EVALUATION OF AUTOMATIC CLASS III DESIGNATION FOR
Lumipulse *G* β-Amyloid Ratio (1-42/1-40)
DECISION SUMMARY

I Background Information:

A De Novo Number

DEN200072

B Applicant

Fujirebio Diagnostics, Inc.

C Proprietary and Established Names

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

D Regulatory Information

Product Code(s)	Classification	Regulation Section	Panel
QSE	Class II (special controls)	21 CFR §866.5840	Immunology (82)

II Submission/Device Overview:

A Purpose for Submission:

De Novo request for evaluation of automatic class III designation for Lumipulse ***G*** β-Amyloid Ratio (1-42/1-40)

B Measurand:

β-amyloid ratio of two cerebral spinal fluid (CSF) analytes: β-amyloid $_{1-42}$ and β-amyloid $_{1-40}$

C Type of Test:

Fully automated, chemiluminescent enzyme immunoassays (CLEIA)

III Indications for Use:

A Indication(s) for Use:

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 ***G*** 1200 System. The Lumipulse ***G*** β-Amyloid Ratio (1-42/1-40) is intended to be used in adult patients, aged 55 years and older, presenting with cognitive impairment who are being evaluated for Alzheimer's disease (AD) and other causes of cognitive decline.

A test result ≥ 0.073 is a negative result which is consistent with a negative amyloid positron emission tomography (PET) scan result. A negative result reduces the likelihood that a patient's cognitive impairment is due to AD.

A test result ≤ 0.058 is a positive result which is consistent with a positive amyloid PET scan result. A positive result does not establish a diagnosis of AD or other cognitive disorder.

A test result between 0.059 and 0.072 is considered as a likely positive result as it is more likely consistent with a positive amyloid PET scan result. A likely positive result does not establish a diagnosis of AD or other cognitive disorders and has increased uncertainty in regard to amyloid PET positivity.

The Lumipulse ***G*** β-Amyloid Ratio (1-42/1-40) results must be interpreted in conjunction with other patient clinical information .

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

B Special Conditions for Use Statement(s):

For prescription use only
For *in vitro* diagnostic use

C Special Instrument Requirements:

The LUMIPULSE ***G***1200 System (cleared under K142895)

IV Device/System Characteristics:

A Device Description:

1. Device:
   The Lumipulse ***G*** β-Amyloid Ratio (1-42/1-40) is an *in vitro* cerebral spinal fluid (CSF) test that calculates the ratio of two analytes, Lumipulse ***G*** β-Amyloid 1-42 and Lumipulse ***G*** β-Amyloid 1-40 assays to generate a numeric value between 0.001 to 1.000.

The test system consists of two component assays, Lumipulse ***G*** β-Amyloid 1-42 and the Lumipulse ***G*** β-Amyloid 1-40 assay, running on LUMIPULSE ***G***1200 system, and Lumipulse ***G*** β-Amyloid Ratio (1-42/1-40) Calculator Tool to calculate the Lumipulse ***G*** β-Amyloid Ratio (1-42/1-40). Lumipulse ***G*** β-Amyloid 1-42 and the Lumipulse ***G*** β-Amyloid 1-40 assays are packed individually. Results of individual assays have not been assessed to support the intended use except for determination of the Lumipulse ***G*** β-Amyloid Ratio (1-42/1-40).

a) Lumipulse ***G*** β-Amyloid 1-42:
Lumipulse ***G*** β-Amyloid 1-42 is used for the quantitative measurement of β-amyloid $_{1\text{-}42}$ in human CSF in order to calculate the Lumipulse ***G*** β-Amyloid Ratio (1-42/1-40). The components of Lumipulse ***G*** β-Amyloid 1-42 are as follows:

Lumipulse *G* β-Amyloid 1-42 (per Immunoreaction Cartridge)		
Component	**Volume**	**Contents**
Antibody Coated Particle Solution	250 µL	anti-β-amyloid $_{1\text{-}42}$ monoclonal antibody (mouse)-coated particles and 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.
Biotinylated Antibody Solution	120 µL	biotinylated antibody (mouse), protein (bovine) stabilizers and chemical stabilizers in 50 mM Tris buffer. Preservative: 0.1% ProClin 300.
Enzyme Labeled Streptavidin Solution	350 µL	alkaline phosphatase (ALP)-labeled streptavidin, protein stabilizers (bovine) and chemical stabilizers in 50 mM MES buffer. Preservative: 0.1% ProClin 300.

Additional materials required but sold separately:

- Lumipulse ***G*** β-Amyloid 1-42 Calibrators Set: Three Lumipulse ***G*** β-Amyloid 1-42 calibrators with at concentration level of 0, 129, and 2,335 pg/mL
- Lumipulse β-Amyloid Controls: Three levels of Lumipulse ***G*** β-Amyloid 1-42 Controls with target concentration of 4,000, 10,000, and 20,000 pg/mL, lyophilized.

b) Lumipulse ***G*** β-Amyloid 1-40:
Lumipulse ***G*** β-Amyloid 1-40 is used for the quantitative measurement of β-amyloid 1-40 in human CSF in order to calculate the Lumipulse ***G*** β-Amyloid Ratio (1-42/1-40). The components of Lumipulse ***G*** β-Amyloid 1-40 are as follows:

Lumipulse *G* β -Amyloid 1-40 (per Immunoreaction Cartridge)		
Component	**Volume**	**Contents**
Antibody Coated Particle Solution	150 µL	anti-β-amyloid$_{1\text{-}40}$ monoclonal antibody (mouse)-coated particles and 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	320 µL	alkaline phosphatase (ALP)-labeled anti-β-amyloid antibody (mouse) conjugate, protein stabilizers (bovine) and chemical stabilizers in in 50 mM MES buffer. Preservative: 0.1% ProClin 300

Additional materials required but sold separately:

- Lumipulse ***G*** β-Amyloid 1-40 Calibrators Set: three Lumipulse ***G*** β-Amyloid 1-40 calibrators with at concentration level of 0, 500, and 30,000 pg/mL.

- Lumipulse β-Amyloid Controls: three levels of Lumipulse ***G*** β-Amyloid 1-40 Controls with target concentration of 274, 548, and 1,027 pg/mL, lyophilized.

Both Lumipulse ***G*** β-Amyloid 1-42 and Lumipulse ***G*** β-Amyloid 1-40 are run on the LUMIPULSE ***G***1200 System. LUMIPULSE ***G***1200 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.

2. The Lumipulse ***G*** β-Amyloid Ratio (1-42/1-40) Calculator Tool:
The Lumipulse ***G*** β-Amyloid Ratio (1-42/1-40) Calculator Tool is a web-based software to calculate the ratio of β-amyloid $_{1\text{-}42}$ to β-amyloid $_{1\text{-}40}$ using results of the Lumipulse ***G*** β-Amyloid 1-42 assay and the Lumipulse ***G*** β-Amyloid 1-40 assay in CSF samples. The user is instructed to launch https://www.fujirebio-amyloid-ratio.com/ to perform the result calculation.

3. Result interpretation:
The Lumipulse ***G*** β-Amyloid Ratio (1-42/1-40) is calculated based on the values from Lumipulse ***G*** β-Amyloid 1-42 and Lumipulse ***G*** β-Amyloid 1-40 and generate a numerical ratio in a range of 0.001 to 1.000 as below.

Lumipulse ***G*** β-Amyloid Ratio (1-42/1-40)	Test Result	Interpretation	Clinical Implication
Ratio $\geq$ 0.073	Negative	Consistent with a negative amyloid PET scan result.	Reduced likelihood that a patient's cognitive impairment is due to AD
Ratio $\leq$ 0.058	Positive	Consistent with a positive amyloid PET scan result.	Diagnosis of AD or other cognitive disorder not established
0.059 $\leq$ Ratio $\leq$ 0.072	Likely Positive	Considered as a likely positive result as it is more likely consistent with a positive amyloid PET scan result.	Diagnosis of AD or other cognitive disorders not established and increased uncertainty in regard to amyloid PET positivity

B Principle of Operation

1. Specimen collection
   Refer to the Lumipulse ***G*** β-Amyloid Ratio (1-42/1-40) package insert

2. Lumipulse ***G*** β-Amyloid 1-42
   Lumipulse ***G*** β-Amyloid 1-42 is an assay including a set of immunoassay reagents for the quantitative measurement of β-amyloid$_{1-42}$ in human CSF specimens based on CLEIA technology using the LUMIPULSE ***G*** System as follows:

   1) First reaction step: β-Amyloid (1-42) calibrator or specimen (50 µL) and biotinylated antibody solution are both added to 'antibody-coated particle solution' compartment in the Immunoreaction Cartridge. β-amyloid $_{1-42}$ in specimens or calibrators specifically bind to anti-β-amyloid $_{1-42}$ monoclonal antibody (mouse) on the particles, and biotinylated antibody (mouse). Biotinylated antibody-antigen immuno-complexes are formed.
   2) Washing step: The particles are washed and rinsed to remove unbound materials.
   3) Second reaction step: Alkaline phosphatase (ALP)-labeled streptavidin specifically that binds to biotinylated immuno-complexes on the particles are added.
   4) Washing step: The particles are washed and rinsed to remove unbound materials.
   5) Enzyme reaction step: Substrate Solution is added and mixed with the particles. 3-(2'-spiroadamantane)-4-methoxy-4-(3"-phosphoryloxy) phenyl-1, 2-dioxetane disodium salt (AMPPD) contained in the Substrate Solution is dephosphorylated by the catalysis of ALP indirectly conjugated to particles.

