BIOMARKER DIAGNOSIS OF ALZHEIMER'S DISEASE

Morphometric Imaging Assay

Cut-Off Determination based on the biomarker signal for Non Demented Population and, Sensitivity, Specificity, 95% Confidence Interval for Alzheimer's Disease- Non Alzheimer's Disease Demented Validation Trial

I. Cut-Off Determination based on the biomarker signal for Non Demented (ND) apparently healthy population

The 27 ND's used for this analysis are presented in **Table 1 and Figure 1**. The population data was then ordered according to the Ln(A/N) signal starting with the lowest and ending with the highest value (**Figure 1**). If the number is not an integer, a linear interpolation was made as **X(N) + F*[X(N+1)-X(N)],** where **X(N)** is the N^{th} value, **X(N+1)** is the $N+1^{th}$ value and **F** is the fractional reminder after taking **0.95** of **X(N+1).** Based on this analysis the cut-off is **6.98 (Table 2)**. Once the average cut-off is determined as **6.98** (**Table 2**), the sensitivity, specificity, and confidence intervals can be determined for the trial between AD (N=22) and Non-ADD (N=17).

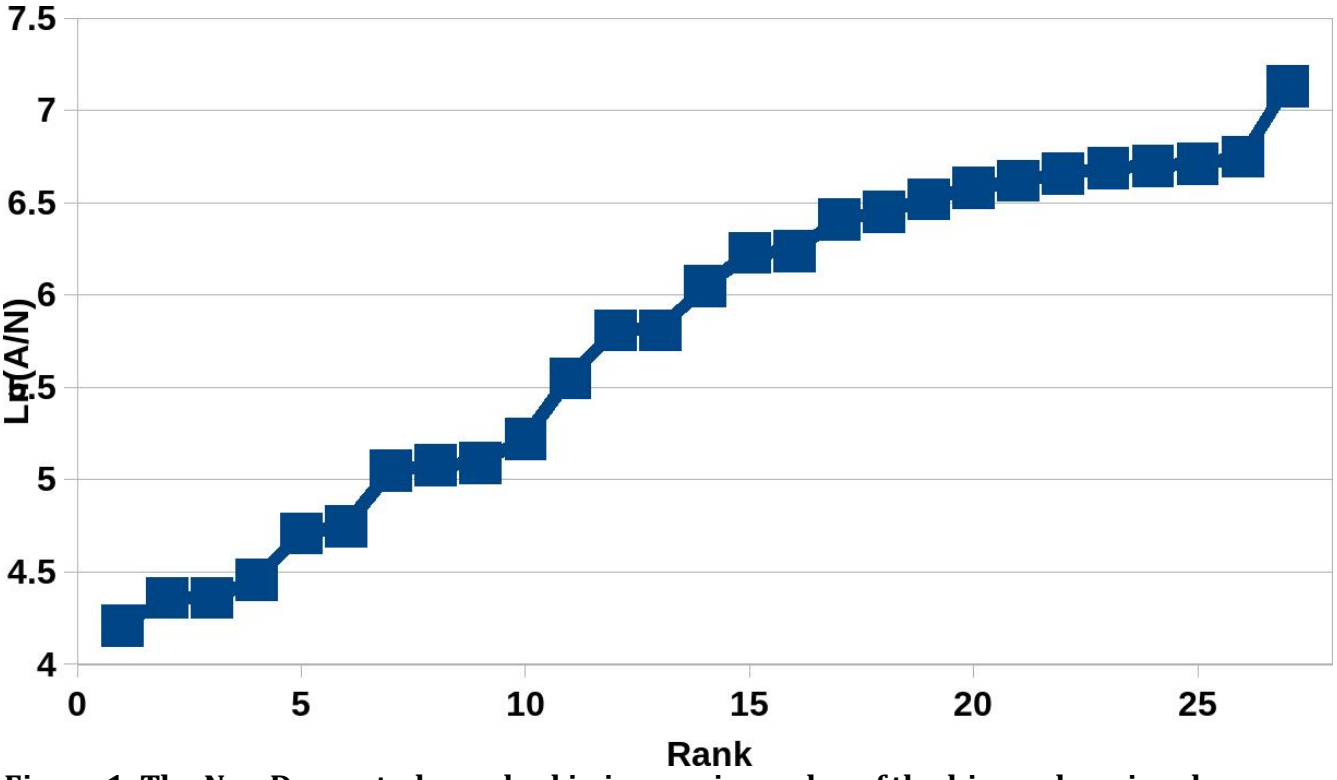

Figure 1. The Non Demented s ranked in increasing order of the biomarker signal, Ln(A/N).

