



**510(k) SUBSTANTIAL EQUIVALENCE DETERMINATION
DECISION SUMMARY
ASSAY ONLY**

I Background Information:

A 510(k) Number

K242706

B Applicant

Fujirebio Diagnostics, Inc.

C Proprietary and Established Names

Lumipulse G pTau217/ β -Amyloid 1-42 Plasma Ratio

D Regulatory Information

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

II Submission/Device Overview:

A Purpose for Submission:

New device

B Measurand:

Phospho-Tau 217 (pTau 217)
 β -Amyloid 1-42

C Type of Test:

Fully automated, chemiluminescent enzyme immunoassay (CLEIA)

III Intended Use/Indications for Use:

A Intended Use(s):

See Indications for Use below.

B Indication(s) for Use:

The Lumipulse **G** pTau217/ β -Amyloid 1-42 Plasma Ratio is an in vitro test using human plasma (K2EDTA) that combines the results of Lumipulse **G** pTau 217 Plasma and Lumipulse **G** β -Amyloid 1-42-N Plasma assays into a ratio of pTau 217 to β -Amyloid 1-42 concentrations using the Lumipulse G1200 System.

The Lumipulse **G** pTau 217/ β -Amyloid 1-42 Plasma Ratio is intended to aid healthcare providers to identify patients with amyloid pathology associated with Alzheimer's disease.

The Lumipulse **G** pTau 217/ β -Amyloid 1-42 Plasma Ratio is indicated for adult patients, aged 50 years and older, presenting at a specialized care setting with signs and symptoms of cognitive decline.

A test result ≤ 0.00370 is a negative result which is consistent with patients who are unlikely to have amyloid pathology. These patients should be investigated for other causes of cognitive decline.

A test result ≥ 0.00738 is a positive result which is consistent with patients who are likely to have amyloid pathology. This result does not establish a diagnosis of Alzheimer's disease or other cognitive disorder.

A test result between 0.00371 and 0.00737 is an indeterminate result which is consistent with patients who are uncertain to have amyloid pathology. These patients should be considered for further testing.

The Lumipulse **G** pTau 217/ β -Amyloid 1-42 Plasma Ratio results must be interpreted in conjunction with other patient clinical information.

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

C Special Conditions for Use Statement(s):

Rx - For Prescription Use Only

D Special Instrument Requirements:

LUMIPULSE G1200 System (K142895)

IV Device/System Characteristics:

A Device Description:

The test system consists of Lumipulse **G** pTau 217 Plasma, Lumipulse **G** β -Amyloid 1-42-N Plasma, and the LUMIPULSE G1200. Lumipulse **G** pTau 217 Plasma and Lumipulse **G** β -Amyloid 1-42-N Plasma are packaged in a box individually and sold separately. The Lumipulse **G** pTau 217 Plasma should be used only with the Lumipulse **G** β -Amyloid 1-42-N Plasma to calculate the ratio of phospho-Tau 217 (pTau 217) to β -Amyloid (1-42). The Lumipulse **G** pTau 217 Plasma and Lumipulse **G** β -Amyloid 1-42-N Plasma immunoassays are not intended to be used individually.

i) Lumipulse **G** pTau 217 Plasma

For in vitro diagnostic use with the LUMIPULSE G1200 for the quantitative measurement of Tau phosphorylated at threonine 217 (pTau 217) in human K₂-EDTA plasma. The Lumipulse **G** pTau 217 Plasma assay should only be used with the Lumipulse **G** β -Amyloid 1-42 Plasma assay to calculate the ratio of pTau 217 to β -amyloid 1-42. This product is for professional use only. The components of Lumipulse **G** pTau 217 Plasma are as follows:

Component	Volume	Contents
Antibody-coated particle solution	Liquid, 150 μ L/ Immunoreaction cartridge	0.025% anti-phosphorylated Tau (217) monoclonal antibody (mouse)-coated particles, protein stabilizers (bovine) in 50 mM Tris buffer. This solution contains gelatin and turns into gel at 15°C or lower. Preservative: 0.1% ProClin 300.
Enzyme-labeled antibody solution	Liquid, 220 μ L/ Immunoreaction cartridge	0.50 μ g/mL alkaline phosphatase (ALP)-labeled anti-Tau monoclonal antibodies (mouse) conjugate, protein (bovine) and chemical stabilizers in 50 mM MOPS buffer. Preservative: 0.1% ProClin 300
Assay specific solution	Liquid, 80 μ L/ Immunoreaction cartridge	3.45% chemical stabilizers in 50mM MOPS buffer. Preservative: 0.1% ProClin 300

The following materials are required for Lumipulse **G** pTau 217 Plasma but provided separately:

- Lumipulse **G** pTau 217 Plasma Calibrators: 1 \times 1.5 mL
Cal1: 0 pg/mL, Cal2: 0.250 pg/mL, Cal3: 1.000 pg/mL, Cal4: 5.000 pg/mL and Cal5:10.000 pg/mL
Contains Tris buffer with protein (bovine) and chemical stabilizers. Preservative: 0.1% ProClin 300 and 0.05% ProClin 950
- Lumipulse **G** Substrate Solution: 6 \times 100 mL
Contains 0.2 mg/mL AMPPD* as a substrate in diethanolamine buffer with a chemical stabilizer. Preservative: sodium azide
- Lumipulse **G** Wash Solution: 1 \times 1000 mL
Contains 342 mM sodium chloride in Tris buffer with a detergent. Preservative: sodium azide
- Sampling tips for LUMIPULSE SYSTEM: 12 \times 96 tips

- Soda lime for LUMIPULSE SYSTEM: 6 × 2 tubes
*AMPPD: 3-(2'-spiroadamantane)-4-methoxy-4-(3''-phosphoryloxy) phenyl-1, 2-dioxetane disodium salt.

Materials required but not provided:

- Lumipulse pTau 217 Plasma Controls: 2 × 2 Concentrations
Level 1 (2 × 1.5 mL) and Level 2 (2 × 1.5 mL)
Contains preservative: 0.1% ProClin 300, 0.05% ProClin 950
- Purified water
- Micropipettes
- Recommended sample cups

ii) Lumipulse G β-Amyloid 1-42-N Plasma

For in vitro diagnostic use with the LUMIPULSE G1200 for the quantitative measurement of β-amyloid1-42 in human K₂-EDTA plasma. The Lumipulse G β-Amyloid 1-42-N Plasma assay should only be use with the Lumipulse G pTau 217 Plasma assay to calculate the ratio of pTau 217 to β-amyloid 1-42. This product is for professional use only. The components of Lumipulse G β-Amyloid 1-42-N Plasma are as follows:

Component	Volume	Contents
Antibody-coated particle solution	Liquid, 150 μL/ Immunoreaction cartridge	0.050% anti-Amyloid1-42 monoclonal antibody (mouse)-coated particles, protein stabilizers (bovine) in 50 mM MES buffer. This solution contains gelatin and turns into gel at 15°C or lower. Preservative: 0.1% Proclin 300.
Enzyme-labeled antibody solution	Liquid, 250μL/ Immunoreaction cartridge	0.50 μg/mL alkaline phosphatase (ALP)-labeled anti-β-amyloid monoclonal antibodies (mouse) conjugate, protein (bovine) and chemical stabilizers in 50 mM MES buffer. Preservative: 0.1% ProClin 300
Assay specific solution	Liquid, 120 μL/ Immunoreaction cartridge	2.05% detergents in 50 mM MES buffer. Preservative: 0.1% ProClin 300

The following materials are required for Lumipulse G β-Amyloid 1-42-N Plasma but sold separately:

- Lumipulse G β-Amyloid 1-42 Plasma Calibrator: 1×1.5 mL
Cal1: 0 pg/mL, Cal2: 30 pg/mL, Cal3: 100 pg/mL and Cal4: 1000 pg/mL
Contains Tris buffer with protein (bovine) and chemical stabilizers. Preservative: 0.1% ProClin 950
- Lumipulse G Substrate Solution: 6 × 100 mL
Contains 0.2 mg/mL AMPPD* as a substrate in diethanolamine buffer with a chemical stabilizer. Preservative: sodium azide
- Lumipulse G Wash Solution: 1 × 1000 mL
Contains 342 mM sodium chloride in Tris buffer with a detergent. Preservative: sodium Azide
- Sampling tips for LUMIPULSE SYSTEM: 12 × 96 tips

- Soda lime for LUMIPULSE SYSTEM: 6 × 2 tubes
*AMPPD: 3-(2'-spiroadamantane)-4-methoxy-4-(3''-phosphoryloxy) phenyl-1, 2-dioxetane disodium salt

Materials required but not provided:

- Lumipulse β-Amyloid Plasma Controls: 2 × 2 Concentrations
Level 1 (2 × 1.5 mL) and Level 2 (2 × 1.5 mL)
Contains preservative: 0.1% ProClin 950
- Purified water
- Micropipettes
- Recommended sample cups

iii) LUMIPULSE G1200 System

LUMIPULSE G1200 is an instrument platform that can perform automated chemiluminescence immunoassays of specimens using LUMIPULSE **G** reagents, conducting various processes such as dispensing, agitation and photometric measurement.

B. Principle of Operation:

1. Specimen collection and preparation

Refer to the Lumipulse **G** pTau 217/β-Amyloid 1-42 Plasma Ratio package insert that is supplied with each individual assay (Lumipulse **G** pTau 217 Plasma assay and Lumipulse **G** β-Amyloid 1-42-N Plasma assay).

2. Lumipulse **G** pTau 217 Plasma:

Lumipulse **G** pTau 217 Plasma is an assay system including a set of immunoassay reagents for the quantitative measurement of pTau 217 in K₂-EDTA plasma specimens based on chemiluminescent enzyme immunoassay (CLEIA) technology by a specific two-step immunoassay method on the LUMIPULSE **G** System as follows:

- a. First reaction step: pTau 217 calibrator or specimen are added to particle solution.
- b. pTau 217 in specimens or calibrators specifically binds to anti- phosphorylated Tau (217) monoclonal antibody (mouse) on the particles, and antigen-antibody immunocomplexes are formed.
- c. Washing step: The particles are washed and rinsed to remove unbound materials.
- d. Second reaction step: Alkaline phosphatase (ALP)-labeled anti-Tau monoclonal antibodies (mouse) are added and specifically bind to the prior formed immunocomplexes on the particles, and additional immunocomplexes are formed.
- e. Washing step: The particles are washed and rinsed to remove unbound materials.
- f. Enzyme reaction step: Substrate Solution is added and mixed with the particles. AMPPD* contained in the Substrate Solution is dephosphorylated by the catalysis of ALP indirectly conjugated to particles.
- g. Luminescence measurement step: Luminescence (at a maximum wavelength of 477 nm) is generated by the cleavage reaction of dephosphorylated AMPPD*. The luminescent signal reflects the amount of pTau 217 present in the sample.

*AMPPD: 3-(2'-spiroadamantane)-4-methoxy-4-(3''-phosphoryloxy) phenyl-1, 2-dioxetane disodium salt.

3. Lumipulse G β-Amyloid 1-42-N Plasma

Lumipulse G β-Amyloid 1-42-N Plasma is an assay system including a set of immunoassay reagents, for the quantitative measurement of β-amyloid₁₋₄₂ in K₂-EDTA plasma specimens based on CLEIA technology by a two-step immunoassay method on the LUMIPULSE G System as follows:

- a. Sampling: β-Amyloid 1-42 Plasma calibrator or specimen are added to the particle solution.
- b. First reaction step: β-amyloid₁₋₄₂ in specimens or calibrators specifically binds to the anti-β-amyloid₁₋₄₂ monoclonal antibody (mouse) on the particles, and antigen-antibody immunocomplexes are formed.
- c. Washing: The particles are washed and rinsed to remove unbound materials.
- d. Second reaction: Alkaline phosphatase (ALP)-labeled anti- β-amyloid monoclonal antibodies (mouse) are added and specifically bind to the prior formed immunocomplexes on the particles, and additional immunocomplexes are formed.
- e. Washing: The particles are washed and rinsed to remove unbound materials.
- f. Enzyme reaction: Substrate Solution is added and mixed with the particles. AMPPD* contained in the Substrate Solution is dephosphorylated by the catalysis of ALP indirectly conjugated to particles.
- g. Luminescence measurement: Luminescence (at a maximum wavelength of 477 nm) is generated by the cleavage reaction of dephosphorylated AMPPD*. The luminescent signal reflects the amount of β-amyloid₁₋₄₂ present in the sample.
*AMPPD: 3-(2'-spiroadamantane)-4-methoxy-4-(3''-phosphoryloxy) phenyl-1, 2-dioxetane disodium salt.