6) 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 β-amyloid $_{1\text{-}42}$ present in the sample.

3. Lumipulse ***G*** β-Amyloid 1-40

Lumipulse ***G*** β-Amyloid 1-40 is an assay including a set of immunoassay reagents, for the quantitative measurement of β-amyloid $_{1\text{-}40}$ in human CSF specimens based on CLEIA technology using the LUMIPULSE ***G*** System as follows:

1) First reaction step: β-Amyloid (1-40) calibrator or specimen (40 µL) and biotinylated antibody solution are both added to 'antibody-coated particle solution' compartment in the Immunoreaction Cartridge. β-amyloid $_{1\text{-}40}$ in specimens or calibrators specifically bind to anti-β-Amyloid $_{1\text{-}40}$ monoclonal antibody (mouse) on the particles and antigen-antibody immunocomplexes are formed.
2) Washing: The particles are washed and rinsed to remove unbound materials.
3) Second reaction: Alkaline phosphatase (ALP)-labeled anti-β-amyloid monoclonal antibody (mouse) fragment specifically binds to anti-β-amyloid $_{1\text{-}40}$ immune-complexes on the particles and additional immunocomplexes are formed.
4) Washing: The particles are washed and rinsed to remove unbound materials.
5) 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.
6) 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 β-amyloid $_{1\text{-}40}$ present in the sample.

4. Lumipulse ***G*** β-Amyloid Ratio (1-42/1-40) Calculator Tool

The Lumipulse ***G*** β-Amyloid Ratio (1-42/1-40) Calculator Tool, using input values produced from Lumipulse ***G*** β-Amyloid 1-42 and Lumipulse ***G*** β-Amyloid 1-40 on the LUMIPULSE ***G***1200 System, is a web-based software available at the following URL (https://www.fujirebio-amyloid-ratio.com/). The calculator software is validated for use on Microsoft Internet Explorer and Edge, Google Chrome, Mozilla Firefox, and Apple Safari. When invalid values are entered, a 'Invalid Value' message is shown with a description of an error (e.g., "The Lumipulse ***G*** β-Amyloid 1-42 assay result value must be less that the Lumipulse ***G*** β-Amyloid 1-40 assay result value").

The Lumipulse ***G*** β-Amyloid Ratio (1-42/1-40) is calculated using the following equation and generate a numerical ratio in a range of 0.001 to 1.000 as below.

$$\text{Lumipulse } \boldsymbol{G} \text{ β-Amyloid Ratio (1-42/1-40)} = \frac{\text{Lumipulse } \boldsymbol{G} \text{ β-Amyloid 1-42 (results in pg/mL)}}{\text{Lumipulse } \boldsymbol{G} \text{ β-Amyloid 1-40 (results in pg/mL)}} = \text{a numerical value ranging 0.001 - 1.000}$$

- The Lumipulse ***G*** β-Amyloid Ratio (1-42/1-40) Calculator Tool reports the following: Lumipulse ***G*** β-Amyloid Ratio (1-42/1-40) result ≥ 0.073 = Negative

- Lumipulse ***G*** β-Amyloid Ratio (1-42/1-40) result ≤ 0.058 = Positive

- 0.059 ≤ Lumipulse ***G*** β-Amyloid Ratio (1-42/1-40) result ≤ 0.072 = Likely Positive

If the concentration of the analytes is outside the measuring range, the following rules apply: If β-amyloid $_{1\text{-}42}$ > 2200 pg/mL, the value should be set to 2200 pg/mL. The ratio is calculated, and the test results are reported as 'Negative'. If β-amyloid $_{1\text{-}42}$ < 38 pg/mL, β-amyloid $_{1\text{-}40}$ < 158 pg/mL, or β-amyloid $_{1\text{-}40}$ > 28,450 pg/mL, the ratio is not calculated using the Lumipulse ***G*** β-Amyloid Ratio (1-42/1-40) Calculator Tool. The results are manually reported as 'ratio undetermined'.

C Instrument Description Information

1. Instrument Name:
   The LUMIPULSE ***G***1200 System (cleared under K142895)

2. Specimen Identification:
   Refer to K142895

3. Specimen Sampling and Handling:
   Refer to K142895

4. Calibration:
   Refer to K142895

5. Quality Control:
   Refer to K142895

V Standards/Guidance Documents Referenced:

- CLSI EP05-A3: Evaluation of Precision of Quantitative Measurement Procedures; Approved Guideline – Third Edition
- CLSI EP06, 2nd ed.: Evaluation of the Linearity of Quantitative Measurement Procedures Second Edition
- CLSI EP07, 3rd ed.: Interference Testing in Clinical Chemistry; – Third Edition
- CLSI EP17-A2: Evaluation of Detection Capability for Clinical Laboratory Measurement Procedures; Approved Guideline -Second Edition
- CLSI EP28-A3c: Defining, Establishing, and Verifying Reference Intervals in the Clinical Laboratory; Approved Guideline Third Edition
- CLSI EP37: Supplemental Tables for Interference Testing in Clinical Chemistry; First Edition

VI Performance Characteristics

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*** β-Amyloid 1-42, Lumipulse ***G*** β-Amyloid 1-40, and Lumipulse ***G*** β-Amyloid Ratio (1-42/1-40). A seven-member panel (Panel members 1–7) was prepared to achieve target concentrations of β-amyloid $_{1-42}$ and β-amyloid $_{1-40}$ that cover the measuring ranges of the individual assays. Panel members 1–4 were prepared by pooling native CSF samples and Panel members 5–7 were prepared by spiking recombinant β-amyloid $_{1-42}$ and β-amyloid $_{1-40}$ proteins into native CSF pools to challenge various ratios of β-amyloid $_{1-42}$ to β-amyloid $_{1-40}$ proteins. 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*** β-Amyloid 1-42

		Within-Run		**Between-Run**		**Between-Day**		**Within-Laboratory**	
Panel member	**Mean (pg/mL)**	**SD**	**%CV**	**SD**	**%CV**	**SD**	**%CV**	**SD**	**%CV**
1	129	2.4	1.9	3.8	2.9	1.4	1.1	4.7	3.6
2	425	6.0	1.4	5.7	1.3	4.6	1.1	9.5	2.2
3	596	7.8	1.3	13.1	2.2	0.0	0.0	15.2	2.6
4	700	9.0	1.3	8.3	1.2	6.2	0.9	13.7	2.0
5	881	13.3	1.5	11.1	1.3	10.7	1.2	20.4	2.3
6	1,274	32.7	2.6	5.2	0.4	35.7	2.8	48.7	3.8
7	2,084	26.3	1.3	25.9	1.2	33.6	1.6	49.9	2.4

b. Lumipulse ***G*** β-Amyloid 1-40

		Within-Run		Between-Run		Between-Day		Within-Laboratory	
Panel member	**Mean (pg/mL)**	**SD**	**%CV**	**SD**	**%CV**	**SD**	**%CV**	**SD**	**%CV**
1	238	18.1	7.6	0.0	0.0	6.2	2.6	19.1	8.0
2	7,700	106.1	1.4	81.8	1.1	36.1	0.5	138.7	1.8
3	10,620	151.0	1.4	204.9	1.9	0.0	0.0	254.5	2.4
4	12,455	154.7	1.2	172.3	1.4	99.0	0.8	251.9	2.0
5	15,515	231.4	1.5	172.8	1.1	187.3	1.2	344.2	2.2
6	20,850	715.2	3.4	410.2	2.0	470.2	2.3	949.1	4.6
7	26,545	422.8	1.6	475.9	1.8	514.6	1.9	818.5	3.1

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

		Within-Run		Between-Run		Between-Day		Within-Laboratory	
Panel member	**Mean Ratio**	**SD**	**%CV**	**SD**	**%CV**	**SD**	**%CV**	**SD**	**%CV**
1	0.542	2.5	4.7	1.2	2.3	1.4	2.5	3.1	5.8
2	0.055	0.1	2.0	0.0	0.7	0.1	1.0	0.1	2.4
3	0.056	0.1	2.0	0.1	1.0	0.1	0.9	0.1	2.4
4	0.056	0.1	1.9	0.0	0.7	0.0	0.7	0.1	2.1
5	0.057	0.1	2.4	0.0	0.0	0.0	0.5	0.1	2.4
6	0.061	0.2	3.7	0.0	0.0	0.1	1.2	0.2	3.9
7	0.079	0.2	2.0	0.1	1.9	0.1	0.9	0.2	2.9

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*** β-Amyloid 1-42 and Lumipulse ***G*** β-Amyloid 1-40. A paired reagent lot was defined as a set of specific lots of Lumipulse ***G*** β-Amyloid 1-42 Immunoreaction Cartridges with Lumipulse ***G*** β-Amyloid 1-42 Calibrators and Lumipulse ***G*** β-Amyloid 1-40 Immunoreaction Cartridges with Lumipulse ***G*** β-Amyloid 1-40 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*** β-Amyloid 1-42