Table 1. Non Demented used for cut-off determination.

#	Sample ID	ln(A/N)	Age	Gender
1	AG09977	4.21	63	F
2	AG11730	4.36	84	M
3	AG12927	4.36	66	F
4	AG12998	4.46	65	M
5	51	4.71	55	M
6	AG04146	4.75	57	M
7	AG07123	5.05	62	M

8	AG13358	5.08	72	F
9	AG04461	5.09	66	M
10	AG07714	5.22	56	F
11	AG12438	5.55	77	M
12	37	5.81	65	F
13	77	5.81	18	M
14	25	6.05	39	M
15	78	6.23	45	F
16	73	6.24	20	F
17	36	6.41	46	M
18	84	6.45	21	M
19	82	6.52	45	F
20	83	6.58	20	M
21	29	6.62	21	M
22	AG05840	6.66	56	F
23	32	6.69	23	M
24	50	6.7	61	M
25	19	6.71	33	M
26	44	6.75	50	F
27	39	7.13	65	F

Table 2. Cut-off determination for N=27.

95 percentiles; N=27			
N	0.95*(N+1)	X(N)+0.60[X(N+1)-X(N)]	Cut-Off
27.00	**26.60**	**6.98**	**6.98**

II. Validation study with Alzheimer's Disease (AD) and Non-Alzheimer's Disease Demented (Non-ADD) populations. Sensitivity, specificity, and confidence intervals for the trial

The AD (N=22) and Non-ADD (N=20) patients for the trial are listed in **Tables 3 A and B**. The sensitivity, specificity, and 95% confidence intervals, for the validation trial, based on the cut-off listed in **Table 2** are presented in **Table 4 and Figure 2.**(Assay Migration Studies for In Vitro Diagnostic Devices Guidance for Industry and FDA Staff Document issued on: April 25, 2013, and Blacker D, Albert MS, Bassett SS, Go RC, Harrell LE, Folstein MF, Reliability and validity of NINCDS-ADRDA criteria for Alzheimer's disease. The National Institute of Mental Health Genetics Initiative, Arch Neurol, 1994 Dec; 51(12):1198-204.)