4. Interpretation of Lumipulse G pTau217/β-Amyloid 1-42 Plasma Ratio results

The LUMIPULSE G1200 reports the results of the Lumipulse G pTau 217 Plasma and the Lumipulse G β-Amyloid 1-42-N Plasma separately. The results from the same patient specimen (K₂-EDTA plasma sample) are combined into a numerical ratio in a range from 0.00000 – 1.00000 as shown below.

$$\text{Lumipulse G pTau217/}\beta\text{-Amyloid 1-42 Plasma Ratio} = \frac{\text{Lumipulse G pTau 217 (results in pg/mL)}}{\text{Lumipulse G }\beta\text{-Amyloid 1-42-N (results in pg/mL)}} = \text{a numerical value ranging 0.00000 to 1.00000}$$

The ratio of pTau 217 to β-Amyloid 1-42 (pTau217/β-Amyloid 1-42) must be manually calculated because the instrument does not report the final result. The numerical ratio must be compared to the cut-offs of 0.00738 and 0.00370. The final result (positive, indeterminate, or negative) must be interpreted by the laboratory professional according to the table below:

Lumipulse G pTau217/ β-Amyloid 1-42 Plasma Ratio	Test Result	Interpretation
Ratio \geq 0.00738	Positive	A positive result is consistent with patients who are likely to have amyloid pathology. This result does not establish a diagnosis of AD or other cognitive disorders.
0.00371 \leq Ratio \leq 0.00737	Indeterminate	An indeterminate result is consistent with patients who are uncertain to have amyloid pathology. These patients should be considered for further testing.
Ratio \leq 0.00370	Negative	A negative result is consistent with patients who are unlikely to have amyloid pathology. These patients should be investigated for other causes of cognitive decline.
<p>Note: The ratio should be rounded to 5 decimal places before comparing against the interpretation of results. If the concentrations of the analytes are outside their respective analytical measuring intervals (AMIs), the following rules apply:</p> <ul style="list-style-type: none"> • If the Lumipulse G pTau 217 Plasma assay result is $<$ 0.047 pg/ml, and the Lumipulse G β-Amyloid 1-42 Plasma assay result is $<$ 12.8 pg/mL, then the ratio cannot be calculated. • If the Lumipulse G pTau 217 Plasma assay result is $<$ 0.047 pg/ml, and the Lumipulse G β-Amyloid 1-42 Plasma assay result is \geq 12.8, then the Lumipulse G pTau 217 Plasma assay result used to calculate the ratio should be “0.047”. • If the Lumipulse G pTau 217 Plasma assay result is $>$ 10.000 pg/mL, then the assay result used to calculate the ratio should be “10.000”. • If the Lumipulse G β-Amyloid 1-42-N Plasma assay result is \geq 500.0 pg/mL and the Lumipulse G pTau 217 Plasma assay result is \leq 1.85 pg/mL, then the Lumipulse G β-Amyloid 1-42-N Plasma assay result used to calculate the ratio should be “500.0”. • If the Lumipulse G β-Amyloid 1-42-N Plasma assay result is \geq 500.0 pg/mL and the Lumipulse G pTau 217 Plasma assay result is $>$ 1.85 pg/mL, then the ratio cannot be calculated. • If the ratio cannot be calculated, then the sample should be re-tested. If upon re-test, one or both individual assay results remain outside the AMI, the ratio cannot be determined. A repeat blood draw is not recommended. 		

V Substantial Equivalence Information:

A Predicate Device Name(s):

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

B Predicate 510(k) Number(s):

DEN200072

C Comparison with Predicate(s):

Device & Predicate Device(s):	<u>Device</u> K242706	<u>Predicate</u> DEN200072
Device Trade Name	Lumipulse G pTau 217/ β-Amyloid 1-42 Plasma Ratio	Lumipulse G β-Amyloid Ratio (1-42/1-40)
General Device Characteristic Similarities		
Intended Use/ Indications For Use	<p>The Lumipulse G pTau 217/β-Amyloid 1-42 Plasma Ratio is an in vitro test using human plasma (K2EDTA) that combines the results of Lumipulse G pTau 217 Plasma and Lumipulse G β-Amyloid 1-42-N Plasma assays into a ratio of pTau 217 to β-Amyloid 1-42 concentrations using the LUMIPULSE G1200 System.</p> <p>The Lumipulse G pTau 217/β-Amyloid 1-42 Plasma Ratio is intended to aid healthcare providers to identify patients with amyloid pathology associated with Alzheimer’s disease.</p> <p>The Lumipulse G pTau 217/β-Amyloid 1-42 Plasma Ratio is indicated for adult patients, aged 50 years and older, presenting at a specialized care setting with signs and symptoms of cognitive decline.</p> <p>A test result ≤ 0.00370 is a negative result which is consistent with patients who are unlikely to have amyloid pathology. These patients should be investigated for other causes of cognitive decline.</p> <p>A test result ≥ 0.00738 is a positive result which is consistent with patients who are likely to have amyloid pathology. This result does not establish a diagnosis of Alzheimer’s disease or other cognitive disorder.</p>	<p>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 1-42 to β-amyloid 1-40 concentrations using the LUMIPULSE G1200 System.</p> <p>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.</p> <p>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.</p> <p>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.</p> <p>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.</p>

	<p>A test result between 0.00371 and 0.00737 is an indeterminate result which is consistent with patients who are uncertain to have amyloid pathology. These patients should be considered for further testing.</p> <p>The Lumipulse G pTau 217/β-Amyloid 1-42 Plasma Ratio results must be interpreted in conjunction with other patient clinical information.</p> <p>This test is not intended as a screening or stand-alone diagnostic test.</p>	<p>The Lumipulse G β-Amyloid Ratio (1-42/1-40) results must be interpreted in conjunction with other patient clinical information.</p> <p>This test is not intended as a screening or stand-alone diagnostic test.</p>
Principle of Operation	Automated Chemiluminescent Enzyme Immunoassay (CLEIA)	Same
Assay Type	Two-step sandwich immunoassays based on chemiluminescent technology	Same
Instrument System	LUMIPULSE G1200 System	Same
Assay Cut-off	Two ratio cut-offs	Same
Sample Volume	40 μ L	Same
Substrate Solution	AMPPD* is added as the substrate	Same
*AMPPD= 3-(2' -spiroadamantane)-4-methoxy-4-(3"-phosphoryloxy) phenyl-I, 2-dioxetane disodium salt		
General Device Characteristic Differences		
Analytes	pTau 217 and β -Amyloid 1-42	β -Amyloid 1-42 and β -Amyloid 1-40
Assay Antibodies	<p><i>pTau 217</i>: anti-phosphorylated Tau (217) monoclonal antibody (mouse)-coated particles and alkaline phosphatase (ALP)-labeled anti-Tau monoclonal antibodies (mouse)</p> <p><i>β-Amyloid 1-42</i>: anti-β-amyloid 1-42 monoclonal antibody (mouse)-coated particles and alkaline-phosphatase (ALP)-labeled anti-β-amyloid 1-42 monoclonal antibodies (mouse) conjugate</p>	<p><i>β-Amyloid 1-42</i>: anti-β-amyloid₁₋₄₂ monoclonal antibody (mouse)-coated particles and biotinylated anti-β-amyloid antibody (mouse)</p> <p><i>β-Amyloid 1-40</i>: anti-β-amyloid₁₋₄₀ monoclonal antibody (mouse)-coated particles and alkaline-phosphatase (ALP)-labeled anti-β-amyloid antibody (mouse) conjugate</p>
Specimen	Plasma (K ₂ -EDTA)	Cerebrospinal fluid (CSF)
Calibrators	<p><i>pTau 217</i>: Lumipulse G pTau 217 Plasma Calibrator Set: 0, 0.250, 1.000, 5.000 and 10.000 pg/mL</p>	<p><i>β-Amyloid 1-42</i>: Lumipulse G β-Amyloid 1-42 Calibrator Set: 30, 129, and 2,335 pg/mL</p>

	<p><i>β-Amyloid 1-42:</i> Lumipulse G <i>β-Amyloid 1-42</i> Plasma Calibrators Set: 0, 30, 100 pg/mL and 1000 pg/mL</p>	<p><i>β-Amyloid 1-40:</i> Lumipulse G <i>β-Amyloid 1-40</i> Calibrator Set: 0, 500, and 30,000 pg/mL</p>
Traceability/ Standardization	<p><i>pTau 217:</i> Traceable to in-house reference calibrators</p> <p><i>β-Amyloid 1-42:</i> Traceable to Lumipulse G <i>β-Amyloid 1-42</i> CSF assay (DEN200027)</p>	<p><i>β-Amyloid 1-42:</i> Standardized against three certified reference materials: ERM-DA480/IFCC, ERM-DA481/IFCC and ERM-DA482/IFCC</p> <p><i>β-Amyloid 1-40:</i> Traceable to INNTEST <i>β-Amyloid 1-40</i></p>
Measuring Range	<p><i>pTau 217:</i> 0.047–10.000 pg/mL <i>β-Amyloid 1-42:</i> 0.8–500.0 pg/mL</p>	<p><i>β-Amyloid 1-42:</i> 38–2203.5 pg/mL <i>β-Amyloid 1-40:</i> 156.3–28450.3 pg/mL</p>
No high-dose hook effect	<p><i>pTau 217:</i> Up to 610 pg/mL <i>β-Amyloid 1-42:</i> Up to 2200 pg/mL</p>	<p><i>β-Amyloid 1-42:</i> Up to 159,000 pg/mL <i>β-Amyloid 1-40:</i> Up to 150,000 pg/mL</p>
Detection Limit	<p><i>pTau 217:</i> Limit of Blank = 0.025 pg/mL Limit of Detection = 0.041 pg/mL Limit of Quantitation = 0.047 pg/mL</p> <p><i>β-Amyloid 1-42:</i> Limit of Blank = 0.128 pg/mL Limit of Detection = 0.207 pg/mL Limit of Quantitation = 0.8 pg/mL</p>	<p><i>β-Amyloid 1-42:</i> Limit of Blank = 2.2 pg/mL Limit of Detection = 11.6 pg/mL Limit of Quantitation = 38.0 pg/mL</p> <p><i>β-Amyloid 1-40:</i> Limit of Blank = 0.97 pg/mL Limit of Detection = 33.0 pg/mL Limit of Quantitation = 158.0 pg/mL</p>
Ratio Calculation	<p>Operator calculates the ratio of pTau 217 to <i>β-Amyloid 1-42</i>. The final test result (negative, indeterminate, or positive) must be interpreted by the laboratory professional according to the instructions provided in the package insert.</p>	<p>A web-based calculator tool calculates the ratio of <i>β-Amyloid 1-42</i> to <i>β-Amyloid 1-40</i> and reports the final test result (negative, likely positive or positive). If the ratio is not calculated by the Calculator Tool, the result is manually reported as ‘ratio undetermined’</p>
Reagent Stability	<p>Unopened: 9 months at 2–8°C On-board: 30 days</p>	<p>Unopened: 19 months at 2–10°C On-board: 15 days</p>
Sample Stability	<p>3 hours on-board 1 day at 2–8°C Not recommended to store at 23–28°C 1 month at -30– -10°C 1 month at -60°C Up to three freeze/thaw cycles</p>	<p>3 hours on-board 8 days at 2–8°C 48 hours at 23–27°C 2 weeks at -30– -10°C 1 month at -80°C Up to three freeze/thaw cycles</p>

VI Standards/Guidance Documents Referenced:

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

- CLSI EP05-A3: Evaluation of Precision of Quantitative Measurement Procedures; Approved Guideline – Third Edition
- CLSI EP06, 2nd ed.: Evaluation of the Linearity of Quantitative Measurement Procedures – Second Edition
- CLSI EP07, 3rd ed.: Interference Testing in Clinical Chemistry; Approved Guideline – Third Edition
- CLSI EP12, 3rd ed.: Evaluation of Qualitative, Binary Output Examination Performance – Third Edition
- CLSI EP17-A2: Evaluation of Detection Capability for Clinical Laboratory Measurement Procedures; Approved Guideline – Second Edition
- CLSI EP28-A3c: Defining, Establishing, and Verifying Reference Intervals in the Clinical Laboratory; Approved Guideline – Third Edition
- CLSI EP34, 1st ed.: Establishing and Verifying an Extended Measuring Interval Through Specimen Dilution and Spiking - First Edition
- CLSI EP35, 1st ed.: Assessment of Equivalence or Suitability of Specimen Types for Medical Laboratory Measurement Procedures - First Edition
- CLSI EP37, 1st ed.: Supplemental Tables for Interference Testing in Clinical Chemistry: Approved Guideline, First Edition
- CLSI EP39, 1st ed.: A Hierarchical Approach to Selecting Surrogate Samples for the Evaluation of In Vitro Medical Laboratory Tests - First Edition

VII Performance Characteristics (if/when applicable):

A Analytical Performance:

All results met the pre-determined acceptance criteria.

1. Precision/Reproducibility:

A study was conducted per CLSI guideline EP05-A3 to evaluate the precision including reproducibility of the Lumipulse **G** pTau 217 Plasma, Lumipulse **G** β -Amyloid 1-42-N Plasma, and Lumipulse **G** pTau217/ β -Amyloid 1-42 Plasma Ratio. A five-member panel (Panel members 1–5) was prepared to achieve target concentrations of pTau 217 and β -Amyloid 1-42 that cover the measuring ranges of the individual assays and to challenge the established cut-offs of 0.00370 and 0.00738 for Lumipulse **G** pTau217/ β -Amyloid 1-42 Plasma Ratio. Panel members 1 and 2 were prepared by pooling native plasma samples and panel member 3 was prepared by spiking β -Amyloid 1-42 protein into pooled patient plasma. Panel members 4 and 5 were prepared by spiking both recombinant pTau 217 and β -Amyloid 1-42 proteins into native plasma pools. The sample panel was used to evaluate 1) within-laboratory precision, 2) lot-to-lot precision and 3) site-to-site reproducibility, as described below:

1) Within-laboratory precision:

To evaluate the within-laboratory precision, each panel member was tested for 20 days, two runs per day, two replicates per run at a single site, using one instrument and one reagent lot for a total of 80 measurements per sample. The results are summarized in the tables below for each analyte and the ratio of the analytes:

a. Lumipulse **G** pTau 217 Plasma

		Within-Run		Between-Run		Between-Day		Within-Laboratory	
Panel member	Mean (pg/mL)	SD	%CV	SD	%CV	SD	%CV	SD	%CV
1	0.083	0.008	10.1	0.002	1.9	0.000	0.0	0.009	10.3
2	0.210	0.006	3.0	0.006	3.1	0.003	1.6	0.010	4.6
3	0.100	0.006	6.5	0.000	0.0	0.003	2.7	0.007	7.0
4	4.492	0.075	1.7	0.044	1.0	0.053	1.2	0.102	2.3
5	8.606	0.147	1.7	0.090	1.0	0.082	0.9	0.190	2.2

b. Lumipulse **G** β -Amyloid 1-42-N Plasma

		Within-Run		Between-Run		Between-Day		Within-Laboratory	
Panel member	Mean (pg/mL)	SD	%CV	SD	%CV	SD	%CV	SD	%CV
1	22.4	0.5	2.2	0.3	1.3	0.1	0.7	0.6	2.6
2	38.8	1.3	3.4	0.7	1.8	0.1	0.3	1.5	3.8
3	19.4	0.5	2.6	0.2	0.9	0.2	1.1	0.6	2.9
4	195.5	2.1	1.1	5.1	2.6	1.0	0.05	5.6	2.9
5	451.1	2.6	1.0	8.9	2.0	5.1	1.1	11.3	2.5

c. Lumipulse **G** pTau217/ β -Amyloid 1-42 Plasma Ratio

		Within-Run		Between-Run		Between-Day		Within-Laboratory	
Panel member	Mean Ratio	SD	%CV	SD	%CV	SD	%CV	SD	%CV
Ratio 1	0.00372	0.00036	9.8	0.00011	2.9	0.00000	0.0	0.00038	10.2
Ratio 2	0.00542	0.00021	3.8	0.00021	3.9	0.00008	1.5	0.00031	5.7
Ratio 3	0.00514	0.00037	7.2	0.00000	0.0	0.00014	2.8	0.00040	7.7
Ratio 4	0.02298	0.00050	2.2	0.00053	2.3	0.00000	0.0	0.00073	3.2
Ratio 5	0.01909	0.00040	2.1	0.00027	1.4	0.00034	1.8	0.00059	3.1

The ratio of pTau 217 to β -Amyloid 1-42 for each panel member was further analyzed to evaluate qualitative agreement. The % correct call was calculated for each panel member based on the number of positive Lumipulse **G** pTau 217/ β -Amyloid 1-42 Plasma Ratio results and summarized in the table below.

