Biomarkers Consortium Project
Use of Targeted Multiplex Proteomic Strategies to Identify Novel Cerebrospinal Fluid (CSF) Biomarkers in Alzheimer's Disease (AD)

Data Primer

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Background:

The data described within this document represents the work of the Biomarkers Consortium Project "**Use of Targeted Multiplex Proteomic Strategies to Identify Novel CSF Biomarkers in AD** This project was submitted to the Biomarkers Consortium Neuroscience Steering Committee by a subgroup of the Alzheimer's Disease Neuroimaging Initiative (ADNI) Industry Private Partner Scientific Board (PPSB) for execution and was managed by a Biomarkers Consortium Project Team that includes members from academia, government and the pharmaceutical industry (see Appendix I). Funding for this project was provided by the Alzheimer's Drug Discovery Foundation, Eisai, Lilly, Merck, Pfizer, and Takeda. This project is the second part of a multi-phased effort seeking to utilize samples collected by ADNI to qualify multiplex panels in both plasma and cerebrospinal fluid (CSF) to diagnose patients with Alzheimer's Disease (AD) and monitor disease progression. An earlier phase of the program focused on analysis of data from ADNI plasma samples run on a multiplex panel (Soares et al, in prep, data available on the ADNI website, www.adni.loni.edu).The first part of this series of analyses was similar to an ongoing study of ADNI plasma samples which used a similar set of multiplex panels (Hu et al, Neurol., Submitted, 2011).

The aim of the project is to determine the ability of a multiplex based immunoassay panel to discriminate among disease states and to monitor disease progression over a one year period in a CSF matrix. The multiplex panel is based upon Luminex immunoassay technology and has been developed by Rules Based Medicine (MyriadRBM) to measure a range of inflammatory, metabolic, lipid and other disease relevant indices. Prior studies using an older version of the MyriadRBM panel (an 89 analyte version) suggested some analytes on the panel differed between AD and controls. The panel has been expanded to include analytes from a recent study (Soares et al, submitted) describing plasma based biomarkers of AD. For this project a 159-analyte version of the panel (discovery MAP) selective for analytes believed to be relevant to AD was chosen.

The analysis of CSF samples on the multiplex panel referred to as the Human Discovery Map by Myriad is available on a commercial fee-for-service basis. The current document describes the technology and experimental design of the CSF multiplex biomarker pilot study.

Description of Multiplex Technology:

The Luminex xMAP technology uses a flow-based laser apparatus to detect fluorescent polystyrene microspheres which are loaded with different ratios of two spectrally distinct fluorochromes (**see Figure 1A, Appendix II**). Using a precise ratio of the fluorochromes, up to 100 different beads can be generated such that each contains a unique color-coded signature. The beads serve as a solid phase matrix that can then be coated with either ligand or capture antibodies (Figure 1B) after which standard sandwich or competitive assay formats are applied to detect the analytes of interest. Signal is typically amplified via a reporter streptavidin-phycoerythrin conjugate. The beads are read one at a time as they pass through a flow cell on the Luminex laser instrument using a dual laser system (see **Figures 1C and D, Appendix II**). One laser records the color code for individual beads (e.g. analyte ID) and the other quantitates the reporter signal (e.g. biomarker concentration). In theory, up to 100 different analytes can be

measured per well per 250 ul of sample. However, dynamic range, matrix interference and cross-reactivity limit the number of analytes that can be multiplexed in one well. The actual MyriadRBM panel consists of several panels with between 3 and 24 multiplexed analytes. The combination of analytes per panel is proprietary to MyriadRBM. In addition, the dilution of samples per plate is also proprietary information.

MyriadRBM has attempted to validate each of the analytes on the 159 analyte panel up to clinical laboratory improvement amendment (CLIA) standards, but the assays themselves are not CLIA approved. Each analyte has an individual standard curve with between 6-8 reference standards. Each plate is run with 3 levels of QCs (low, medium and high) for each analyte. A total of 16 of the CSF samples were retested using a separate never before thawed replicate aliquot on the fifth of the five 96 well plates to provide blinded test/re-test quality control data. Assays are qualified based on least detectable dose (LDD - see below), precision, cross-reactivity, dilutional linearity, spike recovery (assessment of accuracy), and test/re-test performance. Cross validation to alternative methods is reported for some assays where feasible. The assays themselves should be considered exploratory and are not in full compliance with diagnostic standards for assays. For example, reference calibrators are diluted in a buffer and not in matrix (i.e. CSF) and measurement bias is a component of the platform. Linearity of dilution and stability were not evaluated. In addition, the magnitude of batch-batch variation is not defined. MyriadRBM uses the following criteria for assay qualification:

Least Detectable Dose

The LDD is the concentration of target analyte that produces a signal that can be distinguished from that produced by a blank with 99% confidence. It is determined from the average and standard deviation of the signal for a minimum of 20 replicate determinations of the standard curve blank for each assay. Three standard deviations are added to the average of the signal, and this value is converted to concentration as interpolated from the dose response curve. The LDD is considered the most reliable lowest point for the individual assays.

Precision

Precision is defined by the agreement between replicate measurements of the same material when measured within Run (intra-assay CV) and over a series of Runs (inter-assay or Total CV). It is determined by measuring 3 levels of controls in duplicate over a minimum of 5 Runs and provides information concerning random error expected in a test result caused by factors that vary under normal laboratory operating conditions such as pipeting, timing, mixing, and temperature. The second type of precision is the test/re-test (plate-to-plate) reproducibility for 16 randomly selected replicate never before thawed CSF samples.

Cross-reactivity

Cross-reactivity is the ability of an assay to differentiate and quantify the analyte of interest in the presence of other similar analytes in the sample that could have a positive or negative effect on the assay value. It is determined by testing high concentrations of each MAP analyte across all multiplexes. However, true specificity against highly related proteins is not well described in some cases.

Spike Recovery

Spike recovery is performed as an assessment of accuracy, although this often is not possible for biological products due to the unavailability of pure "gold" standards. It is used to account for interference caused by compounds introduced from the physical composition of the sample or sample matrix that may affect the accurate measurement of the analyte. It is performed by spiking different amounts of standard spanning the assay range into standard curve diluent (control spike) and known samples. The average % recovery is calculated as the proportion of spiked standard in the sample (observed) to that of the control spike (expected) following analysis.

Correlation

Agreement of MyriadRBM multiplexed assay values to other methods is assessed by testing samples in an alternate commercial immunoassay system, when available. This comparison of methods is performed to estimate inaccuracy or systematic error. Data from the two methods are graphed in a comparison plot and the correlation coefficient is determined. Further testing of any biomarker that is significantly increased or decreased compared to cognitively normal controls in this study using an alternate commercially available test method, for example a commercially available ELISA method, is an essential requirement in the process of further assessment of the reproducibility of such findings.

Dynamic Range

The dynamic range is defined as the range of standard used to produce the standard curve. It is initially realized during assay development when standards are analyzed in a wide range above and below the expected concentrations using full-log dilutions. The standards are subsequently retested using reduced serial dilutions that target the useful part of the standard curve.

MyriadRBM provides reports of analytes with the LDD and range for that particular run. Values that are below LDD are typically reported as LOW. In some instances however, concentration values are reported that are below the LDD because they were readable on the calibration curve. Such values usually have poor precision, and should be used with caution if at all. High values may be reported as >top of analyte range concentration. If there is not sufficient volume, MyriadRBM will report as quantity not sufficient (QNS).

Analyte Quality Control (QC) results from the 2011 ADNI CSF Analysis:

QC data that is specific for the CSF samples included in this study are the test/retest results for the 16 randomly selected CSF samples (summarized in **Table 1, Appendix II**). For these 16 CSF samples (test/retest samples), a never before thawed second aliquot, blinded to the MyriadRBM analytical staff, was included on the 5^{th} plate, so that for the majority of analytes in the CSF samples studied here, there was a re-test concentration determined that serves as an independent CSF-specific QC assessment. This table provides statistical parameters that are useful for characterizing the precision performance for each analyte. A limitation in this data, as in the patient CSF dataset, is the occurrence in some instances of low results such that there are some analytes for which the CSF test/retest data is sparse or nonexistent. We suggest that for analytes with test/re-test N<7 **OR** mean %difference >35 **OR** mean absolute %difference >60% **OR** Bland Altman slope **and** intercept significantly different from 0 should be treated with caution.