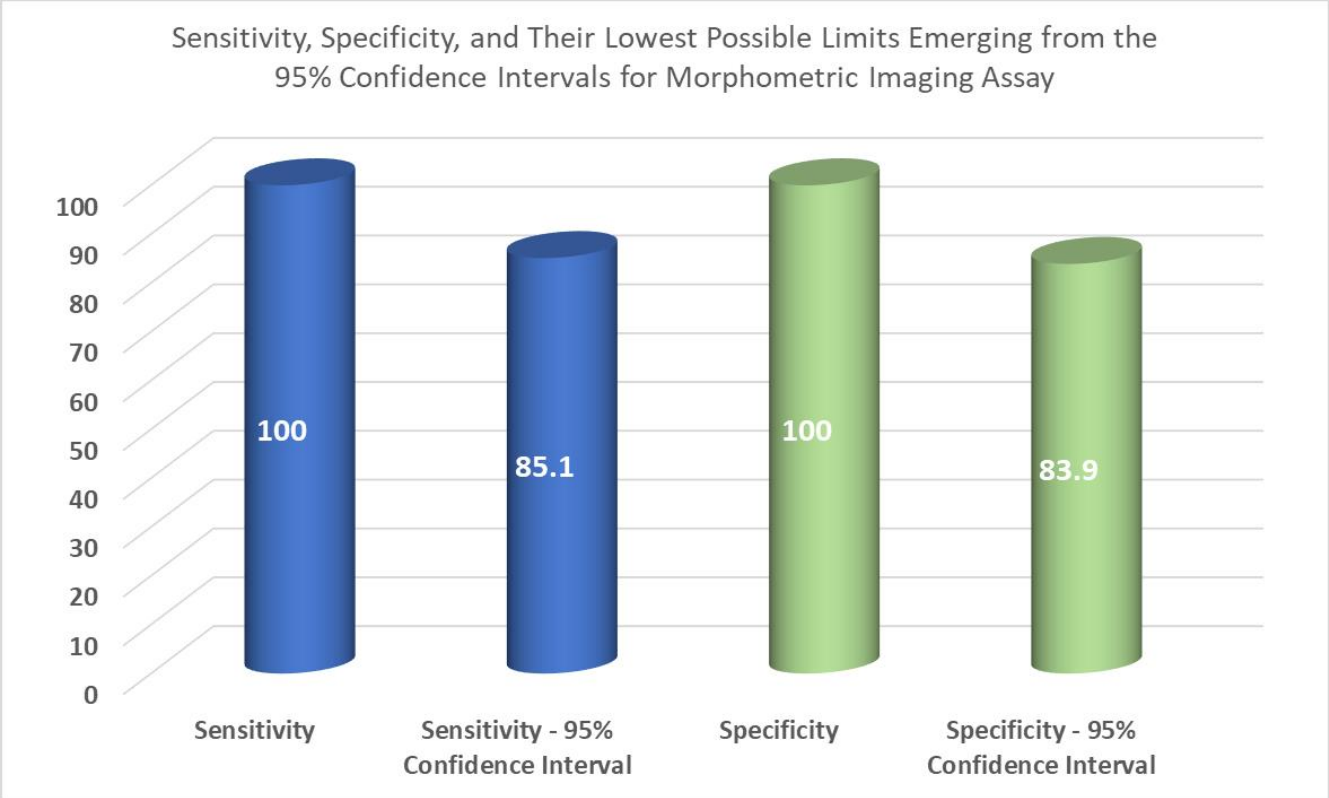

Figure 2. Sensitivity, Specificity, and Their Lowest Possible Limits Emerging from the 95% Confidence Intervals from the Morphometric Imaging Assay.

Table 3A. Alzheimer's Disease (AD) patients in the trial with the Morphometric Imaging Assay. The complete list of AD patients with autopsy or equivalent, disease duration greater than 4 years, independent validations, and cross-correlation in section 3).

#	Sample ID	ln(A/N)	Age	Gender
1	539	7.10	77	M
2	AG11368	7.16	77	M
3	557	7.55	78	M
4	AG08527	7.57	61	M
5	60	7.81	81	M
6	61	7.89	79	M
7	AG06869	8.03	60	F
8	AG10788	8.08	87	NA
9	AG05770	8.11	70	M
10	AG08170	8.15	56	M

11	538	8.39	79	M
12	AG07374	8.40	73	M
13	AG06840	9.20	56	M
14	AG08245	9.78	75	M
15	AG06844	9.79	59	M
16	42	9.85	78	M
17	AG06263	10.43	67	F
18	43	10.44	88	M
19	AG05810	10.49	79	F
20	AG04159	10.67	52	F
21	554	11.18	85	M
22	40	11.93	59	M

Table 3B Non-ADD patients in the trial with the Morphometric Imaging Assay

#	Sample ID	ln(A/N)	Age	Gender
1	ND27760	4.60	55	F
2	GM02173	4.77	52	F
3	GM04715	4.93	40	M
4	GM05031	5.10	60	M
5	AG08395	5.15	85	F
6	GM00305	5.36	56	F
7	GM02165	5.40	67	M
8	GM04210	5.58	59	M
9	GM05030	5.61	56	M
10	GM04222	5.89	59	M
11	GM02167	5.91	59	F
12	ND34265	6.01	62	M
13	GM04476	6.04	57	M
14	GM02038	6.18	22	M
15	GM04198	6.48	63	F
16	ND31618	6.75	58	F
17	AG06274	6.77	65	F
18	564	5.80	67	F

19	574	5.66	90	F
20	586	6.50	69	M

Table 4. The sensitivity, specificity, and 95% confidence intervals, for the trial.

	AD (Positive: N=22) vs. Non-ADD(Negative; N=20)		
Morphometric Imaging Ln(A/N)	**Positive**	**Negative**	**Total**
Positive	**22**	**0**	**22**
Negative	**0**	**20**	**20**
Total	**22**	**20**	**42**
Sensitivity 100.0% 95% CI: 85.1% to 100.0%			
Specificity 100.0% 95% CI: 83.9% to 100.0%			

This report shows that the 95 percentile approach to determine the cut-off produces very similar results with the normal distributions of the same data for the morphometric imaging assay, i.e., 100% sensitivity and 100% specificity. The lowest boundaries for these measures as determined by the 95% confidence intervals are 85.1 % for the Sensitivity and 83.9% for the specificity. The cut-off determination by using this 95 percentile method is also very similar with the cut-off value determined using the Gaussian distributions, 6.98 versus 6.96.