Panel member	Mean Ratio	Total Replicates (N)	Qualitative agreement	
			Number of Positive Lumipulse G pTau 217/ β -Amyloid 1-42 Plasma Ratio results at > 0.00738	%Ratio Positive Result
Ratio 1	0.00372	80	0	0.0 (0/80)
Ratio 2	0.00542	80	0	0.0 (0/80)
Ratio 3	0.00514	80	0	0.0 (0/80)
Ratio 4	0.02298	80	80	100.0 (80/80)
Ratio 5	0.01909	80	80	100.0 (80/80)

The % correct call was also calculated for each panel member based on the number of negative Lumipulse **G** pTau 217/ β -Amyloid 1-42 Plasma Ratio results and summarized in the table below.

Panel member	Mean Ratio	Total Replicates (N)	Qualitative agreement	
			Number of Negative Lumipulse G pTau 217/ β -Amyloid 1-42 Plasma Ratio results at \leq 0.00370	%Ratio Negative Result
Ratio 1	0.00372	80	38	47.5 (38/80)*
Ratio 2	0.00542	80	0	0.0 (0/80)
Ratio 3	0.00514	80	0	0.0 (0/80)
Ratio 4	0.02298	80	0	0.0 (0/80)
Ratio 5	0.01909	80	0	0.0 (0/80)
*The qualitative agreement was calculated based on shift from the “negative” to “indeterminate” category. None of the ratio results shifted from “negative” into “positive” test result.				

2) Lot-to-lot precision:

To evaluate between-lot precision, the panel members were tested at a single site, using one instrument and three reagent lots which were paired between Lumipulse **G** pTau 217 Plasma and Lumipulse **G** β -Amyloid 1-42-N Plasma. A paired reagent lot was defined as a set of specific lots of Lumipulse **G** pTau 217 Plasma Immunoreaction Cartridges with Lumipulse **G** pTau 217 Plasma Calibrators and Lumipulse **G** β -Amyloid 1-42-N Plasma Immunoreaction Cartridges with Lumipulse **G** β -Amyloid 1-42-N Plasma Calibrators. Each panel member was tested for five days, two runs per day, three replicates per run using three lots, for a total of 90 measurements for each sample. The results are summarized in the tables below for each analyte and the ratio of the analytes.

a. Lumipulse **G** pTau 217 Plasma

		Within-Run		Between-Run		Between-Day		Between-Lot		Total	
Panel member	Mean (pg/mL)	SD	%CV	SD	%CV	SD	%CV	SD	%CV	SD	%CV
1	0.079	0.006	7.7	0.005	6.9	0.000	0.0	0.006	7.6	0.010	12.8
2	0.210	0.010	4.9	0.005	2.3	0.000	0.0	0.011	5.4	0.016	7.6
3	0.095	0.007	7.9	0.002	1.9	0.001	1.1	0.006	6.3	0.010	10.3
4	4.445	0.075	1.7	0.017	0.4	0.044	1.0	0.126	2.8	0.154	3.5
5	8.702	0.168	1.9	0.033	0.4	0.048	0.6	0.242	2.8	0.300	3.4

b. Lumipulse **G** β -Amyloid 1-42-N Plasma

		Within-Run		Between-Run		Between-Day		Between-Lot		Total	
Panel member	Mean (pg/mL)	SD	%CV	SD	%CV	SD	%CV	SD	%CV	SD	%CV
1	23.3	0.5	2.3	0.6	2.4	0.4	1.7	1.2	5.3	1.5	6.4
2	40.5	1.4	3.5	0.5	1.3	0.6	1.6	1.4	3.5	2.2	5.4
3	20.2	0.5	2.5	0.5	2.2	0.2	1.0	1.0	5.1	1.3	6.2
4	199.9	3.1	1.6	3.5	1.7	2.5	1.3	13.4	6.7	14.4	7.2
5	449.7	6.4	1.4	6.5	1.4	8.4	1.9	16.7	3.7	20.8	4.6

c. Lumipulse **G** pTau217/ β -Amyloid 1-42 Plasma Ratio

		Within-Run		Between-Run		Between-Day		Between-Lot		Total	
Panel member	Mean Ratio	SD	%CV	SD	%CV	SD	%CV	SD	%CV	SD	%CV
Ratio 1	0.00342	0.00028	8.2	0.00020	5.9	0.00000	0.0	0.00044	12.8	0.00056	16.3
Ratio 2	0.00520	0.00030	5.8	0.00012	2.2	0.00013	2.5	0.00037	7.2	0.00051	9.8
Ratio 3	0.00469	0.00038	8.0	0.00011	2.3	0.00011	2.3	0.00051	10.9	0.00065	13.9
Ratio 4	0.02234	0.00052	2.3	0.00039	1.7	0.00044	2.0	0.00210	9.4	0.00224	10.0
Ratio 5	0.01939	0.00046	2.4	0.00029	1.4	0.00029	1.5	0.00127	6.5	0.00141	7.3

The ratio of pTau 217 to β -Amyloid 1-42 for each panel member was further analyzed to evaluate qualitative agreement. The % correct call was calculated for each panel member

based on the number of positive Lumipulse **G** pTau 217/ β -Amyloid 1-42 Plasma Ratio results and summarized in the table below.

Panel member	Mean Ratio	Total Replicates (N)	Qualitative agreement	
			Number of Positive Lumipulse G pTau 217/ β -Amyloid 1-42 Plasma Ratio results at > 0.00738	%Ratio Positive Result
Ratio 1	0.00367	90	0	0.0 (0/90)
Ratio 2	0.00541	90	0	0.0 (0/90)
Ratio 3	0.00509	90	0	0.0 (0/90)
Ratio 4	0.02115	90	90	100.0 (90/90)
Ratio 5	0.01838	90	90	100.0 (90/90)

The % correct call was also calculated for each panel member based on the number of negative Lumipulse **G** pTau 217/ β -Amyloid 1-42 Plasma Ratio results and summarized in the table below.

Panel member	Mean Ratio	Total Replicates (N)	Qualitative agreement	
			Number of Negative Lumipulse G pTau 217/ β -Amyloid 1-42 Plasma Ratio results at ≤ 0.00370	%Ratio Negative Result
Ratio 1	0.00367	90	46	51.1 (46/90)*
Ratio 2	0.00541	90	0	0.0 (0/90)
Ratio 3	0.00509	90	0	0.0 (0/90)
Ratio 4	0.02115	90	0	0.0 (0/90)
Ratio 5	0.01838	90	0	0.0 (0/90)

*The qualitative agreement was calculated based on shift from the “negative” to “indeterminate” category. None of the ratio results shifted from “negative” into “positive” test result.

The Lumipulse **G** pTau 217/ β -Amyloid 1-42 Plasma Ratio Package Insert under ‘Limitations of the Procedure’ states that “*results that border the presumed lower cutoff of 0.00370 should be interpreted with caution as lot-to-lot differences can be observed that could potentially re-categorize a borderline patient result from a “negative” into an “indeterminate”.*”

3) Site-to-site reproducibility

To evaluate site-to-site reproducibility, each panel member was tested for five days, two runs per day, three replicates per run at three sites (one instrument at each site) using one reagent lot, for a total of 90 measurements for each sample. The results are summarized in the tables below for each analyte and the ratio of the analytes:

a. Lumipulse *G* pTau 217 Plasma

		Within-Run		Between-Run		Between-Day		Between-Site		Total	
Panel member	Mean (pg/mL)	SD	%CV	SD	%CV	SD	%CV	SD	%CV	SD	%CV
1	0.085	0.007	8.7	0.004	5.1	0.003	3.6	0.002	2.7	0.009	11.0
2	0.219	0.011	5.0	0.006	2.7	0.000	0.0	0.009	4.2	0.016	7.1
3	0.104	0.007	6.6	0.001	1.3	0.003	3.1	0.006	5.4	0.009	9.1
4	4.375	0.075	1.7	0.276	6.3	0.017	0.4	0.057	1.3	0.292	6.7
5	8.624	0.129	1.5	0.137	1.6	0.093	1.1	0.057	0.7	0.217	2.5

b. Lumipulse *G* β -Amyloid 1-42-N Plasma

		Within-Run		Between-Run		Between-Day		Between-Site		Total	
Panel member	Mean (pg/mL)	SD	%CV	SD	%CV	SD	%CV	SD	%CV	SD	%CV
1	23.3	0.6	2.5	0.7	2.8	0.8	3.6	0.8	3.5	1.5	6.3
2	40.7	2.0	5.0	0.3	0.8	1.6	3.9	1.5	3.8	3.0	7.4
3	20.5	0.5	2.6	0.4	2.0	1.0	4.8	0.9	4.6	1.5	7.4
4	207.5	4.0	1.9	9.4	4.5	9.8	4.7	5.1	2.5	15.0	7.2
5	470.4	5.2	1.1	9.8	2.1	24.0	5.1	12.1	2.6	29.1	6.2

c. Lumipulse *G* pTau217/ β -Amyloid 1-42 Plasma Ratio

		Within-Run		Between-Run		Between-Day		Between-Site		Total	
Panel member	Mean Ratio	SD	%CV	SD	%CV	SD	%CV	SD	%CV	SD	%CV
Ratio 1	0.00367	0.00032	8.8	0.00016	4.4	0.00020	5.5	0.00016	4.2	0.00044	12.1
Ratio 2	0.00541	0.00035	6.5	0.00012	2.2	0.00020	3.7	0.00008	1.4	0.00043	8.0
Ratio 3	0.00509	0.00037	7.3	0.00000	0.0	0.00019	3.7	0.00000	0.0	0.00042	8.2
Ratio 4	0.02115	0.00049	2.3	0.00090	4.3	0.00083	3.9	0.00119	5.6	0.00177	8.4
Ratio 5	0.01838	0.00035	1.9	0.00035	1.9	0.00065	3.5	0.00046	2.5	0.00094	5.1

The ratio of pTau 217 to β -Amyloid 1-42 for each panel member was further analyzed to evaluate qualitative agreement. The % correct call was calculated for each panel member

based on the number of positive Lumipulse **G** pTau 217/ β -Amyloid 1-42 Plasma Ratio results and summarized in the table below.

Panel member	Mean Ratio	Total Replicates (N)	Qualitative agreement	
			Number of Positive Lumipulse G pTau 217/ β -Amyloid 1-42 Plasma Ratio results at > 0.00738	%Ratio Positive Result
Ratio 1	0.00342	90	0	0.0 (0/90)
Ratio 2	0.00520	90	0	0.0 (0/90)
Ratio 3	0.00469	90	0	0.0 (0/90)
Ratio 4	0.02234	90	90	100.0 (90/90)
Ratio 5	0.01939	90	90	100.0 (90/90)

The % correct call was also calculated for each panel member based on the number of negative Lumipulse **G** pTau 217/ β -Amyloid 1-42 Plasma Ratio results and summarized in the table below.

Panel member	Mean Ratio	Total Replicates (N)	Qualitative agreement	
			Number of Negative Lumipulse G pTau 217/ β -Amyloid 1-42 Plasma Ratio results at \leq 0.00370	%Ratio Negative Result
Ratio 1	0.00342	90	59	65.6 (59/90)*
Ratio 2	0.00520	90	0	0.0 (0/90)
Ratio 3	0.00469	90	3	3.3 (3/90)
Ratio 4	0.02234	90	0	0.0 (0/90)
Ratio 5	0.01939	90	0	0.0 (0/90)

*. The qualitative agreement was calculated based on shift from the “negative” to “indeterminate” category. None of the ratio results shifted from “negative” into “positive” test result.

4) Lumipulse **G** pTau217/ β -Amyloid 1-42 Plasma Ratio Precision Simulation

The within-laboratory precision and site-to site reproducibility of Lumipulse **G** β pTau217/ β -Amyloid 1-42 Plasma Ratio was further evaluated by simulating *i*) %CV for the ratio of two variables when %CVs are evaluated for each variable, Lumipulse **G** pTau 217 Plasma and Lumipulse **G** β -Amyloid 1-42-N Plasma, separately, and *ii*) percent positive calls at seven different ratio levels. For a ratio assay as U/V, the precision performance can be different at the same ratio value when the combinations for Lumipulse **G** pTau 217 Plasma (U variable) and Lumipulse **G** β -Amyloid 1-42-N Plasma (V variable) results are different. Because the assay random measurement error changes with the analyte concentration (%CV), different combinations for Lumipulse **G** pTau 217 Plasma and Lumipulse **G** β -Amyloid 1-42-N Plasma results correspond to different random measurement errors. To evaluate the precision characteristics of Lumipulse **G** pTau217/ β -Amyloid 1-42 Plasma Ratio numerical values under different combinations of pTau 217 and

β -Amyloid 1-42, the variance profiles of Lumipulse **G** pTau 217 Plasma and Lumipulse **G** β -Amyloid 1-42-N Plasma were considered. The simulated probability of a positive call was estimated for the mean ratio of Panel member 1–5 tested in within-laboratory precision and site-to-site reproducibility above. Random measurement error was considered normally distributed, and a generator of random normally distributed numbers was used. The %CV of the Lumipulse **G** β pTau217/ β -Amyloid 1-42 Plasma Ratio was calculated for different combinations of pTau 217 and β -Amyloid 1-42 and the results met acceptable precision criteria.

