.60	47.44	47.54	50.27	46.89	49.75	48.56	49.77	46.21

SD	10.38	12.84	8.67	10.43	9.97	10.51	10.42	10.55	8.85
Median	47.53	44.37	46.86	49.1	45.87	48.39	47.6	49.69	44.68
Minimum	14.17	20.97	14.17	27.60	14.17	31.36	14.17	33.36	36.51
Maximum	90.51	85.73	83.93	90.51	83.93	90.51	90.51	83.93	68.07
2.5th Percentile	32.93	29.04	34.08	35.18	29.42	34.93	32.58	35.96	N/A*
97.5th Percentile	76.66	82.12	66.5	77.40	69.03	79.04	76.78	75.85	N/A*
	Aβ42/Aβ40 Ratio								
Mean	0.1014	0.111	0.1017	0.0961	0.1002	0.1022	0.1006	0.1071	0.1039
SD	0.0135	0.0186	0.0113	0.009	0.0127	0.014	0.0136	0.0108	0.0123
Median	0.1005	0.1081	0.102	0.096	0.0997	0.1009	0.0995	0.1061	0.1023
Minimum	0.075	0.075	0.0775	0.0764	0.075	0.0764	0.075	0.0809	0.0893
Maximum	0.19	0.19	0.1523	0.1277	0.19	0.1748	0.19	0.1358	0.1397
2.5th Percentile	0.0814	0.0831	0.082	0.0813	0.0817	0.0812	0.0813	0.0856	N/A*
97.5th Percentile	0.13	0.1696	0.12	0.1139	0.1195	0.1365	0.1266	0.1306	N/A*
	p-tau217								
Mean	10.99	44.63	3.31	1.8	12.77	9.79	10.28	15.32	16.56

SD	19.41	14.76	10.87	1.66	20.74	18.4	19.05	20.3	24.22
Median	1.35	41.61	0.65	0.65	1.53	0.65	1.34	1.67	1.1
Minimum	0.65	15.35	0.65	0.65	0.65	0.65	0.65	0.65	0.65
Maximum	86.34	86.34	85.71	7.70	81.44	86.34	86.34	63.72	60.33
2.5th Percentile	0.65	19.45	0.65	0.65	0.65	0.65	0.65	0.65	N/A*
97.5th Percentile	64.35	73.16	43.44	6.62	68.41	61.43	67.4	60.24	N/A*
	np-tau217								
Mean	37.98	0.81	44.47	50.22	35.51	39.66	38.85	30.28	38.49
SD	26.38	0.47	20.99	20.43	24.81	27.30	25.59	27.70	38.27
Median	40.13	0.68	42.67	45.02	39.29	40.65	40.44	33.27	41.57
Minimum	0.32	0.32	0.47	14.53	0.33	0.32	0.32	0.34	0.4
Maximum	201.72	3.59	164.21	201.72	116.21	201.72	201.72	116.21	149.4
2.5th Percentile	0.47	0.35	0.85	26.43	0.47	0.5	0.48	0.5	N/A*
97.5th Percentile	89.84	1.91	90.29	95.86	84.02	95.94	87.34	68.27	N/A*
	%p-tau217								
Mean	2.81	1.89	2.51	3.59	2.87	2.78	2.89	2.37	2.13

SD	2.4	0.90	1.98	3.02	2.54	2.31	2.49	1.68	1.81
Median	1.84	1.61	1.75	2.15	1.83	1.91	1.84	1.81	1.55
Minimum	0.44	0.60	0.44	0.68	0.77	0.44	0.60	0.95	0.44
Maximum	17.48	5.75	12.96	17.48	17.48	14.33	17.48	7.52	8.01
2.5th Percentile	0.94	0.78	0.92	1.05	0.93	0.95	0.96	1.02	N/A*
97.5th Percentile	10.26	4.03	8.10	12.34	10.01	10.26	10.5	7.40	N/A*
	APS2 % (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.									

Aβ40 = β-Amyloid 1-40; Aβ42 = β-Amyloid 1-42; APS2 = Amyloid Probability Score 2; np-tau217 = non-phosphorylated tau protein at amino acid threonine, position 217; p-tau217 = phosphorylated tau protein at amino acid threonine, position 217

In this cognitively normal cohort, APS2 values were predominantly low, with the majority of participants falling below the Negative threshold (<27.5). This concentration of values in the Negative range indicates that the PrecivityAD2 test reliably identifies individuals at low likelihood of Alzheimer's-related pathology within the intended-use population. Approximately 20% of participants were classified as Positive or Likely Positive, while 80% were classified as Negative, which is consistent with prior published reports[9-12]. APS2 positive classifications demonstrated an expected increase with advancing age, also in line with previous findings. No meaningful differences were observed between males and females. Due to limited sample sizes in certain subgroups, the data was not sufficient to draw definitive conclusions regarding racial or other demographic differences.

12.2. Expected Values

The expected values for the PrecivityAD2 test in individuals with Alzheimer's disease-related neurological diseases and other medical conditions commonly observed in the intended use population were evaluated. The analysis is based on data from the BioIVT[8] cohort, which included 47 individuals.

Data from individuals with AD-related neurological diseases and other medical conditions were analyzed to determine the expected values of the PrecivityAD2 test for each disease group.

Table 53.33: PrecivityAD2 Test Results by Disease in the BioIVT Cohort

Disease	APS2 Result						APS2 Result % (n)		
	n	Mean	SD	Median	Min, Max	2.5th Percentile, 97.5th Percentile	Positive (APS2 > 66.5)	Likely Positive (27.5< APS2< 66.5)	Negative (APS2 < 27.5)
Behavioral Frontotemporal Dementia	3	45	46.90	22	14, 99	N/A*, N/A*	33.30% (1)	0.0% (0)	66.70% (2)
Lewy Body Dementia	1	28	-	28	28, 28	N/A*, N/A*	0.0% (0)	100.0% (1)	0.0% (0)
Frontotemporal Dementia	3	12.70	11.50	13	1, 24	N/A*, N/A*	0.0% (0)	0.0% (0)	100.0% (3)
Parkinson's	40	32.20	26	23	5, 98	7, 97	12.50% (5)	35.00% (14)	52.50% (21)

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

APS2 = Amyloid Probability Score 2

VI. PROPOSED LABELING:

The labeling supports the finding of equivalence for this device.

VII. CONCLUSION:

The submitted information in this premarket notification is complete and supports a substantial equivalence decision.

VIII. REFERENCES

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7) The Bio-Hermes reference range cohort drew from the Bio-Hermes study, a blood and digital platform study with 956 participants aged 55- 85 with mild cognitive impairment, suspected Alzheimer's disease, or normal cognition. The reference range study drew from Cohort 1 of the Bio-Hermes study: individuals with normal cognition

8) The BioIVT expected values cohort in this study drew from the BioIVT cohort (PrecisionMed Protocol 1009 and PrecisionMed Protocol 8008), which consists of donors >50 years with neurodegenerative and neuropsychiatric diseases.

9) Mohs RC, Beauregard D, Dwyer J, et al. The Bio-Hermes Study: Biomarker database developed to investigate blood-based and digital biomarkers in community-based, diverse populations clinically screened for Alzheimer's disease. *Alzheimers Dement*. 2024;20(4):2752-2765. doi:10.1002/alz.13722

10) Kuhn E, Klinger HM, Amariglio RE, et al. SCD-plus features and AD biomarkers in cognitively unimpaired samples: A meta-analytic approach for nine cohort studies. *Alzheimers Dement*. 2025;21(5):e14307. doi:10.1002/alz.14307

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