III. The complete list of AD patients with autopsy or equivalent

, disease duration greater than 4 years, independent validations, and cross-correlation.

There are 22 AD patients in the validation trial, 14 of which are autopsy or autopsy equivalent (64%). Eight patients (36%) have clinical diagnosis.
Out of the eight clinically diagnosed patients:7 are hypervalidated:

a) 3 are confirmed AD cases by having a disease duration greater than 4 years (see Khan and Alkon 2010, Figure 2 below),
b) 2 are confirmed AD cases by independent research,
c) 2 are cross- correlation with AD Index or PKCε (**Table 5**), the majority of which were studied with autopsy confirmed AD cases.
Sponsor has submitted a summary of the foundational science in past interactions with the agency. The agency had stated that Sponsors science is not being debated. Sponsor believes the science developed over 35 years and supported by over 50 peer reviewed publications supports the fact that all the assays are measuring the molecular activity through skin fibroblasts that mirrors brain synapses.
It is with this logic that sponsor believes that the autopsy supported study of the greater than 4 year AD patient clinical diagnosis, the cross correlating assays, and the autopsy confirmed data results are similar.

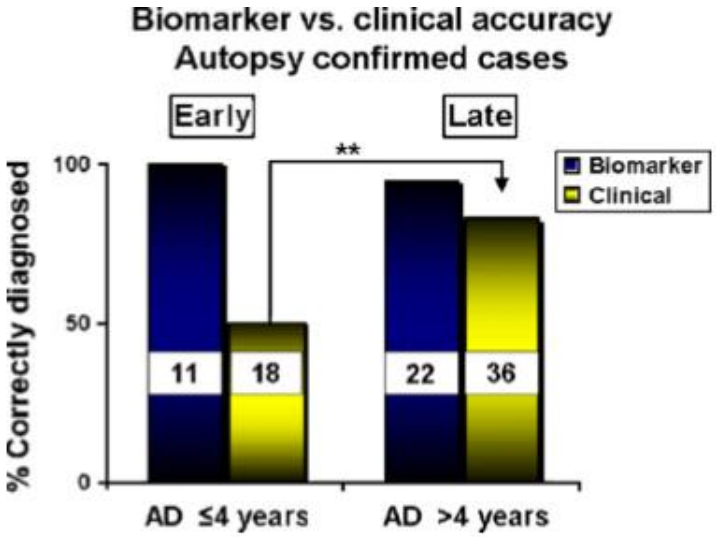

Fig. 2. AD-Biomarker vs. clinical accuracy of autopsy-confirmed patients: the patients were categorized into two groups: (i) early (≤4 years of disease duration) and (ii) late (>4 years of disease duration). Disease duration of the patients was estimated from the date of biopsy and the day that the first symptoms of dementia were observed. In the case of early onset patients (≤4 years of disease duration), AD-Biomarker outperformed clinical diagnosis. For the category >4 years of disease duration, clinical diagnostic performance was improved, but remained somewhat lower than that of AD-Biomarker performances. The number of patients is indicated in the bar for each group.

Table 5. The complete list of AD patients with autopsy or equivalent, disease duration greater than 4 years, independent validations, and cross-correlation.