2. Linearity:

The linearity of Lumipulse **G** pTau 217 Plasma and Lumipulse **G** β -Amyloid 1-42-N Plasma was evaluated in accordance with the CLSI guideline EP06, 2nd edition. For each analyte, a high sample pool was diluted with a low sample pool to create a series of 14 dilutions for pTau217 or a series of 15 dilutions for β -Amyloid 1-42 that span across the measuring ranges of the respective assays. Each sample dilution was measured in one run with six replicates using one reagent lot of each assay and the mean of the replicates was calculated for each sample. A weighted regression analysis was performed to determine the predicted values. Percent deviation from linearity (DL) between the observed values and the best linear fit (predicted values) was calculated. The % DL was within $\pm 10\%$ for each dilution level. The best linear regression fits are summarized in the table below:

Lumipulse G pTau217 Plasma	$y = 0.985x - 0.001; R^2 = 0.9993$
Lumipulse G β -Amyloid 1-42-N Plasma	$y = 1.01x + 0.02; R^2 = 0.9997$

The Lumipulse **G** pTau 217 Plasma is linear across the range from 0.037 pg/mL to 11.326 pg/mL. The claimed analytical measuring interval is from 0.047 pg/mL to 10.000 pg/mL.

The Lumipulse **G** β -Amyloid 1-42-N Plasma is linear across the range from 0.8 pg/mL to 1012.0 pg/mL. The claimed analytical measuring interval is from 0.8 pg/mL to 500.0 pg/mL.

High-Dose Hook Effect:

High-dose hook effect was evaluated for the Lumipulse **G** pTau 217 Plasma and the Lumipulse **G** β -Amyloid 1-42-N Plasma on the LUMIPULSE G1200 analyzer. The expected and measured concentrations were compared for the K₂-EDTA plasma samples spiked with a high concentration stock of pTau 217 (~600 pg/mL) or β -Amyloid 1-42 (2435 pg/mL) and further serially diluted. All dilutions were tested with the undiluted sample in replicates of four in one single run for pTau 217 or in triplicates in one single run for β -Amyloid 1-42. No high-dose hook effect was observed up to approximately 610 pg/mL for the Lumipulse **G** pTau 217 Plasma and up to approximately 2200 pg/mL for the Lumipulse **G** β -Amyloid 1-42-N Plasma.

3. Analytical Specificity/Interference:

1) *Interference:*

The effect of potential endogenous and exogenous substances to the Lumipulse **G** pTau 217/ β -Amyloid 1-42 Plasma Ratio and its component assays, Lumipulse **G** pTau 217 Plasma and Lumipulse **G** β -Amyloid 1-42-N Plasma, was evaluated in accordance with the CLSI guidelines EP07, 3rd ed. and EP37. Three human plasma samples with various concentrations of pTau 217 (0.079 pg/mL–5.145 pg/mL) and β -amyloid 1-42 (20.5 pg/mL–498.6 pg/mL) were prepared for targeting the pTau217/ β -Amyloid 1-42 Plasma Ratio between 0.00317–0.1039, a range that spans the two ratio cutoffs of the Lumipulse **G** pTau217/ β -Amyloid 1-42 Plasma Ratio. Test samples were created by spiking with the potential endogenous and exogenous interfering substances. Control samples were spiked only with the appropriate solvent used to create the interfering substances panel. The pTau 217 and β -amyloid 1-42 in the test samples and control samples were measured in three replicates. The recovery was calculated by comparing measurements of the test and control samples. No significant interference was observed ($\leq \pm 10\%$ difference of test from control) for the Lumipulse **G** pTau 217 Plasma, the Lumipulse **G** β -Amyloid 1-42-N Plasma, and the Lumipulse **G** pTau217/ β -Amyloid 1-42 Plasma Ratio up to the concentrations of the potentially interfering substances tested as shown in the table below:

Endogenous Interferents	Test Concentration
Biotin	3500 ng/mL
Conjugated Bilirubin	40 mg/dL
Free Bilirubin (Unconjugated)	40 mg/dL
Hemoglobin	400 mg/dL
Human Anti-mouse Antibodies (HAMA IgG)	1000 ng/mL
Human Serum Albumin	14 g/dL
Immunoglobulin A (IgA)	500 mg/dL
Immunoglobulin G (IgG)	2000 mg/dL
Immunoglobulin M (IgM)	300 mg/dL
Rheumatoid Factor (RF IgM)	200 IU/mL
Triglycerides	1350 mg/dL

Exogenous Interferents	Test Concentration
Acetaminophen	1324 μ mol/L
Acetazolamide	5.7 mg/dL
Acetylcysteine	15.0 mg/dL
Acetylsalicylic Acid	3.62 mmol/L
Aducanumab (Aduhelm)	2 mg/mL
Alendronate Sodium	0.04 mg/mL
Ampicillin	215 μ mol/L
Aripiprazole	45 mg/dL
Ascorbic Acid	342 μ mol/L
Aspirin	0.39 mg/mL
Atorvastatin	0.075 mg/dL
Bisacodyl	0.009 mg/mL
Caffeine	556 μ mol/L
Calcium Dobesilate	6.0 mg/dL
Cefoxitin	660 mg/dL
Cetirizine HCL	0.435 mg/dL

Exogenous Interferents	Test Concentration
Chloramphenicol	139.2 µmol/L
Chlorothiazide	2.7 mg/dL
Clopidogrel	0.045 mg/mL
Cyclosporine	0.18 mg/dL
Desipramine HCL	0.204 mg/dL
Diazepam	3.00 mg/dL
Digoxin	49.9 nmol/L
Donanemab (Kisunla)	0.84 mg/mL
Donepezil (Aricept)	30 mg/dL
Doxycycline	1.8 mg/dL
Escitalopram	0.0192 mg/dL
Esomeprazole	0.69 mg/dL
Estradiol	0.00000075 mg/dL
Famotidine	0.244 mg/dL
Furosemide	1.59 mg/dL
Gabapentin	2.67 mg/dL
Galantamine (Reminyl)	250 mg/dL
Heparin	330 U/dL
Hydralazine HCL	1.44 mg/dL
Hydrochlorothiazide	3.79 µmol/L
Ibuprofen	21.9 mg/dL
Insulin	115 mU/L
Lactitol	12 mg/mL
Lecanemab (Leqembi)	0.475 mg/mL
Levodopa	0.5 mg/dL
Levothyroxine	0.0429 mg/dL
Lisinopril	0.0246 mg/dL
Loperamide HCL	0.0090 mg/mL
Losartan	0.06 mg/mL
Memantine (Namenda)	250 mg/dL
Metformin	1.2 mg/dL
Methyldopa	2.25 mg/dL
Metoprolol	18.7 µmol/L
Minocycline	0.1 mg/mL
Mirtazapine	0.025 mg/mL
Nifedipine	5.4 mg/dL
Phenylbutazone	32.1 mg/dL
Prednisone	0.00950 mg/dL
Quetiapine (Seroquel)	800 mg/L
Rifampicin	4.8 mg/dL
Rivastigmine (Exelon)	45 mg/dL
Sacubitril Calcium Salt	0.915 mg/dL
Simvastatin	0.168 mg/dL
Spironolactone	0.0555 mg/dL
Sulfasalazine	7.5 mg/dL
Theophylline	333 µmol/L

Exogenous Interferents	Test Concentration
Tramadol HCL	0.314 mg/dL
Valsartan	1.17 mg/dL
Venlafaxine HCL	0.135 mg/mL
Warfarin	243 μ mol/L
Zaleplon	0.01 mg/mL

The Lumipulse **G** pTau 217/ β -Amyloid 1-42 Plasma Ratio Package Insert under ‘Limitations to the Procedure’ states that “*The addition of endogenous substances shown in the Performance Characteristics section does not affect the measured values. Other interfering substances may affect measurement results*”.

2) *Cross-reactivity:*

The performance of the Lumipulse **G** pTau 217/ β -Amyloid 1-42 Plasma Ratio in the presence of potential cross-reactive pTau and β -amyloid species is based on the effect of these potential cross-reactants on the performance of the two component assays, the Lumipulse **G** pTau 217 Plasma and the Lumipulse **G** β -Amyloid 1-42-N Plasma. The study was conducted by preparing four human plasma samples with-varying pTau 217 concentration (0.142–7.518 pg/mL) and two human plasma samples with varying β -amyloid 1-42 concentration (29.7–43.8 pg/mL). Test samples were created by spiking with the potential cross-reactive pTau and β -amyloid species at the concentration listed in the tables below. Control samples were spiked only with the appropriate solvent used to create the cross-reactive pTau and β -amyloid species. The pTau 217 and β -amyloid 1-42 in the test samples and control samples were measured in three to four replicates. The % cross-reactivity of each analyte was calculated by comparing measurements of the test and control samples using the equation below.

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

The highest observed % cross-reactivity values for each cross-reactive pTau and β -amyloid species are summarized for each analyte:

a. Lumipulse **G** pTau 217 Plasma

Cross-Reactant	Test Concentration (pg/mL)	Highest Observed Cross-Reactivity (%)
pTau 181	100	0.050
pTau 205	100	0.090
pTau 212	100	0.020
pTau 231	100	0.040
Total Tau	100	0.040

The four human plasma samples were tested in three replicates. The peptides analyzed are the ones described in literature as being the clinically most relevant ones related to Alzheimer’s disease.

b. Lumipulse **G** β -Amyloid 1-42-N Plasma

Cross-Reactant	Test Concentration (pg/mL)	Highest Observed Cross-Reactivity (%)
β -Amyloid (3p $\epsilon^{\#}$) ₃₋₄₂	10000	0.007*
β -Amyloid ₁₋₃₇	1000	0.100**
β -Amyloid ₁₇₋₄₂	1000	0.000**
β -Amyloid ₁₋₃₈	1000	0.000**
β -Amyloid ₁₋₄₀	1000	0.000**
β -Amyloid ₁₋₄₃	1000	0.200**
[#] 3p ϵ indicates the N-terminus of the amyloid beta protein has been modified with pyroglutamate The two human plasma samples were tested in three replicates* or four replicates**		

The Lumipulse **G** pTau 217 Plasma and Lumipulse **G** β -Amyloid 1-42-N Plasma assay showed no significant cross-reactivity (within $\pm 10\%$ difference of test from Control) up to 100 pg/mL for the pTau cross-reactants and up to 1000 pg/mL for the β -amyloid 1-42 cross-reactants (with the exception of β -Amyloid (3p ϵ)₃₋₄₂ which was tested at 10000 pg/mL).

To assess the specificity of the anti-pTau217 capture monoclonal antibody towards all other possible epitopes and threonine phosphorylation sites across the entire human protein, peptide sequences from regions of human Tau441 around all threonine phosphorylation sites was analyzed and compared to the epitope region of the anti-pTau217 mAb. For each potential phosphorylated threonine, a peptide with 5 amino acids before and after the potentially phosphorylated T were selected. Both the amino acids in the pTau peptides and their position towards the pTau217 peptide were analyzed. Results showed that of the 35 pTau peptides analyzed, only the peptide covering the phosphorylated threonine at amino acid 217 matches the sequence corresponding with the epitope of the capture antibody.

4. Assay Reportable Range:

The Lumipulse **G** pTau217/ β -Amyloid 1-42 Plasma Ratio combines the results of the Lumipulse **G** pTau 217 Plasma and the Lumipulse **G** β -Amyloid 1-42-N Plasma from the same patient plasma specimen into a numerical value. The established measuring intervals for each individual analyte support the calculation of the Lumipulse **G** pTau 217/ β -Amyloid 1-42 Plasma Ratio test up to 1.00000.

Lumipulse G pTau217 Plasma	
Analytical Measuring Interval (AMI) ^a	0.047 pg/mL to 10.000 pg/mL
Reportable Range ^b	0.041 pg/mL to 10.000 pg/mL

Lumipulse G β-Amyloid 1-42-N Plasma	
Analytical Measuring Interval (AMI) ^a	0.8 pg/mL to 500.0 pg/mL
Reportable Range ^b	0.2 pg/mL to 500.0 pg/mL
^a The range of values in pg/mL which meets the limits of acceptable performance for linearity, imprecision, and bias. The AMI extends from the lower limit of the linear range to the upper limit of the assay parameter ^b The lower limit of detection to the upper limit of the assay parameter	

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

1) Traceability

There is no international reference material or method for β -Amyloid 1-42 in plasma or serum. The calibrators for use with Lumipulse G pTau 217 Plasma were prepared gravimetrically under internal quality system and are traceable to in-house reference calibrators (i.e. Master Golden Standard of Lumipulse G pTau 217 Plasma). No certified reference material is currently available. The calibrators for use with the Lumipulse G β -Amyloid 1-42-N Plasma were prepared gravimetrically and are traceable to Lumipulse G β -Amyloid 1-42 CSF assay (DEN200027).