Panel member	Mean (pg/mL)	Within-Run		Between-Run		Between-Day		Between-Lot		Total	
		SD	%CV	SD	%CV	SD	%CV	SD	%CV	SD	%CV
1	123	2.4	2.0	4.6	3.7	3.2	2.6	2.1	1.7	6.4	5.2
2	413	5.1	1.2	13.2	3.2	7.1	1.7	8.0	1.9	17.7	4.3
3	569	8.7	1.5	22.9	4.0	0.0	0.0	3.9	0.7	24.8	4.4
4	686	8.2	1.2	21.0	3.1	18.8	2.7	7.7	1.1	30.3	4.4
5	868	8.7	1.0	30.8	3.5	4.5	0.5	13.7	1.6	35.1	4.0
6	1,254	16.8	1.3	33.2	2.6	18.7	1.5	18.6	1.5	45.6	3.6
7	2,090	17.8	0.9	39.2	1.9	15.7	0.8	37.4	1.8	59.2	2.8

b. Lumipulse ***G*** β-Amyloid 1-40

Panel member	Mean (pg/mL)	Within-Run		Between-Run		Between-Day		Between-Lot		Total	
		SD	%CV	SD	%CV	SD	%CV	SD	%CV	SD	%CV
1	229	8.0	3.5	4.7	2.1	3.8	1.7	6.5	2.8	11.9	5.2
2	7,343	108.3	1.5	249.7	3.4	0.0	0.0	138.7	1.9	305.5	4.2
3	9,958	150.5	1.5	284.2	2.9	0.0	0.0	0.0	0.0	321.6	3.2
4	11,862	198.3	1.7	412.3	3.5	195.8	1.7	104.4	0.9	508.5	4.3
5	14,892	207.8	1.4	489.1	3.3	0.0	0.0	111.9	0.8	543.0	3.6
6	19,773	265.3	1.3	427.6	2.2	321.7	1.6	0.0	0.0	597.2	3.0
7	25,657	345.4	1.3	372.5	1.5	251.6	1.0	0.0	0.0	566.9	2.2

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

		Within-Run		Between-Run		Between-Day		Between-Lot		Total	
Panel member	**Mean Ratio**	**SD**	**%CV**	**SD**	**%CV**	**SD**	**%CV**	**SD**	**%CV**	**SD**	**%CV**
1	0.538	1.8	3.3	1.5	2.8	1.7	3.2	1.7	3.1	3.4	6.3
2	0.056	0.1	2.0	0.1	0.9	0.1	2.3	0.1	2.2	0.2	3.9
3	0.057	0.1	2.2	0.1	1.8	0.1	1.4	0.0	0.9	0.2	3.3
4	0.058	0.1	2.0	0.1	1.8	0.1	1.1	0.1	1.3	0.2	3.2
5	0.058	0.1	1.5	0.1	1.1	0.1	1.6	0.0	0.3	0.1	2.5
6	0.063	0.1	1.9	0.1	1.2	0.1	2.1	0.0	0.8	0.2	3.2
7	0.081	0.1	1.5	0.2	2.4	0.2	2.2	0.1	1.0	0.3	3.6

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*** β-Amyloid 1-42

		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	124	2.4	2.0	4.3	3.5	3.2	2.6	9.6	7.7	11.3	9.1
2	418	5.2	1.2	13.9	3.3	2.7	0.6	14.5	3.5	20.9	5.0
3	575	8.5	1.5	21.4	3.7	0.0	0.0	24.1	4.2	33.3	5.8
4	689	8.6	1.3	21.6	3.1	15.4	2.2	16.4	2.4	32.4	4.7
5	877	9.5	1.1	20.8	2.4	7.0	0.8	33.8	3.9	41.4	4.7
6	1,257	17.6	1.4	28.2	2.2	0.0	0.0	43.9	3.5	55.0	4.4
7	2,120	16.9	0.8	33.3	1.6	32.8	1.5	46.5	2.2	68.0	3.2

b. Lumipulse ***G*** β-Amyloid 1-40

		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	232	5.9	2.5	9.7	4.2	18.7	8.0	13.0	5.6	25.4	11.0
2	7,284	102.1	1.4	218.4	3.0	222.0	3.0	158.4	2.2	364.0	5.0
3	9,946	134.0	1.3	358.1	3.6	138.7	1.4	284.2	2.9	496.2	5.0
4	11,833	175.3	1.5	400.2	3.4	405.8	3.4	313.2	2.6	673.5	5.7
5	14,845	207.1	1.4	453.7	3.1	263.1	1.8	480.5	3.2	740.8	5.0
6	19,558	247.6	1.3	495.0	2.5	505.5	2.6	561.9	2.9	936.8	4.8
7	25,538	258.4	1.0	582.7	2.3	684.2	2.7	828.5	3.2	1249.4	4.9

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

		Within-Run		Between-Run		Between-Day		Between-Site		Total	
Panel member	**Mean Ratio**	**SD**	**%CV**	**SD**	**%CV**	**SD**	**%CV**	**SD**	**%CV**	**SD**	**%CV**
1	0.539	1.6	3.0	2.4	4.4	4.3	8.0	0.0	0.0	5.2	9.6
2	0.057	0.1	2.0	0.1	1.5	0.2	3.0	0.0	0.0	0.2	3.9
3	0.058	0.1	1.9	0.1	1.6	0.2	2.7	0.0	0.0	0.2	3.6
4	0.058	0.1	1.9	0.2	2.6	0.2	2.8	0.0	0.0	0.3	4.3
5	0.059	0.1	1.4	0.1	1.2	0.2	2.9	0.0	0.6	0.2	3.5
6	0.064	0.1	2.0	0.0	0.7	0.2	3.5	0.0	0.0	0.3	4.1
7	0.083	0.1	1.4	0.2	2.6	0.3	3.5	0.1	1.6	0.4	4.9

4) Lumipulse ***G*** β-Amyloid Ratio (1-42/1-40) Precision Simulation:
The within-laboratory precision and site-tosite reproducibility of Lumipulse ***G*** β-Amyloid Ratio (1-42/1-40) was further evaluated by simulating percent positive calls at seven different ratio levels. For a bivariate ratio assay, the precision performance can be different at the same ratio value when the combinations for Lumipulse ***G*** β-Amyloid 1-42 and Lumipulse ***G*** β-Amyloid 1-40 results are different. Because the assay random measurement error y changes with the analyte concentration (%CV), different combinations for Lumipulse ***G*** β-Amyloid 1-42 and Lumipulse ***G*** β-Amyloid 1-40 results correspond to different random measurement random errors. In order to evaluate the precision characteristics of Lumipulse ***G*** β-Amyloid Ratio (1-42/1-40) numerical values under different combinations of β-amyloid $_{1\text{-}42}$ and β-amyloid $_{1\text{-}40}$, the variance profile of Lumipulse ***G*** β-Amyloid 1-42 and Lumipulse ***G*** β-Amyloid 1-40 was considered. The simulated probability of a positive call was estimated for the mean ratio of Panel member 1 7 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 simulated probability of positive call was

calculated for different combinations of β-amyloid $_{1-42}$ and β-amyloid $_{1-40}$ and the results met acceptable precision criteria.

2. Linearity:

The linearity of the Lumipulse ***G*** β-Amyloid 1-42 and Lumipulse ***G*** β-Amyloid 1-40 was evaluated in accordance with the CLSI guideline EP06, 2nd ed.. For each analyte, a high sample pool was diluted with a low sample pool to create a series of 22 dilutions for β-amyloid $_{1-42}$ or a series of 21 dilutions for β-amyloid $_{1-40}$ that span across the measuring ranges of the respective assays. Each sample dilution was measured in one run with four replicates using one reagent lot of each assay and the mean of the four 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 ± 10% for each dilution level. The best linear regression fits are summarized in the table below:

Assay	**Range (pg/mL)**	**Slope (95%CI)**	**Intercept (95% CI)**	**R^2**
Lumipulse ***G*** β-Amyloid 1-42	37.5 – 2203.5	1.02 (0.96 1.00)	-23.4 (-47.5 0.62)	1.00
Lumipulse ***G*** β-Amyloid 1-40	156.3 – 28450.3	1.03 (1.02 – 1.05)	13.6 (-167.4 – 194.5)	1.00

High-Dose Hook Effect:
The high-dose hook effect was evaluated for the Lumipulse ***G*** β-Amyloid 1-42 and the Lumipulse ***G*** β-Amyloid 1-40 on the LUMIPULSE ***G***1200 analyzer. The expected and measured concentrations were compared for the test samples spiked with a high concentration stock of the β-amyloid $_{1-42}$ (159,072 pg/mL) and β-amyloid $_{1-40}$ (168,426 pg/mL). The recovery was 98 103% for the Lumipulse ***G*** β-Amyloid 1-42 and 93 108% for the Lumipulse ***G*** β-Amyloid. No high-dose hook effect was observed up to approximately 159,000 pg/mL for the Lumipulse ***G*** β-Amyloid 1-42 and up to approximately 150,000 pg/mL for the Lumipulse ***G*** β-Amyloid 1-40.