All Alzheimer's Disease Patients for Morphometric Imaging Assay Validation Trial (N=22)								
No	Sample#	Age	Gender	AD diagnosis		Ln(A/N)	Cross Correlation	Independent Validation - Publications
				Determined by	Duration (Yrs)			
1	42	78	M	Clinical	2	9.85	PKCε and AD Index	
2	538	79	M	**Autopsy**	N/A	8.39	-	
3	539	77	M	**Autopsy**	N/A	7.1	PKCε	
4	554	85	M	**Autopsy**	N/A	11.18	-	
5	557	78	M	**Autopsy**	N/A	7.55	PKCε	
6	40	59	M	Clinical	7	11.04	PKCε and AD Index	
7	43	88	M	Clinical	9	10.74	PKCε and AD Index	
8	60	81	M	Clinical	N/A	8.39	-	
9	61	79	M	Clinical	N/A	7.96	PKCε	
10	AG05770	70	M	**Autopsy**	7.5	8.11	PKCε and AD Index	Ramamoorthy M, Sykora P, Scheibye-Knudsen M, Dunn C, Kasmer C, Zhang Y, Becker KG, Croteau DL, Bohr VA, Sporadic Alzheimer disease fibroblasts display an oxidative stress phenotype Free radical biology & medicine53:1371-80 2012
11	AG06263	67	F	Clinical	7	10.43	AD Index	Khan TK, Alkon DL, An internally controlled peripheral biomarker for Alzheimer's disease: Erk1 and Erk2 responses to the inflammatory signal bradykinin Proceedings of the National Academy of Sciences of the United States of America103:13203-7 2006
12	AG08170	56	M	Clinical	3	8.15	-	Coffey EE, Beckel JM, Laties AM, Mitchell CH, Lysosomal alkalization and dysfunction in human fibroblasts with the Alzheimer's disease-linked presenilin 1 A246E mutation can be reversed with cAMP Neuroscience: 2014
13	AG08245	75	M	**Autopsy**	7	9.78	PKCε	
14	AG08527	61	M	**Autopsy**	N/A	7.57	PKCε and AD Index	Favit A, Grimaldi M, Nelson TJ, Alkon DL, Alzheimer's-specific effects of soluble beta-amyloid on protein kinase C-alpha and -gamma degradation in human fibroblasts. Proc Natl Acad Sci U S A95(10):5562-7 1998
15	AG06840	56	M	**Genetic**	4	9.2	-	Greotti E, Capitanio P, Wong A, Pozzan T, Pizzo P, Pendin D, Familial Alzheimer's disease-linked presenilin mutants and intracellular Ca Cell calcium79:44-56 2018
16	AG04159	52	F	**Genetic**	8	10.67	-	Greotti E, Capitanio P, Wong A, Pozzan T, Pizzo P, Pendin D, Familial Alzheimer's disease-linked presenilin mutants and intracellular Ca Cell calcium79:44-56 2018'

17	AG06844	59	M	**Genetic**	11	9.79	-	Jenkins EC, Ye L, Gu H, Wisniewski HM, Mitotic index and Alzheimer's disease. Neuroreport9(17):3857-61 1998
18	AG10788	87	NA	**Genetic**	17	8.08	PKCε and AD Index	Meyer K, Feldman HM, Lu T, Drake D, Lim ET, Ling KH, Bishop NA, Pan Y, Seo J, Lin YT, Su SC, Church GM, Tsai LH, Yankner BA, REST and Neural -Gene Network Dysregulation in iPSC Models of Alzheimer's Disease Cell reports26:1112-1127.e9 2018
19	AG07374	73	M	Clinical	N/A	8.4	-	Ramamoorthy M, Sykora P, Scheibye-Knudsen M, Dunn C, Kasmer C, Zhang Y, Becker KG, Croteau DL, Bohr VA, Sporadic Alzheimer disease fibroblasts display an oxidative stress phenotype Free radical biology & medicine53:1371-80 2012
20	AG06869	60	F	**Autopsy**	1	8.03	-	Meyer K, Feldman HM, Lu T, Drake D, Lim ET, Ling KH, Bishop NA, Pan Y, Seo J, Lin YT, Su SC, Church GM, Tsai LH, Yankner BA, REST and Neural Gene Network Dysregulation in iPSC Models of Alzheimer's Disease Cell reports26:1112-1127.e9 2018
21	AG11368	77	M	**Autopsy**	N/A	7.16		Mendonsa G, Dobrowolska J, Lin A, Vijairania P, Jong YJ, Baenziger NL, Molecular profiling reveals diversity of stress signal transduction cascades in highly penetrant Alzheimer's disease human skin fibroblasts PLoS ONE4:e4655 2008'
22	AG05810	79	F	**Autopsy**	N/A	10.49	AD Index	Duan L, Bhattacharyya BJ, Belmadani A, Pan L, Miller RJ, Kessler JA, Stem cell derived basal forebrain cholinergic neurons from Alzheimer's disease patients are more susceptible to cell death Mol Neurodegener9(1):3 2014