2) Stability

Stability for the Lumipulse G pTau217/ β -Amyloid 1-42 Plasma Ratio is based on reagent stability for the individual component assays and the ratio. The Lumipulse G pTau217/ β -Amyloid 1-42 Plasma Ratio only uses the assay reagents which are stored under the claimed storage condition and within the claimed expiration date for each assay. The stability of Lumipulse G pTau 217 Plasma and the Lumipulse G β -Amyloid 1-42-N Plasma were evaluated based on the following studies:

a. Reagent shelf-life:

To evaluate reagent shelf-life stability, three lots of the Lumipulse G pTau 217 Plasma and three lots of the Lumipulse G β -Amyloid 1-42-N Plasma reagents were stored at 2–8°C and 2–10°C, respectively. Five human plasma samples with varying pTau 217 concentrations (range 0.144 pg/mL–7.495pg/mL) and three human plasma samples with varying β -amyloid 1-42 (range 42.5 pg/mL–762.2 pg/mL) concentrations were tested in six replicates at 0 (baseline), 2, 4 and 7 months of shelf-life. The test results obtained at the different time points were compared to the test results at baseline (0 month). Results showed that the Lumipulse G pTau 217 Plasma are stable at 2–8°C for up to 9 months and the Lumipulse G β -Amyloid 1-42-N Plasma reagents are stable at 2–10°C for up to 9 months.

b. Reagent on-board stability:

To evaluate reagent on-board stability, one lot of the Lumipulse G pTau 217 Plasma and one lot of the Lumipulse G β -Amyloid 1-42-N Plasma reagents were stored on-board the

LUMIPULSE G1200 System. Five human plasma samples with varying pTau 217 concentrations (range: 0.122 pg/mL–7.719 pg/mL) and three human plasma samples with varying β -amyloid 1-42 concentrations (range: 44.2 pg/mL– 846.9 pg/mL) were tested at Day 1, 15, and 35 after study initiation (for pTau 217) or at Day 0, 15, 31 and 38 (for β -amyloid 1-42). The test results obtained at the different time points were compared to the test results at Day 1. Results showed that the Lumipulse **G** pTau 217 Plasma and the Lumipulse **G** β -Amyloid 1-42-N Plasma reagents are stable on-board the LUMIPULSE G1200 System for up to 30 days.

c. Sample stability:

- i. Standing time from collection to centrifugation: Five K₂-EDTA plasma samples with varying pTau 217 and β -amyloid 1-42 concentrations (i.e., 0.074 pg/mL– 0.821 pg/mL and 24.7 pg/mL–35.6 pg/mL, respectively) were freshly collected to challenge various pTau217/ β -Amyloid 1-42 Plasma Ratio that ranged from 0.00299 – 0.03055. The pTau 217 and β -Amyloid 1-42 concentrations were measured in samples that were stored at ambient temperature for different duration from collection to centrifugation. The time to centrifugation ranges from (i) 30 minutes \pm 2 minutes, (ii) 1 hour \pm 5 minutes, and (iii) 2 hours \pm 5 minutes from collection. After centrifuging, aliquots from the plasma samples were tested on the LUMIPULSE G1200 in triplicate (n=3) for both Lumipulse **G** pTau217 and Lumipulse **G** β -Amyloid 1-42 plasma.
- ii. Sample storage stability: K₂-EDTA plasma samples from the same donors were freshly collected in separate tubes and centrifuged 30 minutes \pm 2 minutes from collection. After centrifuging, aliquots of the plasma samples were tested (Day 0) or stored at four different temperatures: (i) -60°C, (ii) -20 \pm 10°C, (iii) 5°C \pm 3°C, and (iv) 25 \pm 2°C for various storage durations and tested in singlicate on the day the samples were removed from storage. Aliquots from same samples were also subjected to up to four freeze/thaw cycles and tested in singlicate on the day the samples were thawed.
- iii. Sample aboard stability: Two K₂-EDTA plasma pools with varying pTau 217 and β -amyloid 1-42 concentrations were prepared to achieve a pTau217/ β -Amyloid 1-42 Plasma Ratio of 0.0067 (low) and 0.1000 (high). The pTau 217 and β -amyloid 1-42 concentrations were measured in triplicate immediately (Time 0) or in singlicate after one, three and five hours had elapsed from Time 0.

The sample stability for the Lumipulse **G** pTau217/ β -Amyloid 1-42 Plasma Ratio were summarized in the table below.

Plasma Sample Stability						
Time to Centrifugation	Storage Temperature					Freeze/Thaw Cycle
	-60°C	-30 – -10°C	2–8°C	23–28 °C	On-Board	
2 hours \pm 5 minutes from collection	1 month	1 month	1 day	Not recommended	3 hours	Up to 3

The Lumipulse **G** pTau 217/ β -Amyloid 1-42 Plasma Ratio Package Insert under ‘Specimen Collection and Preparation’ states ‘*Specimens used in the Lumipulse **G** pTau 217/ β -Amyloid 1-42 Plasma Ratio degrade rapidly at room temperature. Avoid storage and shipment at room temperature (23-28°C)*’.

6. Detection Limit:

The detection capability for the Lumipulse **G** pTau217/ β -Amyloid 1-42 Plasma Ratio is based on the detection capability for its component assays, the Lumipulse **G** pTau 217 Plasma and Lumipulse **G** β -Amyloid 1-42-N Plasma.

The detection capabilities for the Lumipulse **G** pTau 217 Plasma and Lumipulse **G** β -Amyloid 1-42-N Plasma assays were evaluated by Limit of Blank (LoB), Limit of Detection (LoD), and Limit of Quantitation (LoQ) studies in accordance with the CLSI guideline EP17-A2. The studies evaluated three lots of Lumipulse **G** pTau 217 Plasma and three lots of Lumipulse **G** β -Amyloid 1-42-N Plasma on three LUMIPULSE G1200 Systems. The calculations were carried out separately for each lot. The LoB, LoD and LoQ were set to the highest values provided by the different lots.

1) Lumipulse **G** pTau 217 Plasma:

For LoB, a set of four analyte-depleted plasma samples was tested in 10 replicates per run, two runs per day for three days to reach a total of 60 measurements per sample for each reagent lot. A non-parametric approach was used to calculate the LoB for each reagent lot. The LoB was determined for each lot as 0.018 pg/mL, 0.020 pg/mL, and 0.025 pg/mL. The claimed LoB is the higher value from the three lots as 0.025 pg/mL.

For LoD, a set of five low level plasma samples with target concentrations of pTau 217 between 0.19–0.64 pg/mL was tested in 10 replicates per run, two runs per day for three days to reach a total of 60 measurements per sample for each reagent lot. The LoD was determined for each lot as 0.027 pg/mL, 0.041 pg/mL, and 0.038 pg/mL. The claimed LoD is the higher value from the three lots as 0.041 pg/mL.

The LoQ was estimated based on the lowest value of the test samples at which the variability was below the 20 %CV. The LoQ was determined for each lot as 0.027 pg/mL, 0.047 pg/mL, and 0.041 pg/mL. The claimed LoQ was 0.047 pg/mL at the lower limit of the analytical measuring interval of Lumipulse **G** pTau 217 Plasma.

2) Lumipulse **G** β -Amyloid 1-42-N Plasma:

For LoB, a set of four analyte-depleted plasma samples was tested in 10 replicates per run, two runs per day for three days to reach a total of 60 measurements per sample for each reagent lot. A non-parametric approach was used to calculate the LoB for each reagent lot. The LoB was determined for each lot as 0.056 pg/mL, 0.122 pg/mL, and 0.128 pg/mL. The claimed LoB is the higher value from the three lots as 0.128 pg/mL.

For LoD, a set of five low level plasma samples with target concentrations of β -Amyloid 1-42 between 0.2– 0.6 pg/mL was tested in 10 replicates per run, two runs per day for three days to reach a total of 60 measurements per sample for each reagent lot. The LoD was determined for each lot as 0.0917 pg/mL, 0.199 pg/mL, and 0.207 pg/mL. The claimed LoD is the higher value from the three lots as 0.2 pg/mL.

The LoQ was determined for each lot as 0.257 pg/mL, 0.288 pg/mL, and 0.449 pg/mL. The claimed LoQ (precision was %CV<20%) at the lower limit of the analytical measuring interval of Lumipulse **G** β -Amyloid 1-42-N Plasma is 0.8 pg/mL.

7. Assay Cut-Off:

The Lumipulse **G** pTau217/ β -Amyloid 1-42 Plasma Ratio assay cutoff was determined by analyzing a total of 208 banked K₂EDTA plasma samples from the Eisai MissionAD, BioFINDER 2, BIOCARD cohorts. Of the 208 evaluable samples, 73 (35.1%) were PET positive and 66 (31.7%) were CSF positive.

		Amyloid PET Scan or CSF Ratio Test		Predictive Value % (n/N) (95% CI)*	Frequency of Results % (n/N)	Likelihood Ratio (95% CI**)
		Positive	Total			
Lumipulse G pTau217/β-Amyloid 1-42 Plasma Ratio	Positive	88	93	94.6% (88/93) (88.7%, 97.6%)	44.7% (93/208)	14.51 (6.49, 33.90)
	Indeterminate	20	42	47.6% (20/42) (34.7%, 60.9%)	20.2% (42/208)	0.75 (0.44, 1.28)
	Negative	6	73	8.2% (6/73) (3.9%, 16.0%)	35.1% (73/208)	0.07 (0.03, 0.15)
	Total	114	208	<i>Prevalence of PET positive or CSF Test positive = 54.8%</i>		

*95% CIs are calculated based on 95% CIs of corresponding likelihood ratios
 **95% CIs are calculated using an asymptotic method for ratios of two independent binomial proportions

Lumipulse G pTau217/β-Amyloid 1-42 Plasma Ratio	Test Result	Interpretation
Ratio \geq 0.00738	Positive	A positive result is consistent with patients who are likely to have amyloid pathology. This result does not establish a diagnosis of AD or other cognitive disorders.
0.00371 < Ratio < 0.00737	Indeterminate	An indeterminate result is consistent with patients who are uncertain to have amyloid pathology. These patients should be considered for further testing.

Lumipulse G pTau217/ β-Amyloid 1-42 Plasma Ratio	Test Result	Interpretation
Ratio ≤ 0.00370	Negative	A negative result is consistent with patients who are unlikely to have amyloid pathology. These patients should be investigated for other causes of cognitive decline.

B Comparison Studies:

1. Method Comparison with Predicate Device:

Not applicable

2. Matrix Comparison:

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

C Clinical Studies:

1. Clinical Performance:

A clinical study was conducted to evaluate the performance of the Lumipulse G pTau217/β-Amyloid 1-42 Plasma Ratio as an aid in the assessment of whether a patient presenting with cognitive impairment and being evaluated for AD and other causes of cognitive decline would test positive or negative for amyloid plaques at the time of testing, as measured by amyloid PET using an FDA-approved tracer or with an FDA authorized amyloid CSF Ratio test. The study included 499 subjects between the ages of 52 and 93 years of which 290 (58.1%) were diagnosed with AD, 154 (30.9%) with mild cognitive impairment (MCI) and 49 (9.8%) with subjective cognitive decline (SCD). The pre-clinical AD diagnostic groups (SCD and MCI) represent 40.7% (203/499) of the study population. The numbers of plasma samples obtained from the different study cohorts (sources) are listed in the table below along with the methods used for determining the subject's amyloid status.

Sources	Amyloid Status determined by	Number of samples
<i>Polaris-AD (AriBio):</i> • Plasma samples collected from the screening period of the clinical phase III study of AR1001 (AR1001-ADP3-US01)	CSF Ratio Test	265
<i>Skåne University Hospital Memory Clinic, Malmö, Sweden:</i> • Plasma samples collected from subjects enrolled in BioFINDER 2	CSF Ratio Test	43
<i>University of Wisconsin:</i> Plasma samples from subjects enrolled in Wisconsin Registry for Alzheimer's Prevention (WRAP)	CSF Ratio Test	39

Sources	Amyloid Status determined by	Number of samples
<i>Global Alzheimer's Platform Foundation:</i> • Plasma samples obtained from subjects enrolled in BioHermes-001 “A Biomarker Database to Investigate Blood-Based and Digital Biomarkers in Participants Screened for Alzheimer's Disease”	Amyloid PET Scan	152
Total		499

The 499 subjects were categorized into four diagnostic groups: 58.1% (290/499) AD, 30.9% (154/499) MCI, 9.8% (49/499) SCD, and 1.2% (6/499) other cognitive diagnoses. The pre-clinical AD diagnostic groups (SCD and MCI) represent 40.7% (203/499) of the study population. 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 the table below.

		Diagnostic Groups*			Amyloid PET Scan		CSF Ratio Test		Amyloid Status		Total
		AD	MCI	SCD	Positive	Negative	Positive	Negative	Positive	Negative	
Total N		290 (58.1%)	154 (30.9%)	49 (9.8%)	66 (13.2%)	86 (17.2%)	189 (37.9%)	158 (31.7%)	255 (51.1%)	244 (48.9%)	499
Sex	Male	135 (46.6%)	81 (52.6%)	21 (42.9%)	22 (33.3%)	27 (31.4%)	102 (54.0%)	89 (56.3%)	124 (48.6%)	116 (47.5%)	240 (48.1%)
	Female	155 (53.4%)	73 (47.4%)	28 (57.1%)	44 (66.7%)	59 (68.6%)	87 (46.0%)	69 (43.7%)	131 (51.4%)	128 (52.5%)	259 (51.9%)
Age	52 – 65	56 (19.3%)	34 (22.1%)	12 (24.5%)	6 (9.1%)	27 (31.4%)	24 (12.7%)	47 (29.7%)	30 (11.8%)	74 (30.3%)	104 (20.8%)
	66 – 70	48 (16.6%)	39 (25.3%)	12 (24.5%)	7 (10.6%)	17 (19.8%)	36 (19.0%)	39 (24.7%)	43 (16.9%)	56 (23.0%)	99 (19.8%)
	71 – 75	83 (28.6%)	29 (18.8%)	8 (16.3%)	17 (25.8%)	17 (19.8%)	54 (28.6%)	32 (20.3%)	71 (27.8%)	49 (20.1%)	120 (24.0%)
	76 – 80	74 (25.5%)	34 (22.1%)	14 (28.6%)	23 (34.8%)	12 (14.0%)	54 (28.6%)	35 (22.2%)	77 (30.2%)	47 (19.3%)	124 (24.8%)
	81– 93	29 (10.0%)	18 (11.7%)	3 (6.1%)	13 (19.7%)	13 (15.1%)	21 (11.1%)	5 (3.2%)	34 (13.3%)	18 (7.4%)	52 (10.4%)
	Mean	72	72	71	75	71	73	70	74	70	72
	Median	73	71	72	76	70	74	69	74	69	72
	Range	55–89	56–93	52–88	64–84	60–85	57–93	52–88	57–93	52–88	52–93
Race	White	243 (83.8%)	131 (85.1%)	43 (87.8%)	49 (74.2%)	57 (66.3%)	177 (93.7%)	140 (88.6%)	226 (88.6%)	197 (80.7%)	423 (84.8%)
	Asian	7 (2.4%)	3 (1.9%)	0 (0.0%)	3 (4.5%)	4 (4.7%)	1 (0.5%)	2 (1.3%)	4 (1.6%)	6 (2.5%)	10 (2.0%)
	African American	37 (12.8%)	18 (11.7%)	5 (10.2%)	14 (21.2%)	21 (24.4%)	10 (5.3%)	15 (9.5%)	24 (9.4%)	36 (14.8%)	60 (12.0%)
	Others**	3 (1.0%)	2 (1.3%)	1 (2.0%)	0 (0.0%)	4 (4.7%)	1 (0.5%)	1 (0.6%)	1 (0.4%)	5 (2.0%)	6 (1.2%)