3. Analytical Specificity/Interference:

1) Interference:

The effect of potential endogenous and exogenous substances to the Lumipulse ***G*** β-Amyloid Ratio (1-42/1-40) and its component assays, Lumipulse ***G*** β-Amyloid 1-42 and Lumipulse ***G*** β-Amyloid 1-40, was evaluated in accordance with the CLSI guidelines EP07, 3rd ed. and EP37. Six pooled human CSF samples with various concentrations of β-amyloid $_{1-42}$ (303–1,980 pg/mL) and β-amyloid $_{1-40}$ (5,997–26,744 pg/mL) were

prepared for targeting the β-Amyloid $_{(1\text{-}42/1\text{-}40)}$ ratios between 0.050–0.084. Test samples were created by spiking with the potential endogenous and exogenous interfering substances at two different levels. Control samples were spiked only with the appropriate solvent used to create the interfering substances panel. The β-amyloid $_{1\text{-}42}$ and β-amyloid $_{1\text{-}40}$ 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 (≤ ±10% difference of test from control) for the Lumipulse ***G*** β-Amyloid 1-42, the Lumipulse ***G*** β-Amyloid 1-40, and the Lumipulse ***G*** β-Amyloid Ratio (1-42/1-40) up to the concentrations of the potential interfering substances tested as shown in the table below:

Endogenous Interferent	**Concentration**
Biotin	1300 ng/mL
Conjugated Bilirubin	0.5 mg/dL
Free Bilirubin (unconjugated)	0.5 mg/dL
Hemoglobin	12.5 mg/dL
Human anti-mouse Antibodies, HAMA (IgG)	1.5 ng/mL
Human Serum Albumin	0.18 g/dL
Immunoglobulin A (IgA)	0.018 g/dL
Immunoglobulin G (IgG)	0.025 g/dL
Immunoglobulin M (IgM)	0.005 g/dL
Rheumatoid Factor	15.8 IU/mL
Triglycerides	40 mg/dL
Whole Blood	2.5% v/v

Exogenous Interferent	**Concentration**
Acetaminophen	20 mg/dL
Acetylcysteine	15 mg/dL
Acetylsalicylic Acid	100 mg/dL
Ampicillin	100 mg/dL
Aripiprazole	0.45 mg/dL
Ascorbic Acid	30 mg/dL
Atorvastatin	0.6 mg/dL
Caffeine	10.8 mg/dL
Calcium Dobesilate	20 mg/dL
Cefoxitin	500 mg/dL
Chloramphenicol	7.8 mg/dL
Clopidogrel	18 mg/dL
Cyclosporine	0.5 mg/dL
1Digoxin	0.5 mg/dL
Donepezil (Aricept)	3 mg/dL
Doxycycline	1.8 mg/dL
Escitalopram	1.5 mg/dL
Esomeprazole	17 mg/dL
Furosemide	9 mg/dL

Exogenous Interferent	Concentration
Galantamine (Reminyl)	25 mg/dL
Heparin	500 U/dL
Hydrochlorothiazide	12 mg/dL
Ibuprofen	50 mg/dL
Levodopa	2 mg/dL
Lisinopril	50 mg/dL
Memantine (Namenda)	25 mg/dL
Metformin	400 mg/dL
Methyldopa	2.25 mg/dL
Metoprolol	90 mg/dL
Phenylbutazone	32.1 mg/dL
Rifampicin	6 mg/dL
Rivastigmine (Exelon)	4.5 mg/dL
Simvastatin	1.4 mg/dL
Quetiapine (Seroquel)	90 mg/dL
Theophylline	10 mg/dL
Warfarin	7.5 mg/dL

2) *Cross-reactivity:*

The performance of the Lumipulse ***G*** β-Amyloid Ratio (1-42/1-40) in the presence of potential cross-reactive β-amyloid species is based on the effect of these potential cross-reactants to the performance of the two component assays, the Lumipulse ***G*** β-Amyloid 1-42 and the Lumipulse ***G*** β-Amyloid 1-40. The study was conducted by preparing four pooled human CSF samples with various concentration of β-amyloid $_{1\text{-}42}$ (349.5 1,808.9 pg/mL) and β-amyloid $_{1\text{-}40}$ (6,594.3 23,28.0 pg/mL) for challenging the β-amyloid $_{(1\text{-}42/1\text{-}40)}$ ratios between 0.056–0.077. Test samples were created by spiking with the potential cross-reactive β-amyloid species. Control samples were spiked only with the appropriate solvent used to create the cross-reactive β-amyloid species panel. The β-amyloid $_{1\text{-}42}$ and β-amyloid $_{1\text{-}40}$ in the test samples and control samples were measured in three replicates. The % cross-reactivity of each analyte was calculated by comparing measurements of the test and control samples using the equation below.

% cross-reactivity = {1- [(mean concentration (pg/mL-Test) / (mean concentration (pg/mL-Control)]} x 100

The mean % cross-reactivity values for each cross-reactive β-amyloid species tested at four levels of pooled human CSF samples are summarized for each analyte:

a. Lumipulse ***G*** β-Amyloid 1-42

Cross-Reactant	Test Concentration (pg/mL)	Mean % Cross-Reactivity
β-amyloid $_{1-37}$	10,000	1.4
β-amyloid $_{1-38}$	10,000	2.0
β-amyloid $_{1-40}$	10,000	-0.3
β-amyloid $_{17-42}$	10,000	-0.6
β-amyloid $_{3-42}$	10,000	0.5
β-amyloid $_{1-43}$	5,000	-49.9

b. Lumipulse ***G*** β-Amyloid 1-40

Cross-Reactant	Test Concentration (pg/mL)	Mean % Cross-Reactivity (N=4)
β-amyloid $_{1-37}$	200,000	-1.3
β-amyloid $_{1-38}$	200,000	-2.0
β-amyloid $_{1-39}$	200,000	0.0
β-amyloid $_{1-42}$	200,000	-2.0
β-amyloid $_{11-40}$	200,000	6.1
β-amyloid $_{17-40}$	200,000	13.4
β-amyloid $_{3-40}$	200,000	1.6
β-amyloid $_{1-43}$	200,000	-0.6

The Lumipulse ***G*** β-Amyloid 1-42 assay showed no significant cross-reactivity (within ±10% difference of test from Control) up to 10,000 pg/mL for the tested cross-reactants except β-Amyloid $_{1-43}$. The Lumipulse ***G*** β-Amyloid 1-40 assay showed no noticeable cross-reactivity up to 200,000 pg/mL for the tested cross-reactants except β-amyloid $_{17-40}$.

4. Assay Reportable Range:

The Lumipulse ***G*** β-Amyloid Ratio (1-42/1-40) combines the results of the Lumipulse ***G*** β-Amyloid 1-42 assay and the Lumipulse ***G*** β-Amyloid 1-40 assay from the same patient CSF specimen into a numerical value in a range of 0.001 to 1.000.

The analytical measuring interval (AMI) for the two component assays are:

- 38 – 2,200 pg/mL for the Lumipulse ***G*** β-Amyloid 1-42
- 158 28,450 pg/mL for the Lumipulse ***G*** β-Amyloid 1-40

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

1) Traceability
The calibrators for use with the Lumipulse ***G*** β-Amyloid 1-42 were prepared gravimetrically under internal quality system and the levels were assigned to the average

value of ERM-DA480/IFCC, ERM-DA481/IFCC and ERM-DA482/IFCC. The calibrators for use with Lumipulse ***G*** β-Amyloid 1-40 were prepared gravimetrically under internal quality system and are traceable to INNOTEST β-Amyloid 1-40.

2) Stability
Stability for the Lumipulse ***G*** β-Amyloid Ratio (1-42/1-40) is based on reagent stability for the individual component assays and the ratio. The Lumipulse ***G*** β-Amyloid Ratio (1-42/1-40) 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*** β-Amyloid 1-42 and the Lumipulse ***G*** β-Amyloid 1-40 were evaluated based on the following studies:

a. Reagent shelf-life:
To evaluate reagent shelf-life stability, the Lumipulse ***G*** β-Amyloid 1-42 and the Lumipulse ***G*** β-Amyloid 1-40 reagents were stored at 2–10°C. Three pooled human CSF samples with varying β-amyloid $_{1-42}$ and β-amyloid $_{1-40}$ concentrations were tested at Day 2, 3, 4, 5, 6, 7, 10, 13, 19, and 25 months after study initiation. The test results obtained at the different time points were compared to the test results at Day 0. Results showed that the Lumipulse ***G*** β-Amyloid 1-42 and the Lumipulse ***G*** β-Amyloid 1-40 reagents are stable at 2–10°C for up to 19 months.

b. Reagent on-board stability:
To evaluate reagent on-board stability, the Lumipulse ***G*** β-Amyloid 1-42 and the Lumipulse ***G*** β-Amyloid 1-40 reagents were stored on-board the LUMIPULSE ***G*** 1200 System for a maximum of 30 days. Three pooled human CSF samples with varying β-amyloid $_{1-42}$ and β-amyloid $_{1-40}$ concentrations were tested at Day 1, 8, 15, and 31 after study initiation. The test results obtained at the different time points were compared to the test results at Day 1. Results showed that the Lumipulse ***G*** β-Amyloid 1-42 and the Lumipulse ***G*** β-Amyloid 1-40 reagents are stable on-board the LUMIPULSE ***G*** 1200 System for up to 15 days.