		Diagnostic Groups*			Amyloid PET Scan		CSF Ratio Test		Amyloid Status		Total
		AD	MCI	SCD	Positive	Negative	Positive	Negative	Positive	Negative	
MMSE	<18	1 (0.3%)	0 (0.0%)	0 (0.0%)	0 (0.0%)	0 (0.0%)	2 (1.1%)	0 (0.0%)	2 (0.8%)	0 (0.0%)	2 (0.4%)
	18-23	118 (40.7%)	2 (1.3%)	0 (0.0%)	21 (31.8%)	14 (16.3%)	50 (26.5%)	37 (23.4%)	71 (27.8%)	51 (20.9%)	122 (24.4%)
	24-30	166 (57.2%)	143 (92.9%)	45 (91.8%)	45 (68.2%)	72 (83.7%)	124 (65.6%)	116 (73.4%)	169 (66.3%)	188 (77.0%)	357 (71.5%)
	Unknown	5 (1.7%)	9 (5.8%)	4 (8.2%)	0 (0.0%)	0 (0.0%)	13 (6.9%)	5 (3.2%)	13 (5.1%)	5 (2.0%)	18 (3.6%)
CDR Global Score	N	220	64	11	0	0	155	140	155	140	295
	Mean	0.6	0.5	0.2	N/A	N/A	0.6	0.5	0.6	0.5	0.6
	Median	0.5	0.5	0	N/A	N/A	0.5	0.5	0.5	0.5	0.5
	Range	0.0-2.0	0.0-1.0	0.0-0.5	N/A	N/A	0.0-2.0	0.0-1.0	0.0-2.0	0.0-1.0	0.0-2.0
FAQ	N	65	87	34	66	86	28	12	94	98	192
	Mean	8.0	5	3	7	5	8	9	7	5	6.0
	Median	7	4	2	6	2	6	5	6	3	4
	Range	0-30	0-22	0-12	0-30	0-19	1-23	2-27	0-30	0-27	0-30
Apolipoprotein E (ApoE) Status	$\epsilon 2\epsilon 3$	3 (1.0%)	0 (0.0%)	2 (4.1%)	0 (0.0%)	0 (0.0%)	4 (2.1%)	3 (1.9%)	4 (1.6%)	3 (1.2%)	7 (1.4%)
	$\epsilon 2\epsilon 4$	6 (2.1%)	1 (0.6%)	0 (0.0%)	0 (0.0%)	0 (0.0%)	6 (3.2%)	1 (0.6%)	6 (2.4%)	1 (0.4%)	7 (1.4%)
	$\epsilon 3\epsilon 3$	40 (13.8%)	13 (8.4%)	17 (34.7%)	0 (0.0%)	0 (0.0%)	53 (28.0%)	19 (12.0%)	53 (20.8%)	19 (7.8%)	72 (14.4%)
	$\epsilon 3\epsilon 4$	69 (23.8%)	14 (9.1%)	11 (22.4%)	0 (0.0%)	0 (0.0%)	89 (47.1%)	7 (4.4%)	89 (34.9%)	7 (2.9%)	96 (19.2%)
	$\epsilon 4\epsilon 4$	19 (6.6%)	6 (3.9%)	2 (4.1%)	0 (0.0%)	0 (0.0%)	25 (13.2%)	2 (1.3%)	25 (9.8%)	2 (0.8%)	27 (5.4%)
	Unknown	53 (52.8%)	120 (77.9%)	17 (34.7%)	66 (100.0%)	86 (100.0%)	12 (6.3%)	126 (79.7%)	78 (30.6%)	212 (86.9%)	290 (58.1%)
Years of Education	≤ 13	28 (9.7%)	32 (20.8%)	15 (30.6%)	24 (36.4%)	23 (26.7%)	21 (11.1%)	10 (6.3%)	45 (17.6%)	33 (13.5%)	78 (15.6%)
	> 13	40 (13.8%)	76 (49.4%)	34 (69.4%)	42 (63.6%)	63 (73.3%)	25 (13.2%)	23 (14.6%)	67 (26.3%)	86 (35.2%)	153 (30.7%)
	Unknown	222 (76.6%)	46 (29.9%)	0 (0.0%)	0 (0.0%)	0 (0.0%)	143 (75.7%)	125 (79.1%)	143 (56.1%)	125 (51.2%)	268 (53.7%)
Diagnostic Group*	AD				27 (40.9%)	33 (38.4%)	143 (75.7%)	87 (55.1%)	170 (66.7%)	120 (49.2%)	290 (58.1%)
	MCI				36 (54.5%)	40 (46.5%)	27 (14.3%)	51 (32.3%)	63 (24.7%)	91 (37.3%)	154 (30.9%)
	SCD				3 (4.5%)	13 (15.1%)	16 (8.5%)	17 (10.8%)	19 (7.5%)	30 (12.3%)	49 (9.8%)

*Table does not include "Other" Diagnostic Group because there were only 6 subjects in this category

**Includes American Indian/Alaskan and Unknown Races

MMSE: Mini Mental State Examination, FAQ: Functional Activities Questionnaire.

The study population consisted of 48.1% (240/499) males with a mean age of 72 years (range 55-93 years with a median age of 73 years), and 51.9% females (259/499) with a mean age of 72 years (range 52-86 years with a median 72 years). The study subjects were 84.8% Whites (423/499). Blacks represented 12.0% (60/499) and Asians and other races comprised 3.2% (16/499).

The amyloid PET scans from the subjects were blindly read by two trained independent readers, with a third independent reader used for adjudication where necessary. An independent PET adjudication was performed utilizing the services of a third party. The subject's adjudicated amyloid PET visual read or result from a CSF Ratio test (Lumipulse $G\beta$ -Amyloid Ratio (1-42/1-40); refer to DEN200072) was used to determine the amyloid status. There were no subjects with both an adjudicated visual PET read and Lumipulse CSF Ratio test result. A breakdown of the PET scan and CSF Ratio test results by clinical status is provided in the table below.

	Diagnostic Groups				Total
	AD	MCI	SCD	Other	
Amyloid PET Scan	60 (20.7%)	76 (49.4%)	16 (32.7%)	0 (0.0%)	152 (30.5%)
CSF Ratio Test	230 (79.3%)	78 (50.6%)	33 (67.3%)	6 (100%)	347 (69.5%)
Total	290	154	49	6	499

Of the 152 subjects with an adjudicated PET visual read, 66 (43.4%) had a positive result while of the 347 subjects with a CSF Ratio test result, 189 (54.5%) had a positive result. In the 189 subjects with a positive CSF Ratio test result, there were three subjects in the "Other" Diagnostic group and in the 158 subjects with a negative CSF Ratio test result, there were three subjects in the "Other" diagnostic group. The percentages of positive PET scan and CSF Ratio test results were 13.2% (66/499) and 37.9% (189/499), respectively, in the study population. Overall, including the six subjects in the "Other" category, 51.1% (255/499) of the evaluable subjects had a positive amyloid status.

Patients with AD presented with a positive amyloid PET scan or CSF Ratio test result more frequently than MCI patients, who in turn presented with a positive PET scan or CSF Ratio test result more frequently than those with SCD. The prevalence of amyloid positivity among AD was 58.6% (170/290) compared to 40.9% (63/154) and 38.8% (19/49) among those with MCI and SCD, respectively.

Results:

1) Clinical Performance

To estimate the clinical performance measures of the Lumipulse $G\beta$ pTau217/ β -Amyloid 1-42 Plasma Ratio, the test result was compared to the adjudicated amyloid PET scan or CSF Ratio test result for each patient. The adjudicated amyloid PET scan positive or CSF Ratio test result positive is called "Amyloid positive". The data are summarized in the table below.

		Amyloid PET Scan [^] or CSF Ratio Test [#]		
		Positive	Negative	Total
Lumipulse G pTau217/β- Amyloid 1-42 Plasma Ratio	Positive (Ratio \geq 0.00738)	201	18	219
	Indeterminate (0.00371 \leq Ratio \leq 0.00737)	49	49	98
	Negative (Ratio \leq 0.00370)	5	177	182
	Total	255	244	499

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

The performance estimates of the Lumipulse G pTau217/ β -Amyloid 1-42 Plasma Ratio is described by PET-positive predictive value (indicated as Predictive Value, PV) and frequency of positive test results of the tested samples (indicated as Frequency of Results, FR) for positive, indeterminate, and negative test results and it is summarized in the table below.

		Amyloid PET Scan [^] or CSF Ratio Test [#]		Predictive Value % (n/N) (95% CI*)	Frequency of Results % (n/N)	Likelihood Ratio (95% CI**)
		Positive	Total			
Lumipulse G pTau217/ β-Amyloid 1-42 Plasma Ratio	Positive (Ratio \geq 0.00738)	201	219	91.8% (201/219) (87.8%, 94.6%)	43.9% (219/499)	10.68 (6.90, 16.79)
	Indeterminate (0.00371 \leq Ratio \leq \leq 0.00737)	49	98	50.0% (49/98) (41.3%, 58.8%)	19.6% (98/499)	0.96 (0.67, 1.36)
	Negative (Ratio \leq 0.00370)	5	182	2.7% (5/182) (1.2%, 6.1%)	36.5% (182/499)	0.03 (0.01, 0.06)
	Total	255	499	<i>Prevalence of amyloid positive= 51.1%</i>		

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

Of the 255 subjects with a positive amyloid PET scan or CSF Ratio result, 201 subjects had a positive Lumipulse **G** pTau217/ β -Amyloid 1-42 Plasma Ratio result. The PV for positive test results was 91.8% (201/219) and the FR was 43.9% (219/500). Of the 244 subjects with an amyloid negative PET scan or CSF Ratio test result, 177 subjects had a negative Lumipulse **G** pTau217/ β -Amyloid1-42 Plasma Ratio result. The PV for negative test results was 2.7% (5/182) and the FR was 36.5% (182/499). Of the 98 subjects with an indeterminate Lumipulse **G** pTau217/ β -Amyloid1-42 Plasma Ratio result, 49 had a positive PET scan or CSF Ratio test result. The PV for indeterminate test results was 50.0% (49/98) and the FR was 19.6% (98/499). The prevalence of PET scan and CSF Ratio test positivity was 51.1% (255/499) overall in the study population.

In conclusion, the data of clinical performance study support that positive Lumipulse **G** pTau217/ β -Amyloid 1-42 Plasma Ratio results are consistent with amyloid PET scan or CSF Ratio test positive results and Lumipulse **G** pTau217/ β -Amyloid 1-42 Plasma Ratio negative results are consistent with amyloid PET scan or CSF Ratio test negative results.

2) Sub-group Analysis

a. Amyloid PET Scan or CSF Ratio Test used for determining amyloid status.

The performance measures of the Lumipulse **G** pTau217/ β -Amyloid 1-42 Plasma Ratio stratified by the method used for determining amyloid status are summarized in the table below.

	Test Result	Amyloid Status		Predictive Value % (n/N) (95% CI*)	Frequency of Results % (n/N)	Likelihood Ratio (95% CI**)
		Positive	Total			
Amyloid PET Scan [^]	Positive (Ratio \geq 0.00738)	56	62	90.3% (56/62) (81.7%, 95.3%)	40.8% (62/152)	12.16 (5.83, 26.43)
	Indeterminate (0.00371 \leq Ratio \leq 0.00737)	9	29	31.0% (9/29) (18.0%, 47.4%)	19.1% (29/152)	0.59 (0.29, 1.17)
	Negative (Ratio \leq 0.00370)	1	61	1.6% (4/69) (0.3%, 8.2%)	40.1% (69/152)	0.022 (0.004, 0.117)
	Total	66	152	<i>Prevalence of PET positive = 43.4%</i>		
CSF Ratio Test [#]	Positive (Ratio \geq 0.00738)	145	157	92.4% (145/157) (87.7%, 95.5%)	45.2% (157/347)	10.10 (5.95, 17.55)
	Indeterminate (0.00371 \leq Ratio \leq 0.00737)	40	69	58.0% (40/69) (47.4%, 67.9%)	19.9% (69/347)	1.15 (0.75, 1.77)

	Test Result	Amyloid Status		Predictive Value % (n/N) (95% CI*)	Frequency of Results % (n/N)	Likelihood Ratio (95% CI**)
		Positive	Total [^]			
	Negative (Ratio ≤ 0.00370)	4	121	3.3% (4/121) (1.3%, 7.9%)	34.9% (121/347)	0.029 (0.011, 0.072)
	Total	189	347	<i>Prevalence of CSF positive = 54.5%</i>		

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

For both amyloid PET scan and CSF Ratio test, the predictive values (PVs) were highest for the positive test results with a decrease for the indeterminate test results and lowest in the negative test results with each amyloid status. The estimates of PV for the positive test results were higher for the CSF Ratio test (92.4%) compared to amyloid PET scan (90.3%). The estimate of PV for the indeterminate test results decreased to 31.0% and 58.0% for the amyloid PET scan and CSF Ratio test, respectively. The estimates of PV for the negative test results were 1.6% for the amyloid PET scan and 3.3 % for the CSF Ratio test. The Frequency of Results (FR) values for the indeterminate test results were 19.1% for the amyloid PET scan and 19.9% for the CSF Ratio test.

b. Diagnostic group

The performance measures of the Lumipulse **G** pTau217/β-Amyloid 1-42 Plasma Ratio stratified by diagnostic groups are summarized in the table below.