c. Transport stability:
To evaluate reagent transport stability, the Lumipulse ***G*** β-Amyloid 1-42 and the Lumipulse ***G*** β-Amyloid 1-40 reagents were stored at two stressed conditions: *(i)* 25 ± 2°C for 6 hours and *(ii)* -5 ± 2°C for 6 hours. Three pooled human CSF samples with varying β-amyloid $_{1-42}$ and β-amyloid $_{1-40}$ concentrations were tested. The test results obtained at stressed conditions *(i)* and *(ii)* were compared to the test results at normal storage condition (2−10°C). The Lumipulse ***G*** β-Amyloid 1-42 and the Lumipulse ***G*** β-Amyloid 1-40 reagents are stable at 25 °C and -5 °C for up to 6 hours.

d. Sample stability:
CSF samples were evaluated for stability under storage conditions and freeze/thaw cycles.

i. Sample storage stability: Seven CSF samples with varying β-amyloid $_{1-42}$ and β-amyloid $_{1-40}$ concentrations were freshly collected to challenge various β-amyloid $_{(1-42/1-40)}$ ratios ranged 0.041 to 0.108 . The β-amyloid $_{1-42}$ concentration and the β-amyloid $_{1-40}$ concentration were measured at Day 0. The CSF samples were stored at four different temperatures: *(i)* -80 °C, (*ii)* -20 ± 10°C, *(iii)* 2–10°C, and *(iv)* 25 ± 2°C for various storage durations.

ii. Sample freeze/thaw stability: Fifteen CSF samples with varying β-amyloid $_{1-42}$ and β-amyloid $_{1-40}$ concentrations were freshly collected to challenge various β-amyloid $_{(1-42/1-40)}$ ratios ranged 0.034 to 0.110). The β-amyloid $_{1-42}$ concentration and the β-amyloid $_{1-40}$ concentration were measured before the test samples were initially frozen. The CSF samples underwent repeated freeze/thaw cycles: frozen at -80 °C and thawed at room temperature (15–25 °C).

The CSF sample stability for the Lumipulse ***G*** β-Amyloid Ratio (1-42/1-40) were summarized in the table below.

CSF Sample Stability				
Storage Temperature				Freeze/Thaw Cycle
-80 °C	-30 – -10°C	2 – 8°C	23 – 27°C	
1 month	2 weeks	8 days	48 hours	Up to 3

6. Detection Limit:

The detection capability for the Lumipulse ***G*** β-Amyloid Ratio (1-42/1-40) is based on the detection capability for its component assays, the Lumipulse ***G*** β-Amyloid 1-42 and Lumipulse ***G*** β-Amyloid 1-40. The results below the lower limit of the measuring interval of each assay are not imported and do not yield a ratio result.

The detection capabilities for the Lumipulse ***G*** β-Amyloid 1-42 and Lumipulse ***G*** β-Amyloid 1-40 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 two lots of Lumipulse ***G*** β-Amyloid 1-42 and two lots of Lumipulse ***G*** β-Amyloid 1-40 on one LUMIPULSE ***G*** 1200 System.

1) Lumipulse ***G*** β-Amyloid 1-42:

For LoB, a set of four analyte-depleted CSF 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. The LoB was determined for each lot as 2.18 pg/mL and 1.06 pg/mL. The claimed LoB is the higher value from the two lots as 2.18 pg/mL.

For LoD, a set of seven low level CSF samples with target concentrations of β-amyloid $_{1-42}$ between 10 22 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 calculated using a precision profile approach. The LoD was determined for each lot as

10.6 pg/mL and 11.6 pg/mL. The claimed LoD is the higher value from both lots as 11.6 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 and the higher LoQ value from both lots was determined. The claimed LoQ was 38 pg/mL at the low limit of the analytical measuring interval of Lumipulse ***G*** β-Amyloid 1-42.

2) Lumipulse ***G*** β-Amyloid 1-40:

For LoB, a set of four analyte-depleted CSF 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. The LoB was determined for each lot as 0.97 pg/mL and 0.35 pg/mL. The claimed LoB is the higher value from the two lots as 0.97 pg/mL.

For LoD, a set of six low level CSF samples with target concentrations of β-amyloid $_{1\text{-}40}$ between 25–50 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 calculated using precision profile. The LoD was determined for each lot as 29.4 pg/mL and 33.0 pg/mL. The claimed LoD is the higher value from both lots as 33.0 pg/mL.

The claimed LoQ was 158 pg/mL (precision was %CV<20%) at the low limit of the analytical measuring interval of Lumipulse ***G*** β-Amyloid 1-40.

7. Assay Cut-Off:

The Lumipulse ***G*** β-Amyloid Ratio (1-42/1-40) assay cutoff was determined by analyzing a total of 235 patients with both CSF and positron emission tomography (PET) data from the Amsterdam Dementia Cohort (ADC) which are distinct from the subjects (from the Alzheimer's Disease Neuroimaging Initiative (ADNI) sample bank) evaluated in the pivotal clinical validation study (see Section C. Clinical Studies below). Among 235 patients, 105 patients with AD dementia, 25 with mild cognitive impairment (MCI), 53 with subjective cognitive decline (SCD) and 52 with other causes of cognitive decline. The procedures of CSF collection, processing and handling were conducted according to a standardized ADC protocol. Three PET tracers (Florbetaben (^{18}F), N=156); Florbetapir (^{18}F), N=8; Flutemetamol (^{18}F), N=71) were used for PET imaging of amyloid deposits in patients. Images were analyzed by visual read and scored as either amyloid PET positive or amyloid PET negative. Amyloid PET positivity was found in 137 of 235 patients (58.3%) and amyloid PET negativity was found in 98 of 235 patients (41.7%). The Receiver Operating Characteristic (ROC) distinguishing the amyloid PET positive and amyloid PET negative groups was computed from the Lumipulse ***G*** β-Amyloid Ratio (1-42/1-40) results. Sensitivity and specificity analyses were used to define the Lumipulse ***G*** β-Amyloid Ratio (1-42/1-40) positivity at 0.058 for amyloid PET positivity by visual read.

Because CSF sample collection and handling variations between the ADC and ADNI standard protocols (see Section C, 10. Clinical Performance, using patient samples obtained

from the ADNI study) can impact the Lumipulse ***G*** β-Amyloid Ratio (1-42/1-40) measurements, a pre-analytical bridging study was conducted using fresh prospectively collected individual CSF samples. Considering the bridging study results and additional potential factors that may impact the Lumipulse ***G*** β-Amyloid Ratio (1-42/1-40) measurements, the test cutoff was established in the table below.

Lumipulse ***G*** β-Amyloid Ratio (1-42/1-40)	Test Result	Interpretation
Ratio ≤ 0.058	Positive	Result is consistent with a positive amyloid PET scan result.
0.059 ≤ Ratio ≤ 0.072	Likely Positive	Result is more likely consistent with a positive amyloid PET scan result.
Ratio ≥ 0.073	Negative	Result is consistent with a negative amyloid PET scan result.

B Comparison Studies:

8. Method Comparison:

   Not applicable

9. Matrix Comparison:

   Not applicable; CSF is the only claimed sample matrix.

C Clinical Studies:

10. Clinical Performance:

    A clinical study was conducted to evaluate the performance of the Lumipulse ***G*** β-Amyloid Ratio (1-42/1-40) 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 an amyloid PET imaging agent. The study included 292 patients aged 55 to 90 years and with sufficient volume of banked CSF samples obtained from the Alzheimer's Disease Neuroimaging Initiative (ADNI) sample bank. A standardized ADNI pre-analytical protocol was used for collection, processing and handling the CSF samples. All study subjects had a clinical diagnosis and had undergone Florbetapir (^{18}F) PET evaluation. The time interval between CSF sampling and PET evaluation was found for 267 subjects within 30 days (91.4%, 267/292), 281 subjects within 60 days (96.2%, 281/292), and 288 subjects within 90 days (98.6%, 288/292).

    The study population consists of 56.8% (166/292) males with a mean age of 73 years (range 55 90 years with a median 73 years) and 43.2% females (126/292) with a mean age of 72

years (range 55–90 years with a median 72 years). The majority of study subjects were White (92.8%, 271/292).

Among 292 study subjects, 6.2% (18/292) were diagnosed with subjective cognitive decline (SCD), 38.0% (111/292) with early mild cognitive impairment (EMCI), 20.2% (59/292) with late mild cognitive impairment (LMCI), and 35.6% (104/292) with AD.

The amyloid PET status of each patient was determined by a minimum of three trained independent readers (two readers and an adjudicator when the first two readers disagree) who were blinded to all other clinical data. Among 292 study subjects, 9.9% (29/292) patients whose PET determinations went to adjudication, and the adjudicated PET read was used in the analysis. PET-positivity was found in 68.2% (199/292) of the study population.

The demographic and clinical characteristics of the study subjects according to diagnostic groups and PET scan status are presented in the table below.