Diagnosis ^{&}	Test Result	Amyloid PET Scan [^] or CSF Ratio Test [#]		Predictive Value % (n/N) (95% CI*)	Frequency of Results % (n/N)	Likelihood Ratio (95% CI**)
		Positive (n)	N			
AD (N=290)	Positive (Ratio ≥ 0.00738)	136	147	92.5% (136/147) (87.8%, 95.6%)	50.7 % (147/290)	8.73 (5.07, 15.48)
	Indeterminate (0.00371 ≤ Ratio ≤ 0.00737)	30	57	52.6% (30/57) (41.2%, 63.9%)	19.7% (57/290)	0.78 (0.50, 1.25)
	Negative (Ratio ≤ 0.00370)	4	86	4.7% (4/86) (1.9%, 11.0%)	29.7% (86/290)	0.03 (0.01, 0.09)

Diagnosis ^{&}	Test Result	Amyloid PET Scan [^] or CSF Ratio Test [#]		Predictive Value % (n/N) (95% CI*)	Frequency of Results % (n/N)	Likelihood Ratio (95% CI**)
		Positive (n)	N			
MCI (N=154)	Positive (Ratio \geq 0.00738)	52	58	89.7% (52/58) (80.5%, 95.0%)	37.7% (58/154)	12.52 (5.98, 27.26)
	Indeterminate (0.00371 \leq Ratio \leq 0.00737)	10	24	41.7% (10/24) (25.7%, 59.6%)	15.6% (24/154)	1.03 (0.49, 2.13)
	Negative (Ratio \leq 0.00370)	1	72	1.4% (1/72) (0.2%, 7.0%)	46.8% (72/154)	0.02 (0.004, 0.109)
SCD (N=49)	Positive (Ratio \geq 0.00738)	11	12	91.7% (11/12) (67.3%, 98.5%)	24.5% (12/49)	17.37 (3.24, 102.00)
	Indeterminate (0.00371 \leq Ratio \leq 0.00737)	8	16	50.0% (8/16) (30.9%, 68.7%)	32.7% (16/49)	1.58 (0.71, 3.46)
	Negative (Ratio \leq 0.00370)	0	21	0.0% (0/21) (0.0%, 13.5%)	42.9% (21/49)	0.00 (0.00, 0.25)

[&]Diagnostic Group does not include "Other" because there were only 6 subjects in this category
[^]Amyloid PET scan result based on visual read
[#] CSF Ratio test result based on Lumipulse $G \beta$ -Amyloid Ratio (1-42/1-40) (DEN200072)
*95% CIs are calculated based on 95% CIs of corresponding likelihood ratios
**95% CIs are calculated using an asymptotic method for ratios of two independent binomial proportions

Across diagnostic groups, the PVs were highest for the positive test results with a decrease for the indeterminate test results and lowest in the negative test results within each diagnostic group. The estimate of PV was consistently high for AD (92.5%), SCD (91.7%), and MCI (89.7%) for the positive test results. The estimate of PV for the intermediate test results decreased to 41.7%, 50.0% and 52.6%, for MCI, SCD, and AD, respectively. The estimate of PV for the negative test results was 0.0% for SCD, 1.4% for AD, and 4.7% for MCI. The FR values for the indeterminate test results were 15.6% for MCI, 19.7% for AD, and 32.7% for SCD, comprising 19.7% across the diagnostic groups.

c. Sex

The performance measures of the Lumipulse $G \beta$ pTau217/ β -Amyloid 1-42 Plasma Ratio were also stratified by sex and summarized in the table below.

Sex	Test Result	Amyloid PET Scan [^] or CSF Ratio Test [#]		Predictive Value % (n/N) (95% CI*)	Frequency of Results % (n/N)	Likelihood Ratio (95% CI**)
		Positive (n)	N			
Male (N=240), Prevalence =51.7%	Positive (Ratio \geq 0.00738)	91	100	91.0% (91/100) (84.6%, 95.0%)	41.7% (100/240)	9.46 (5.15, 17.91)
	Indeterminate (0.00371 \leq Ratio \leq 0.00737)	32	57	56.1% (31/57) (44.9%, 66.9%)	23.8% (57/240)	1.20 (0.76, 1.89)
	Negative (Ratio \leq 0.00370)	1	83	1.2% (1/83) (0.2%, 6.3%)	34.6% (83/240)	0.011 (0.002, 0.063)
Female (N=259), Prevalence =50.6%	Positive (Ratio \geq 0.00738)	110	119	92.4% (110/119) (86.9%, 95.8%)	45.9% (110/259)	11.94 (6.51, 22.55)
	Indeterminate (0.00371 \leq Ratio \leq 0.00737)	17	41	41.5% (17/41) (28.6%, 55.4%)	15.8% (41/259)	0.69 (0.39, 1.22)
	Negative (Ratio \leq 0.00370)	4	99	4.0% (4/99) (1.6%, 9.5%)	38.2% (99/259)	0.041 (0.016, 0.103)
[^] Amyloid PET scan result based on visual read [#] CSF Ratio test result based on Lumipulse G β -Amyloid Ratio (1-42/1-40) (DEN200072) *95%CIs are calculated based on 95%CIs of corresponding likelihood ratios **95%CIs are calculated using an asymptotic method for ratios of two independent binomial proportions						

For both male and female groups, the PVs were highest for the positive test results with a decrease for the indeterminate test results and lowest in the negative test results. The estimates of PV for the positive test results were similar for males (91.0%) and females (92.4%). The estimate of PV for the indeterminate test results decreased to 56.1% and 41.5% for males and females, respectively. The estimates of PV for the negative test results were 1.2% for males and 4.0% for females. The FR values for the indeterminate test results were 23.8% for males and 15.8% for females.

d. Age

The performance measures of the Lumipulse **G** pTau217/ β -Amyloid 1-42 Plasma Ratio were also stratified by age and summarized in the table below.

Age (years)	Test Result	Amyloid PET Scan [^] or CSF Ratio Test [#]		Predictive Value % (n/N) (95% CI*)	Frequency of Results % (n/N)	Likelihood Ratio (95% CI**)
		Positive (n)	N			
52-65 (N=104), Prevalence =28.8%	Positive (Ratio ≥ 0.00738)	22	23	95.7% (22/23) (80.1%, 99.2%)	22.1% (23/104)	54.27 (9.90, 311.60)
	Indeterminate (0.00371 \leq Ratio ≤ 0.00737)	7	19	36.8% (7/19) (20.2%, 56.2%)	18.3% (19/104)	1.44 (0.62, 3.17)
	Negative (Ratio ≤ 0.00370)	1	62	1.6% (1/62) (0.3%, 7.6%)	59.6% (62/104)	0.040 (0.007, 0.204)
66-70 (N=99), Prevalence =43.4%	Positive (Ratio ≥ 0.00738)	35	37	94.6% (40/42) (83.6%, 98.5%)	37.4% (42/99)	22.79 (6.62, 83.61)
	Indeterminate (0.00371 \leq Ratio ≤ 0.00737)	6	15	40.0% (6/15) (20.7%, 62.5%)	15.2% (25/99)	0.87 (0.34, 2.17)
	Negative (Ratio ≤ 0.00370)	2	47	4.3% (2/47) (1.2%, 13.0%)	47.5% (68/120)	0.058 (0.016, 0.195)
71-75 (N=120), Prevalence =59.2%	Positive (Ratio ≥ 0.00738)	55	63	87.3% (55/63) (79.1%, 93.0%)	52.5% (157/120)	4.75 (2.62, 9.22)
	Indeterminate (0.00371 \leq Ratio ≤ 0.00737)	15	24	62.5% (15/24) (44.9%, 77.8%)	20.0% (56/120)	1.15 (0.56, 2.42)
	Negative (Ratio ≤ 0.00370)	1	33	3.0% (1/33) (0.5%, 14.6%)	27.5% (49/262)	0.022 (0.004, 0.118)
76-80 (N=124), Prevalence =62.1%	Positive (Ratio ≥ 0.00738)	61	65	93.8% (61/65) (86.5%, 97.5%)	52.4% (46/124)	9.31 (3.93, 23.81)
	Indeterminate (0.00371 \leq Ratio ≤ 0.00737)	16	30	53.3% (16/30) (38.4%, 68.0%)	24.2% (14/124)	0.70 (0.38, 1.30)
	Negative (Ratio ≤ 0.00370)	0	29	0.0% (0/29) (0.0%, 11.3%)	23.4% (16/124)	0.00 (0.00, 0.08)
81-93 (N=52), Prevalence =65.4%	Positive (Ratio ≥ 0.00738)	28	31	90.3% (28/31) (79.4%, 96.4%)	59.6% (46/52)	4.94 (2.04, 14.38)
	Indeterminate (0.00371 \leq Ratio ≤ 0.00737)	5	10	50.0% (5/10) (25.8%, 74.7%)	19.2% (14/52)	0.53 (0.18, 1.56)

	Test Result	Amyloid PET Scan [^] or CSF Ratio Test [#]		Predictive Value % (n/N) (95% CI*)	Frequency of Results % (n/N)	Likelihood Ratio (95% CI**)
		Positive (n)	N			
	Negative (Ratio ≤ 0.00370)	1	11	9.1% (1/11) (1.7%, 35.3%)	21.2% (16/52)	0.053 (0.008, 0.289)

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

Across age groups, the PVs were highest for the positive test results with a decrease for the indeterminate test results and lowest in the negative test results. The estimates of PV for the positive test results were 95.7%, 94.6%, and 93.9% for ages 51–65, 66–70 and 76–80, respectively. PV values were 90.3% for ages 81–93 and 87.3% for the ages 71–75. The % of the indeterminate test results was highest (24.2%) for the ages 76–80 compared to 66–70 (15.2%), 51–64 (18.3%), 81–93 (19.2%), and 71–75 (20.0%). The estimates of PV for the negative test results were 0.0% for the ages 76–80, 1.6% for the ages 51–65, 3.0% for the ages 71–75, 4.3% for the ages 66–70, and 9.1% for the ages 81–93. The FR value for the indeterminate test results was 15.2% for the ages 66-70, 18.3% for the ages 51–65, 19.2% for the ages 81–93, 20.0% for the ages 71– 75, and 24.2 % for the ages 76–80.

e. Race

The performance measures of the Lumipulse **G** pTau217/β-Amyloid 1-42 Plasma Ratio were also stratified by race and summarized in the table below.

Race	Test Result	Amyloid PET Scan [^] or CSF Ratio Test [#]		Predictive Value % (n/N) (95% CI*)	Frequency of Results % (n/N)	Likelihood Ratio (95% CI**)
		Positive (n)	N			
White (N=423)	Positive (Ratio ≥ 0.00738)	181	196	92.3% (181/196) (88.2%, 95.2%)	46.3% (196/423)	10.52 (6.54, 17.23)
	Indeterminate (0.00371 ≤ Ratio ≤ 0.00737)	41	79	51.9% (38/80) (42.1%, 61.6%)	18.7% (80/423)	0.94 (0.63, 1.40)
	Negative (Ratio ≤ 0.00370)	4	148	2.7% (4/148) (1.1%, 6.6%)	35.0% (148/423)	0.024 (0.009, 0.061)

Race	Test Result	Amyloid PET Scan [^] or CSF Ratio Test [#]		Predictive Value % (n/N) (95% CI*)	Frequency of Results % (n/N)	Likelihood Ratio (95% CI**)
		Positive (n)	N			
African American (N=60)	Positive (Ratio \geq 0.00738)	15	17	88.2% (15/17) (68.4%, 96.6%)	28.3% (17/60)	11.25 (3.25, 42.17)
	Indeterminate (0.00371 \leq Ratio \leq 0.00737)	8	15	53.3% (8/15) (32.5%, 72.9%)	25.0% (15/60)	1.71 (0.72, 4.04)
	Negative (Ratio \leq 0.00370)	1	28	3.6% (2/28) (0.6%, 15.6%)	46.7% (28/60)	0.056 (0.009, 0.277)
Asian and Other (N=16)	Positive (Ratio \geq 0.00738)	5	6	83.3% (5/6) (53.3%, 96.7%)	37.5% (6/16)	11.00 (2.51, 64.50)
	Indeterminate (0.00371 \leq Ratio \leq 0.00737)	0	4	0.0% (0/4) (0.0%, 41.4%)	25.0% (4/16)	0.00 (0.00, 1.55)
	Negative (Ratio \leq 0.00370)	0	6	0.0% (0/6) (0.0%, 30.2%)	37.5% (6/16)	0.00 (0.00, 0.95)

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

Across race groups, the PVs were highest for the positive test results with a decrease for the indeterminate test results and lowest in the negative test results. For white patients, the estimate of PV was 92.3%, 51.9%, and 2.7% for the positive, intermediate, and negative test results, respectively. The FR values for white patients were 46.3%, 18.7% and 35.0% for the positive, indeterminate, and negative test results, respectively. For African American patients, the estimate of PV was 88.2%, 53.3%, and 3.6% for the positive, indeterminate, and negative test results, respectively. The FR values for African American patients were 28.3%, 25.0% and 46.7% for the positive, indeterminate, and negative test results, respectively. For Asian patients, the estimate of PV was 83.3%, 0.0%, and 0.0% for the positive, indeterminate, and negative test results, respectively. The FR values for Asian patients were 37.5%, 25.0% and 37.5% for the positive, indeterminate, and negative results, respectively.

The estimates of PV for Asians and other races have high uncertainty due to limited numbers of patients evaluated in the study.

D Clinical Cut-Off:

Refer to Assay Cut-off

E Expected Values/Reference Range:

1) Reference Interval

A reference interval of the Lumipulse **G** pTau217/ β -Amyloid 1-42 Plasma Ratio was determined in cognitively normal subjects in accordance with the CLSI guideline EP28-A3c. Subjects were diagnosed as cognitively normal via a combination of Mini Mental State Examination (MMSE), Montreal Cognitive Assessment (MoCA), and Clinical Dementia Rating (CDR) scores. A total of 240 evaluable human plasma samples obtained from research biobanks or commercial vendors, and clinical sites at the U.S. Department of Veterans Affairs (VA Puget Sound Health Care System, Seattle) and University of Nevada, Las Vegas (UNLV) were collected from 115 males and 125 females aged 51 to 84 years old (mean of 68 years and median of 68 years). The numbers of cognitively normal subjects between 76 and 80 and between 81-84 years of age were 28 and 10 respectively, which are smaller in comparison to the numbers in the other age groups. The results of the cognitively normal subjects tested with Lumipulse **G** pTau 217 Plasma, Lumipulse **G** β -Amyloid 1-42-N Plasma and Lumipulse **G** pTau217/ β -Amyloid 1-42 Plasma Ratio are summarized in the table below:

	Cognitively Normal					
Age Group (years)	All	Age 51–65	Age 66–70	Age 71–75	Age 76–80	Age 81–84
N	240	90	68	44	28	10
Lumipulse G pTau 217 Plasma						
Mean (SD)	0.144 (0.251)	0.078 (0.044)	0.163 (0.401)	0.189 (0.247)	0.185 (0.116)	0.294 (0.201)
Median	0.089	0.064	0.093	0.129	0.172	0.250
Range	0.047– 3.357	0.047– 0.293	0.047– 3.357	0.047– 1.570	0.047– 0.529	0.102– 0.648
2.5th Percentile, 97.5th Percentile	0.047, 0.529	0.047, 0.264	0.048, 1.291	0.047, 1.457	N/A*, N/A*	N/A*, N/A*
Lumipulse G β-Amyloid 1-42-N Plasma						
Mean (SD)	25.0 (7.3)	24.0 (4.0)	25.7 (11.5)	24.9 (5.6)	25.5 (4.6)	27.9 (7.3)
Median	24.0	24.0	23.5	23.5	26.1	24.5
Range	10.0–110.5	10.0–37.5	14.9–110.5	17.7–43.6	17.0–33.0	18.9–42.1
2.5th Percentile, 97.5th Percentile	17.1, 37.5	16.2, 32.9	16.5, 59.5	17.7, 42.9	N/A*, N/A*	N/A*, N/A*

Age Group (years)	All	Age 51–65	Age 66–70	Age 71–75	Age 76–80	Age 81–84
N	240	90	68	44	28	10
Lumipulse G pTau217/β-Amyloid 1-42 Plasma Ratio						
Mean (SD)	0.00603 (0.01055)	0.00342 (0.00312)	0.00695 (0.01673)	0.00791 (0.01045)	0.00729 (0.00430)	0.01138 (0.00844)
Median	0.00366	0.00274	0.00373	0.00495	0.00680	0.00983
Range	0.00087– 0.13815	0.00156– 0.02930	0.00087– 0.13815	0.00181– 0.06356	0.00187– 0.01928	0.00284– 0.02734
2.5th Percentile, 97.5th Percentile	0.00179, 0.02609	0.00173, 0.01033	0.00154, 0.05694	0.00184, 0.05982	N/A*, N/A*	N/A*, N/A*
Lumipulse G pTau217/β-Amyloid 1-42 Plasma Ratio (% , n)						
Negative (Ratio ≤ 0.00370)	50.4% (121)	74.4% (67)	50.0% (34)	31.8% (14)	14.3% (4)	20.0% (2)
Indeterminate (0.00371 ≤ Ratio ≤ 0.00737)	31.7% (76)	23.3% (21)	32.4% (22)	45.5% (20)	39.3% (11)	20.0% (2)
Positive (Ratio ≥ 0.00738)	17.9% (43)	2.2% (2)	17.6% (12)	22.7% (10)	46.4% (13)	60.0% (6)
*Results listed as not applicable (N/A) are due to an insufficient number of samples to properly calculate the value						

The established 95% reference intervals are 0.047–0.529 pg/mL for pTau217, 17.1–37.5 pg/mL for β-Amyloid 1-42, and 0.00179–0.02608 for pTau217/β-Amyloid 1-42 ratio. Across the age groups, 17.9% have a positive Lumipulse **G** pTau 217/β-Amyloid 1-42 Plasma Ratio test result, 31.7% have an indeterminate Lumipulse **G** pTau 217/β-Amyloid 1-42 Plasma Ratio test result, and 50.4% have a negative Lumipulse **G** pTau 217/β-Amyloid 1-42 Plasma Ratio test result. The percentage of cognitively normal subjects who tested positive at a ratio of ≥ 0.00738 with Lumipulse **G** pTau 217/β-Amyloid 1-42 Plasma Ratio increased with age from 2.2% at age 50–65 years to 17.6% at age 66–70 years and 22.7% at age 71–75 years. The percentages of positive Lumipulse **G** pTau 217/β-Amyloid 1-42 Plasma Ratio test results reach 46.4% at age 76–80 years and 60% at age 81-84 years but the sample numbers in these two age groups are small.

The medians for Lumipulse **G** pTau217/β-Amyloid 1-42 Plasma Ratio did not differ significantly across race and the 95% reference interval of Lumipulse **G** pTau217/β-Amyloid 1-42 Plasma Ratio is comparable in males and females as shown in the table below.

	Cognitively Normal				
	Race			Sex	
	White	Black	Other	Male	Female
N	216	18	6	115	125
Lumipulse G pTau 217 Plasma					
Mean (SD)	0.146 (0.263)	0.139 (0.109)	0.080 (0.033)	0.138 (0.114)	0.149 (0.331)
Median	0.088	0.100	0.075	0.101	0.076
Range	0.047–3.357	0.047–0.434	0.047–0.132	0.047–0.663	0.047–3.357
2.5th Percentile, 97.5th Percentile	0.047, 0.549	N/A*, N/A*	N/A*, N/*A	0.047, 0.541	0.047, 0.556
Lumipulse G β-Amyloid 1-42-N Plasma					
Mean (SD)	24.1 (4.3)	35.4 (19.6)	26.7 (6.3)	23.8 (5.1)	26.1 (8.8)
Median	23.9	31.0	24.5	23.2	24.7
Range	10.0–42.1	18.8–110.5	20.2–37.5	10.0–43.6	17.3–110.5
2.5th Percentile, 97.5th Percentile	17.0, 33.2	N/A*, N/A*	N/A*, N/A*	16.0, 37.8	19.1, 37.7
Lumipulse G pTau217/β-Amyloid 1-42 Plasma Ratio					
Mean (SD)	0.00625 (0.01106)	0.00432 (0.00326)	0.00300 (0.00099)	0.00615 (0.00576)	0.00591 (0.01357)
Median	0.00378	0.00304	0.00306	0.00428	0.00316
Range	0.00173– 0.13815	0.00087– 0.01199	0.00156– 0.00444	0.00180– 0.03365	0.00087– 0.13815
2.5th Percentile, 97.5th Percentile	0.00180, 0.02683	N/A*, N/A*	N/A*, N/A*	0.00191, 0.02754	0.00173, 0.02254
Lumipulse G pTau217/β-Amyloid 1-42 Plasma Ratio (% , n)					
Negative (Ratio ≤ 0.00370)	48.6% (105)	61.1% (11)	83.3% (5)	38.3% (44)	61.6% (77)
Indeterminate (0.00371 ≤ Ratio ≤ 0.00737)	32.9% (71)	22.2% (4)	16.7% (1)	41.7% (48)	22.4% (28)
Positive (Ratio ≥ 0.00738)	18.5% (40)	16.7% (3)	0.0% (0)	20.0% (23)	16.0% (20)
*Results listed as not applicable (N/A) are due to an insufficient number of samples to properly calculate the value					

2) Expected Values

The expected values for non-AD dementia and other neurological conditions were investigated. A total of 296 evaluable human plasma samples with clinical and diagnostic information obtained from research biobanks or commercial vendors were included in analyzing expected values. The test samples were collected from individuals 22 years and older who presented with a diagnosis of non-AD dementia and other neurological conditions. The test results are summarized in the tables below:

	Disease Conditions			
	All Conditions Combined	Brain Hemorrhage or Ischemia	Frontotemporal Dementia	Lewy Body Dementia
N	269	24	41	39
Lumipulse G pTau 217 Plasma				
Mean (SD)	0.311 (0.440)	0.190 (0.154)	0.393 (0.355)	0.735 (0.782)
Median	0.154	0.146	0.287	0.421
Range	0.047–3.343	0.051–0.697	0.047–1.345	0.070–3.343
2.5th Percentile, 97.5th Percentile	0.047, 1.686	N/A*, N/A*	0.047, 1.335	0.070, 3.343
Lumipulse G β-Amyloid 1-42-N Plasma				
Mean (SD)	18.1 (9.8)	14.2 (10.1)	19.0 (7.4)	20.6 (9.1)
Median	18.7	11.2	17.6	20.9
Range	0.7–50.2	2.2–40.0	5.3–36.0	5.2–50.2
2.5th Percentile, 97.5th Percentile	1.9, 39.7	N/A*, N/A*	5.4, 35.8	5.2, 50.2
Lumipulse G pTau217/β-Amyloid 1-42 Plasma Ratio				
Mean (SD)	0.02814 (0.04592)	0.02655 (0.04174)	0.02699 (0.02857)	0.03958 (0.03851)
Median	0.00992	0.01610	0.01360	0.02642
Range	0.00173–0.45182	0.00173–0.20909	0.00222–0.11113	0.00295–0.17443
2.5th Percentile, 97.5th Percentile	0.00211, 0.17086	N/A*, N/A*	0.00223, 0.10979	0.00295, 0.17443
Lumipulse G pTau217/β-Amyloid 1-42 Plasma Ratio (% , n)				
Negative (Ratio ≤ 0.00370)	23.0% (62)	12.5% (3)	12.2% (5)	5.1% (2)
Indeterminate (0.00371 ≤ Ratio ≤ 0.00737)	21.2% (57)	16.7% (4)	22.0% (9)	10.3% (4)
Positive (Ratio ≥ 0.00738)	55.8% (150)	70.8% (17)	65.9% (27)	84.6% (33)
*Results listed as not applicable (N/A) are due to an insufficient number of samples to properly calculate the value				

	Disease Conditions				
	Multiple Sclerosis	Parkinson's Disease	Stroke	Traumatic Brain Injury	Vascular Dementia
N	39	40	33	30	23
Lumipulse G pTau 217 Plasma					
Mean (SD)	0.111 (0.118)	0.196 (0.244)	0.319 (0.466)	0.210 (0.199)	0.230 (0.291)
Median	0.071	0.115	0.176	0.156	0.120
Range	0.047–0.653	0.047–1.220	0.047–2.458	0.050–0.994	0.047–1.279
2.5th Percentile, 97.5th Percentile	0.047, 0.653	0.047, 1.211	N/A*, N/A*	N/A*, N/A*	N/A*, N/A*
Lumipulse G β-Amyloid 1-42-N Plasma					
Mean (SD)	21.6 (6.8)	19.8 (7.1)	14.9 (11.8)	8.9 (8.6)	24.2 (10.7)
Median	21.0	18.9	12.3	5.1	25.6
Range	2.1–49.4	7.3–39.4	0.7–46.7	1.1–25.5	2.4–43.7
2.5th Percentile, 97.5th Percentile	2.1, 49.4	7.3, 39.3	N/A*, N/A*	N/A*, N/A*	N/A*, N/A*
Lumipulse G pTau217/β-Amyloid 1-42 Plasma Ratio					
Mean (SD)	0.00578 (0.00672)	0.01032 (0.01203)	0.03958 (0.05044)	0.06593 (0.09111)	0.01564 (0.02891)
Median	0.00367	0.00592	0.01851	0.03739	0.00421
Range	0.00175– 0.03238	0.00242– 0.04729	0.00213– 0.22429	0.00235– 0.45182	0.00174– 0.13606
2.5th Percentile, 97.5th Percentile	0.00175, 0.03238	0.00242, 0.04725	N/A*, N/A*	N/A*, N/A*	N/A*, N/A*
Lumipulse G pTau217/β-Amyloid 1-42 Plasma Ratio (% , n)					
Negative (Ratio ≤ 0.00370)	51.3% (20)	32.5% (13)	12.1% (4)	20.0% (6)	39.1% (9)
Indeterminate (0.00371 ≤ Ratio ≤ 0.00737)	33.3% (13)	30.0% (12)	18.2% (6)	6.7% (2)	30.4% (7)
Positive (Ratio ≥ 0.00738)	15.4% (6)	37.5% (15)	69.7% (23)	73.3% (22)	30.4% (7)
*Results listed as not applicable (N/A) are due to an insufficient number of samples to properly calculate the value					

The percentage of positive Lumipulse G pTau 217/β-Amyloid 1-42 Plasma Ratio test results for plasma samples of patients diagnosed with Lewy Body Dementia is 84.6%. The high number of positive Lumipulse G pTau 217/β-Amyloid 1-42 Plasma Ratio test results in patients diagnosed with Lewy Body Dementia is likely due to Lewy body disease often co-occurring with Alzheimer's disease neuropathological change which can be detected using plasma pTau181

and pTau217¹. The numbers of positive Lumipulse **G** pTau 217/ β -Amyloid 1-42 Plasma Ratio test results are also high in acute ischemic stroke patients^{2,3} and patients with frontotemporal dementia and traumatic brain injury^{4,5}.

VIII Proposed Labeling:

The labeling supports the finding of substantial equivalence for this device.

IX Conclusion:

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

¹ Smith, A., *et al.*, (2024). Comparing the diagnostic performance of plasma pTau181 and pTau217 for the identification of Alzheimer's disease co-pathology in Lewy Body Disease. *Alzheimer's Dement.*, 20: e091458.

² Gundersen, J.K., *et al.*, (2024). Clinical value of plasma brain-derived tau and p-tau217 in acute ischemic stroke, *Cerebral Circulation - Cognition and Behavior*, 6:100291.

³ Mielke, M.M., *et al.*, (2020) Performance of plasma phosphorylated tau 181 and 217 in the community. *Nat Med*, 28: 1398–1405

⁴ Rojas, J.C., *et al.*, (2021). Diagnostic value of plasma P-tau217 in frontotemporal dementia spectrum disorders. *Alzheimer's Dement.*, 17: e055763.

⁵ Asken, B.M., *et al.*, (2022). Plasma P-tau181 and P-tau217 in Patients With Traumatic Encephalopathy Syndrome With and Without Evidence of Alzheimer Disease Pathology (2022). *Neurology*, 99: e594-e604.