		Diagnostic Groups				**Visual Amyloid PET Read**		**Total = 292** % (N)
		SCD = 18 % (N)	**EMCI = 111** % (N)	**LMCI = 59** % (N)	**AD = 104** % (N)	**Positive =199** % (N)	**Negative =93** % (N)	
Sex	Male	61.1% (11)	53.2% (59)	54.2% (32)	61.5% (64)	58.8% (117)	52.7% (49)	56.8% (166)
	Female	38.9% (7)	46.8% (52)	45.8% (27)	38.5% (40)	41.2% (82)	47.3% (44)	43.2% (126)
Age (years)	55 59	0.0% (0)	4.5% (5)	3.4% (2)	4.8% (5)	4.0 (8)	4.3 (4)	4.1% (12)
	60 69	22.2% (4)	38.7% (43)	27.1% (16)	21.2% (22)	24.1% (48)	39.8% (37)	29.1% (85)
	70 79	55.6% (10)	43.2% (48)	57.6% (34)	51.9% (54)	53.8% (107)	41.9% (39)	50.0% (146)
	≥80	22.2% (4)	13.5% (15)	11.9% (7)	22.1% (23)	18.1% (36)	14.0% (13)	16.8% (49)
	Mean	75	71	73	74	-	-	73
	Median	74	71	74	74	-	-	73
	Min.	66	57	55	55	-	-	55
	Max.	85	88	88	90	-	-	90
Race	White	83.3% (15)	92.8% (103)	96.6% (57)	92.3% (96)	95.5% (190)	87.1% (81)	92.8% (271)
	Asian	0.0% (0)	1.8% (2)	0.0% (0)	4.8% (5)	1.5% (3)	4.3% (4)	2.4% (7)
	African American	5.6% (1)	0.9% (1)	1.7% (1)	1.9% (2)	1.0% (2)	3.2% (3)	1.7% (5)
	Others*	11.1% (2)	4.5% (5)	1.7% (1)	1.0% (1)	2.0% (4)	5.4% (5)	3.1% (9)

MMSE**	<18	0.0% (0)	0.0% (0)	0.0% (0)	1.0% (1)	0.5% (1)	0.0% (0)	0.3% (1)
	18-23	0.0% (0)	0.9% (1)	0.0% (0)	52.9% (55)	25.1% (50)	6.5% (6)	19.2% (56)
	24-30	100.0% (18)	99.1% (110)	100.0% (59)	46.2% (48)	74.4% (148)	93.5% (87)	80.5% (235)
Apolipo-protein E (ApoE) Status	$\varepsilon2\varepsilon3$	16.7% (3)	9.0 % (10)	10.2% (6)	3.8% (4)	3.0% (6)	18.3% (17)	7.9% (23)
	$\varepsilon2\varepsilon4$	0.0% (0)	0.9% (1)	3.4% (2)	1.0% (1)	1.5% (3)	1.1% (1)	1.4% (4)
	$\varepsilon3\varepsilon3$	44.4% (8)	48.6% (54)	39.0% (23)	32.7% (34)	31.7 % (63)	60.2% (56)	40.8% (119)
	$\varepsilon3\varepsilon4$	33.3% (6)	36.0% (40)	39.0% (23)	43.3% (45)	48.7% (97)	18.3% (17)	39.0% (114)
	$\varepsilon4\varepsilon4$	5.6% (1)	5.4% (6)	8.5% (5)	19.2% (20)	15.1% (30)	2.1% (2)	10.9% (32)
Years of Education	≤13	16.7% (3)	26.1% (29)	18.6% (11)	18.3% (19)	23.6% (47)	16.1% (15)	21.2% (62)
	>13	83.3% (15)	73.9% (82)	81.4% (48)	81.7% (85)	76.4% (152)	83.9% (78)	78.8% (230)
Visual Amyloid PET Read	Positive	38.9% (7)	42.3% (47)	55.9% (33)	90.4% (94)	-	-	68.2% (199)
	Negative	61.1% (11)	57.7% (64)	44.1% (26)	9.6% (10)	-	-	31.8% (93)

* including Hawaiian / Pacific Islander

** Mini-Mental State Examination (MMSE)

The above table indicates the majority of the study subjects (80.5%, 235/292) were considered in absence of clinical presentation of dementia based on the Mini-Mental State Examination (MMSE) score between 24 30. The APOE $\varepsilon3\varepsilon4$ and $\varepsilon4\varepsilon4$ genotype, which more often are shown in patients with AD dementia was found in 39% (114/292) and 10.9% (32/292), respectively, of the study population. Most study subjects received >13 years of education (78.8%, 230/292). The diagnostic groups did not differ with respect to age or sex. Patients with AD dementia had lower MMSE scores than those with MCI and SCD. Patients with AD dementia also showed more often a positive PET scan than MCI patients, who in turn had more often a positive PET scan than those with SCD. The prevalence of amyloid positivity among AD was 90.4% (94/104) and it was 55.9% (33/59) and 42.3% (47/111) among those with LMCI and EMCI, respectively.

Results:

1) Clinical Performance

To estimate the clinical performance characteristics of the Lumipulse ***G*** β-Amyloid Ratio (1-42/1-40), the test result was compared to the visual amyloid PET read for each patient. The data are summarized in the table below.

		Visual Amyloid PET Read		
		Positive	**Negative**	**Total**
Lumipulse *G* β-Amyloid Ratio (1-42/1-40)	**Positive (Ratio ≤ 0.058)**	171	6	177
	Likely positive (0.059 ≤ Ratio ≤ 0.072)	13	9	22
	Negative (Ratio ≥ 0.073)	15	78	93
	Total	199	93	292

The performance estimates of the Lumipulse ***G*** β-Amyloid Ratio (1-42/1-40) 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, likely positive and negative test results and it is summarized in the table below.

		Visual Amyloid PET Read		**Predictive Value % (n/N) (95% CI)**	**Frequency of Results % (n/N) (95% CI)**
		Positive	**Total**		
Lumipulse *G* β-Amyloid Ratio (1-42/1-40)	**Positive (Ratio ≤ 0.058)**	171	177	96.6% (171/177) (92.8%; 98.4%)	60.6% (177/292) (54.9%; 66.1%)
	Likely positive (0.059 ≤ Ratio ≤ 0.072)	13	22	59.1% (13/22) (38.7%; 66.7%)	7.5% (22/292) (5.0%; 11.1%)
	Negative (Ratio ≥ 0.073)	15	93	16.1% (15/93) (10.0%; 24.9%)	31.8% (93/292) (26.8%; 37.4%)
	Total	199	292	*Prevalence of PET positive = 68.2%*	

Confidence intervals were calculated as confidence intervals for binomial proportions using a Wilson score method.

*In conclusion, the data of clinical performance study support that positive Lumipulse **G** β-Amyloid Ratio (1-42/1-40) results are consistent with PET scan positive results and negative Lumipulse **G** β-Amyloid Ratio (1-42/1-40) results are consistent with PET scan negative results.*

2) Sub-group Analysis

a. Diagnostic group

The performance measures of the Lumipulse ***G*** β-Amyloid Ratio (1-42/1-40) stratified by diagnostic groups are summarized in the table below.

Diagnosis	**Test Result**	**Visual Amyloid PET Read**		**Predictive Value % (n/N) (95% CI)**	**Frequency of Results % (n/N) (95 %CI)**
		Positive (n)	**N**		
AD (N=104)	Positive (Ratio ≤ 0.058)	89	90	98.9% (89/90) (94.0%; 99.8%)	86.5% (90/104) (78.7%; 91.8%)
	Likely positive (0.059 ≤ Ratio ≤ 0.072)	3	4	75.0% (3/4) (30.1%; 95.4%)	3.8% (4/104) (1.5%; 9.5%)
	Negative (Ratio ≥ 0.073)	2	10	20.0% (2/10) (5.7%; 51.0%)	9.6% (10/104) (5.3%; 16.8%)
LMCI (N=59)	Positive (Ratio ≤ 0.058)	32	33	97.0 (32/33) (84.7%; 99.5%)	55.9% (33/59) (43.3%; 67.8%)
	Likely positive (0.059 ≤ Ratio ≤ 0.072)	4	5	80.0% (4/5) (37.6%; 96.4%)	8.5% (5/59) (3.7%; 18.4%)
	Negative (Ratio ≥ 0.073)	4	21	19.0 (4/21) (7.7%; 40.0%)	35.6% (21/59) (24.6%; 48.3%)
EMCI (N=111)	Positive (Ratio ≤ 0.058)	43	47	91.5% (43/47) (80.1%; 96.6%)	42.3 (47/111) (33.6%; 51.6%)
	Likely positive (0.059 ≤ Ratio ≤ 0.072)	5	10	50.0% (5/10) (23.7%; 76.3%)	9.0% (10/111) (5.0%; 15.8%)
	Negative (Ratio ≥ 0.073)	8	54	14.8% (8/54) (7.7%; 26.6%)	48.6% (54/111) (39.6%; 57.8%)
SCD (N=18)	Positive (Ratio ≤ 0.058)	7	7	100.0% (7/7) (64.6%; 100.0%)	38.9% (7/18) (20.3%; 61.4%)
	Likely positive (0.059 ≤ Ratio ≤ 0.072)	1	3	33.3% (1/3) (6.1% 79.2%)	16.7% (3/18) (5.8%; 39.2%)
	Negative (Ratio ≥ 0.073)	1	8	12.5% (1/8) (2.2%; 47.1%)	44.4% (8/18) (24.6%; 66.3%)

Across diagnostic groups, the Predictive values (PV) were highest for the positive test results with a decrease for the likely positive test results and lowest in the negative test results within each diagnostic group. The estimate of PV was consistently high for AD (99%), LMCI (97%), EMCI (92%), and SCD (100.0%). for the positive test results. The estimate of PV for the negative test results was 12.5% for SCD, 14.8% for EMCI, 19.0% for LMCI, and 20.0% for AD in each diagnostic group. The % of the likely positive test results within each diagnostic group was 3.8% for AD, 8.5% for LMCI, 9.0% for EMCI, and 16.7% for SCD that comprised 7.5% across all diagnostic groups.

b. Sex

The performance measures of the Lumipulse ***G*** β-Amyloid Ratio (1-42/1-40) were also stratified by sex and summarized in the table below.

Sex	**Test Result**	**Visual Amyloid PET Read**		**Predictive Value % (n/N) (95% CI)**	**Frequency of Results % (n/N) (95% CI)**
		Positive (n)	**N**		
Male (N=166)	Positive (Ratio ≤ 0.058)	98	102	96.1% (98/102) (90.3%; 98.5%)	61.4% (102/166) (53.9%; 68.5%)
	Likely positive (0.059 ≤ Ratio ≤ 0.072)	7	11	63.6% (7/11) (35.4%; 84.8%)	6.6% (11/166) (3.7%; 11.5%)
	Negative (Ratio ≥ 0.073)	12	53	22.6% (12/53) (13.5%; 35.5%)	31.9% (53/166) (25.3%; 39.4%)
Female (N=126)	Positive (Ratio ≤ 0.058)	73	75	97.3% (73/75) (90.8%; 99.3%)	59.5% (75/126) (50.8%; 67.7%)
	Likely positive (0.059 ≤ Ratio ≤ 0.072)	6	11	54.5% (6/11) (28.0%; 78.7%)	8.7% (11/126) (4.9%; 15.0%)
	Negative (Ratio ≥ 0.073)	3	40	7.5% (3/40) (2.6%; 19.9%)	31.7% (40/126) (24.3%; 40.3%)

The estimates of PV for the positive test results were similar for males (96%) and females (97%). The estimates of PV for the likely positive test results were 63.6% and 54.5% for males and females, respectively. The estimates of PV for the negative test results were 22.6% for males and 7.5% for females. The % of the likely positive test results was similar for males (6.6%) and females (8.7%).

c. Age

The performance measures of the Lumipulse ***G*** β-Amyloid Ratio (1-42/1-40) were also stratified by age and summarized in the table below.

Age (years)	Result	Visual Amyloid PET Read		Predictive Value % (n/N) (95% CI)	Frequency of Results % (n/N) (95% CI)
		Positive (n)	N		
55-59 (N=12)	Positive (Ratio ≤ 0.058)	6	7	85.7% (6/7) (48.7%; 97.4%)	58.3% (7/12) (32.0%; 80.7%)
	Likely positive (0.059 ≤ Ratio ≤ 0.072)	0	2	0.0% (0/2) (0.0%; 65.8%)	16.7% (2/12) (4.7%; 44.8%)
	Negative (Ratio ≥ 0.073)	2	3	66.7% (2/3) (20.8%; 93.9%)	25.0% (3/12) (8.9%; 53.2%)
60-69 (N=85)	Positive (Ratio ≤ 0.058)	41	43	95.3% (41/43) (84.5%; 98.7%)	50.6% (43/85) (40.2%; 61.0%)
	Likely positive (0.059 ≤ Ratio ≤ 0.072)	2	5	40.0% (2/5) (11.8%; 76.9%)	5.9% (5/85) (2.5%; 13.0%)
	Negative (Ratio ≥ 0.073)	5	37	13.5 (5/37) (5.9%; 28.0%)	43.5% (37/85) (33.5%; 54.1%)
70-79 (N=146)	Positive (Ratio ≤ 0.058)	95	97	97.9% (95/97) (92.8%; 99.4%)	66.4% (97/146) (58.4%; 73.6%)
	Likely positive (0.059 ≤ Ratio ≤ 0.072)	8	11	72.7% (8/11) (43.4%; 90.3%)	7.5% (11/146) (4.3%; 13.0%)
	Negative (Ratio ≥ 0.073)	4	38	10.5% (4/38) (4.2%; 24.1%)	26.0% (38/146) (19.6%; 33.7%)
≥80 (N=49)	Positive (Ratio ≤ 0.058)	29	30	96.7% (29/30) (83.3%; 99.4%)	61.2% (30/49) (47.2%; 73.6%)
	Likely positive (0.059 ≤ Ratio ≤ 0.072)	3	4	75.0% (3/4) (30.1%; 95.4%)	8.2% (4/49) (3.2%; 19.2%)
	Negative (Ratio ≥ 0.073)	4	15	26.7% (4/15) (10.9%; 52.0%)	30.6% (15/49) (19.5%; 44.5%)

Across age groups, the Predictive values (PV) were highest for the positive test results with a decrease for the likely positive test results and lowest in the negative test results within each age group. The estimate of PV was 86% for age group 55 59. PV was above 95% for age groups 60 and older. The % of the likely positive test results was highest 16.7% for age group 55–59 and 6-8% for age groups 60 and older.

d. Race

The performance measures of the Lumipulse ***G*** β-Amyloid Ratio (1-42/1-40) were also stratified by race and summarized in the table below.

Race	**Result**	**Visual Amyloid PET Read**		**Predictive Value % (n/N) (95% CI)**	**Frequency of Results % (n/N) (95% CI)**
		Positive (n)	**N**		
White (N=271)	Positive (Ratio ≤ 0.058)	163	169	96.4% (163/169) (92.5%; 98.4%)	62.4% (169/271) (56.5%; 67.9%)
	Likely positive (0.059 ≤ Ratio ≤ 0.072)	12	20	60.0% (12/20) (38.7%; 78.1%)	7.4% (20/271) (4.8%; 11.1%)
	Negative (Ratio ≥ 0.073)	15	82	18.3% (15/82) (11.4%; 28.0%)	30.3% (82/271) (25.1%; 36.0%)
Asian (N=7)	Positive (Ratio ≤ 0.058)	3	3	100.0% (3/3) (43.9%; 100.0%)	42.9% (3/7) (15.8%; 75.0%)
	Likely positive (0.059 ≤ Ratio ≤ 0.072)	0	0	-	0.0% (0/7) (0.0%; 35.4%)
	Negative (Ratio ≥ 0.073)	0	4	0.0% (0/4) (0.0%; 49.0%)	57.1% (4/7) (25.0%; 84.2%)
African American (N=5)	Positive (Ratio ≤ 0.058)	2	2	100.0% (2/2) (34.2%; 100.0%)	40.0% (2/5) (11.8%; 76.9%)
	Likely positive (0.059 ≤ Ratio ≤ 0.072)	0	0	-	0.0% (0/5) (0.0%; 43.4%)
	Negative (Ratio ≥ 0.073)	0	3	0.0% (0/3) (0.0%; 56.1%)	60.0% (3/5) (23.1%; 88.2%)
Others* (N=9)	Positive (Ratio ≤ 0.058)	3	3	100.0% (3/3) (43.9%; 100.0%)	33.3% (3/9) (12.1%; 64.6%)
	Likely positive (0.059 ≤ Ratio ≤ 0.072)	1	2	50.0% (1/2) (9.5%; 90.5%)	22.2% (2/9) (6.3%; 54.7%)
	Negative (Ratio ≥ 0.073)	0	4	0.0% (0/4) (0.0%; 49.0%)	44.4% (4/9) (18.9%; 73.3%)

* including Hawaiian / Pacific Islander

Across race groups, the Predictive values (PV) were highest for the positive test results with a decrease for the likely positive test results and lowest in the negative test results within each race group. For White patients, the estimate of PV was 96.4%, 60.0%, and 18.3% for the positive test results, likely positive test results, and negative results, respectively. The % of likely positive results was 7.4% for the White group. The estimates of PV and % of the likely positive results for Asian, African American, and other races had high uncertainty due to limited numbers of patients in the study.

D Clinical Cut-Off:

Refer to Assay Cut-off

E Reference Interval / Expected Values:

1) Reference Interval

A reference interval of the Lumipulse ***G*** β-Amyloid Ratio (1-42/1-40) in cognitively normal subjects aged 55 years and above was determined in accordance with the CLSI guideline EP28-A3c. Cognitively normal subjects were defined as individuals who did not meet clinical criteria for a diagnosis of AD and MCI and had an MMSE of 29 30. A total of 281 human CSF samples including 229 samples from a longitudinal population-based study of normal aging (BIOCARD study) and 52 samples from the real-world clinics at the Washington University Medical Campus. After excluding 69 samples from subjects aged <55 years old and samples with no valid result for the Lumipulse ***G*** β-Amyloid Ratio (1-42/1-40) (individual assay results outside AMI of the Lumipulse ***G*** β-Amyloid 1-42 and the Lumipulse ***G*** β-Amyloid 1-40), the remaining 212 evaluable CSF samples were tested. These samples were collected from 98 males and 114 females aged 55 to 92 years (mean: 64 years and median: 62 years) who presented no sign of cognitive impairments within 6 months of clinical evaluation. The results of the Lumipulse ***G*** β-Amyloid Ratio (1-42/1-40) tested in these cognitively normal subjects are summarized in the tables below:

	Cognitively Normal						
	All	**Age**			**Race**		
		55-59	**60-69**	**70-92**	**White**	**African American**	**Other**
N	212	58	109	45	149	55	8
Lumipulse G β-Amyloid Ratio (1-42/1-40)							
Mean	0.082	0.088	0.082	0.076	0.081	0.085	0.084
SD	0.019	0.015	0.017	0.024	0.020	0.016	0.011
Median	0.088	0.091	0.088	0.087	0.087	0.088	0.085
Minimum, Maximum	0.028, 0.112	0.033, 0.112	0.039, 0.110	0.028, 0.112	0.028, 0.112	0.033, 0.112	0.06, 0.099
2.5th Percentile, 97.5th Percentile	0.039, 0.107	0.033, 0.112	0.040, 0.106	0.028, 0.112	0.039, 0.107	0.033, 0.112	0.061, 0.099

Lumipulse G β-Amyloid Ratio (1-42/1-40) (%, n)							
Positive	14.2% (30)	8.6% (5)	11.0% (12)	28.9% (13)	16.8% (25)	9.1 % (5)	0.0% (0)
Likely Positive	11.3% (24)	3.4% (2)	17.4% (19)	6.7% (3)	12.1% (18)	9.1% (5)	12.5% (1)
Negative	74.5% (158)	87.9% (51)	71.6% (78)	64.4% (29)	71.1% (106)	81.8% (45)	87.5% (7)

The number of cognitively normal subjects tested positive at a ratio ≤ 0.058 with Lumipulse ***G*** β-Amyloid Ratio (1-42/1-40) increased with age. The medians for Lumipulse ***G*** β-Amyloid Ratio (1-42/1-40) did not differ significantly across age and race.

2) Expected Values

The expected values for non-AD dementia and other neurological conditions were investigated. A total of 454 evaluable human CSF samples with clinical diagnostic information obtained from longitudinal population-based studies of the Biomarkers for Older Controls at Risk for Dementia (BIOCARD) study were included in analyzing expected values. The test samples were collected from individuals 22 years and older who presented with an appropriate diagnosis of non-AD dementia and other neurological conditions. The test results are summarized in the tables below:

	Alcoholic* Dementia	**Frontotemporal Dementia**	**Hemorrhage**	**Hippocampal Sclerosis**	**Hypoxia**
N	9	40	10	39	8
Lumipulse G β-Amyloid Ratio (1-42/1-40)					
Mean	0.085	0.080	0.082	0.091	0.077
SD	0.020	0.020	0.020	0.016	0.015
Median	0.089	0.087	0.083	0.096	0.079
Minimum, Maximum	0.038, 0.107	0.034, 0.108	0.037, 0.108	0.050, 0.112	0.049, 0.099
2.5th Percentile, 97.5th Percentile	-	0.034, 0.108	-	0.050, 0.112	-
Lumipulse G β-Amyloid Ratio (1-42/1-40) (%, n)					
Positive	11.1% (1)	22.5% (9)	10.0% (1)	10.3% (4)	12.5% (1)
Likely Positive	0.0% (0)	15.0% (6)	0.0% (0)	5.1% (2)	25.0% (2)
Negative	88.9% (8)	62.5% (25)	90.0% (9)	84.6% (33)	62.5% (5)

* including Wernicke-Korsakoff Syndrome

	Ischemia	Lewy Body Dementia	Multiple Sclerosis	Necrosis	Normal Pressure Hydrocephalus
N	10	80	40	10	40
Lumipulse G β-Amyloid Ratio (1-42/1-40)					
Mean	0.077	0.064	0.090	0.090	0.091
SD	0.021	0.021	0.007	0.005	0.017
Median	0.085	0.063	0.090	0.089	0.098
Minimum, Maximum	0.037, 0.099	0.019, 0.104	0.064, 0.106	0.080, 0.098	0.049, 0.110
2.5th Percentile, 97.5th Percentile	-	0.021, 0.104	0.064, 0.106	-	0.049, 0.110
Lumipulse G β-Amyloid Ratio (1-42/1-40) (%, n)					
Positive	20.0% (2)	40% (32)	0.0% (0)	0.0% (0)	7.5% (3)
Likely Positive	0.0% (0)	31.3% (25)	5.0% (2)	0.0% (0)	7.5% (3)
Negative	80.0% (8)	28.8% (23)	95.0% (38)	100.0% (10)	85.0% (34)

	Parkinson's Disease	Stroke	Traumatic Brain Injury	Vascular Dementia	Huntington's Disease
N	40	38	39	48	3
Lumipulse G β-Amyloid Ratio (1-42/1-40)					
Mean	0.082	0.081	0.080	0.087	0.077
SD	0.018	0.018	0.014	0.017	0.004
Median	0.089	0.086	0.081	0.091	0.075
Minimum, Maximum	0.035, 0.102	0.040, 0.107	0.042, 0.113	0.038, 0.117	0.074, 0.081
2.5th Percentile, 97.5th Percentile	0.035, 0.102	-	0.042, 0.113	0.039, 0.115	-
Lumipulse G β-Amyloid Ratio (1-42/1-40) (%, n)					
Positive	15.0% (6)	21.1% (8)	7.7% (3)	6.3% (3)	0.0% (0)
Likely Positive	10.0% (4)	2.6% (1)	15.4% (6)	10.4% (5)	0.0% (0)
Negative	75.0% (30)	76.3% (29)	76.9% (30)	83.3% (40)	100.0% (3)

F Other Supportive Performance Characteristics Data:

None

VII Proposed Labeling

The labeling supports the decision to grant the De Novo request for this device.

VIII Identified Risks to Health and Mitigation Measures

Identified Risks to Health	Mitigation Measures
Failure to correctly interpret test results can lead to false positive results (leading to workup and anxiety regarding a serious diagnosis that is incorrect) or false negative results (leading to delays in getting treatment and delays planning early in the course of this progressive disease)	Special Controls (1) and (2)
Incorrect test results that provide false positive results (leading to workup and anxiety regarding a serious diagnosis that is incorrect) or false negative results (leading to delays in getting treatment and delays planning early in the course of this progressive disease)	Special Controls (1) and (2)

IX Summary of Benefit-Risk and Signal Assessment

A Summary of the Assessment of Benefit:

The benefit of this device is large in magnitude due to the value of evaluating patients who exhibit cognitive impairments as an adjunct to identify the cause of cognitive decline based on the β-amyloid ratio $_{(1\text{-}42/1\text{-}40)}$ in CSF. The high agreement of the test results between Lumipulse ***G*** β-Amyloid Ratio (1-42/1-40) and brain PET imaging is expected to reduce unnecessary PET scanning that is mostly available in large medical centers located in limited geographic areas. In addition, the Lumipulse ***G*** β-Amyloid Ratio (1-42/1-40) is expected to benefit cognitively impaired patients with β-amyloid pathology for determining their therapeutic options.

B Summary of the Assessment of Risk:

Risks associated with the use of this device are mainly due to false positive and false negative test results. False positive test results could lead to unnecessary treatment with medications that are FDA-approved for the treatment of cognitive symptoms for the patients with β-amyloid pathology (e.g., aducanumab for AD), which could lead to expense and inappropriate treatment side effects. False negative test results could result in losing potential for additional workups for correct disease diagnosis or for treatments of diseases with β-amyloid pathology. Focusing on the intended patients most significantly affected by false test results, there was one AD patient (0.3%, 1/292) with a false positive test result (this AD patient had negative PET) and two AD patients (0.7%, 2/292) with a false negative test result (these two AD patients had positive PET) in the clinical performance study. Three PET scan-positive (1.0%, 3/292) and one PET scan-negative (0.3%, 1/292) AD patients had a likely positive test result. This test is not a stand-alone assay and other clinical evaluations, or additional tests are used for determining treatment options.

C Patient Perspectives:

This submission did not include specific information on patient perspectives for this device.

D Summary of the Assessment of Benefit-Risk:

The device is intended to be an aid in the evaluation of AD and other causes of cognitive decline. As such, the assay provides clinicians with an additional assessment tool for the condition of β-amyloid pathology in brain. For adults at age 55 and older, as well as for other people, there is currently no laboratory test other than PET imaging, that allows assessment of amyloid load. This is important to patients because most medical centers do not have the technology to assess individuals using the currently available imaging modalities. Additionally, this test is minimally invasive. It will be helpful to physicians counseling patients who have cognitive impairment that may be Alzheimer's disease related.

There are general risks to the use of this test. This test determines amyloid presence, not a clinical diagnosis (such as Alzheimer's disease). Therefore, even when used correctly, additional other testing is required regarding the actual underlying cause of the cognitive complaints. There is the risk of anxiety of a lumbar puncture (although the actual physical risks are minimal). Additional risks are the risks of false positive, which could lead to workup and anxiety regarding a serious diagnosis that is incorrect, and the risk of a false negative, which could delay getting treatment and delay planning early in the course of this progressive disease. It is unclear that these tests will be used judiciously in clinical practice. Because treatments are limited, and available treatment is of questionable usefulness, it is not clear how useful amyloid burden testing would be at this stage given current disease clinical practice. PET imaging, which provides similar results, has not been widely adopted, mainly because of these issues.

The device displays high agreement to the PET scan results. Like the PET scan results, it is essential that this test be used in combination with clinical exam, medical and natural history of the disorder, and other tests. Furthermore, special controls will allow this field of testing, which is currently limited to this test, and to PET scanning, to grow with clear regulatory expectations. Therefore, the probable benefits appear to outweigh the probable risk in light of the special controls established for this device and in combination with general controls.

X Conclusion

The De Novo request is granted and the device is classified under the following and subject to the special controls identified in the letter granting the De Novo request:

Product Code: QSE
Device Type: Alzheimer's disease pathology assessment test
Class: II
Regulation: 21 CFR 866.5840