The second set of QC samples available was prepared by spiking human plasma with extracts of cell cultures expressing the individual analytes. The purpose of these QC samples is to assure that the mechanical and volumetric functionality of the robotic system is reproducible. The ADNI CSF sample cohort was run on 5 plates. These QC results for each analyte are included in **Table 2 (see Appendix II)**. These QCs were performed in duplicate, but CSF samples were run in singlicate according to the RBM testing protocol. As a result the first QC result from each plate was used to derive the summary QC statistics for each analyte. For purposes of assigning a level of confidence in the quality of performance in the CSF analyses we recommend careful review of the two types of QC data included in this study. The first level of QC performance that reflects the mechanical, volumetric functionality and immunoassay response over the range of calibrators can be estimated from the data in **Table 2 (see Appendix II)**. Analytes with one or more QC CV values above 25% should be treated with caution. Figure 2 (**See Appendix II**) highlights (A) the 31 analytes with QC CVs within the 20-30% range and (B) the 16 analytes with QC CVs >30%. In addition, analytes with numerous sample values close to or below LDD should be treated with caution.

Methodology:

A total of 327 CSF samples from the baseline ADNI sample set was assessed (N= 92 Controls, 69 AD, 149 for amnestic mild cognitive impairment (MCI) and 1 unknown diagnosis, plus 16 technical replicates). One patient was excluded from the final analysis due to a screen failure. These baseline CSF samples have matching aliquots from year 1 CSF so that possible future studies on longitudinal change would be possible if funding becomes available for such a follow-up investigation. Of the 149 MCI subjects, 38 subjects had progressed to dementia as of March 2010. In addition the selected samples have additional biomarker data sets available. For example, samples from AD subjects with associated CSF Aβ42/tau measures and/or Pittsburgh Compound B (PIB) one year data were included in the AD subset. Table 3 summarizes the demographics of the population selected.

CSF samples were obtained in the morning following an overnight fast at the baseline visit in the ADNI 1 study. For the majority of samples, the time from collection to freezing was within 60 minutes. Processing, aliquoting and storage at -80^0C were performed according to the ADNI Biomarker Core Laboratory Standard Operating Procedures.

Listing of the Multiplex Analytes, LDD and Range:

Each analyte on the panel has a validation report that is available through MyriadRBM. Validation reports and dynamic range for serum and plasma in young healthy normal patients are known and can be obtained from MyriadRBM. There are no specific validation reports and dynamic range data using CSF matrix due to the lack of availability to MyriadRBM of normal control CSF samples. The experience to date in measuring CSF biomarkers using MyriadRBM methodology can be found in references 1-3,5. **Table 2 (see Appendix II)** lists the analytes, concentration units, and LDD. In addition, **Table 2 (see Appendix II)** lists summary statistics from the RBM QCs run during the analysis of the ADNI CSF subset.

It should be noted that age was calculated based upon date of birth and upon date of sample draw from baseline visit. Samples were randomized for processing at MyriadRBM and MyriadRBM was blind to the clinical information. A Statistical Analysis Plan (**see Appendix III**) was prepared prior to analysis.

What is posted on the ADNI Website and cautionary notes to data analysis:

There are two datasets posted on the ADNI website relating to the CSF multiplex pilot from the Biomarkers Consortium Project. The first dataset coded ***ADNI CSF Multiplex Raw Data*** includes the original raw data from the run to be intended as reference. The second dataset entitled ***ADNI CSF QC Multiplex data*** is the cleaned, quality controlled data according to methodology described in the statistical analysis plan. See **Tables 4 and 5 (Appendix II)** for definitions of the column headers in these tables. It is recommended that raw data not be used to derive summary statistics as many of the analytes are not normally distributed and there are some analytes with LOW or HIGH values reported. Summary statistics should not be run on data that are not normally distributed. It is recommended that analytes with numerous LOW or HIGH values listed or with majority of values listed below the LDD be treated with caution as deriving reliable results may be challenging. Consultancy with a trained statistician is highly recommended prior to reporting results based upon multiple comparisons. Note that for CSF samples with replicates, data from both aliquots are included in the datasets.

The analyses described in the statistical analysis plan should be regarded as exploratory and meant for hypothesis and model generation, rather than for hypothesis confirmation and model validation. Results from this study will be compared with those from other studies on CSF proteins in AD, and findings will need to be confirmed and expanded upon in subsequent studies using other, independent data sets.

References:

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APPENDIX I

Biomarkers Consortium CSF Proteomics Project Team Members:

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APPENDIX II
Figures and Tables

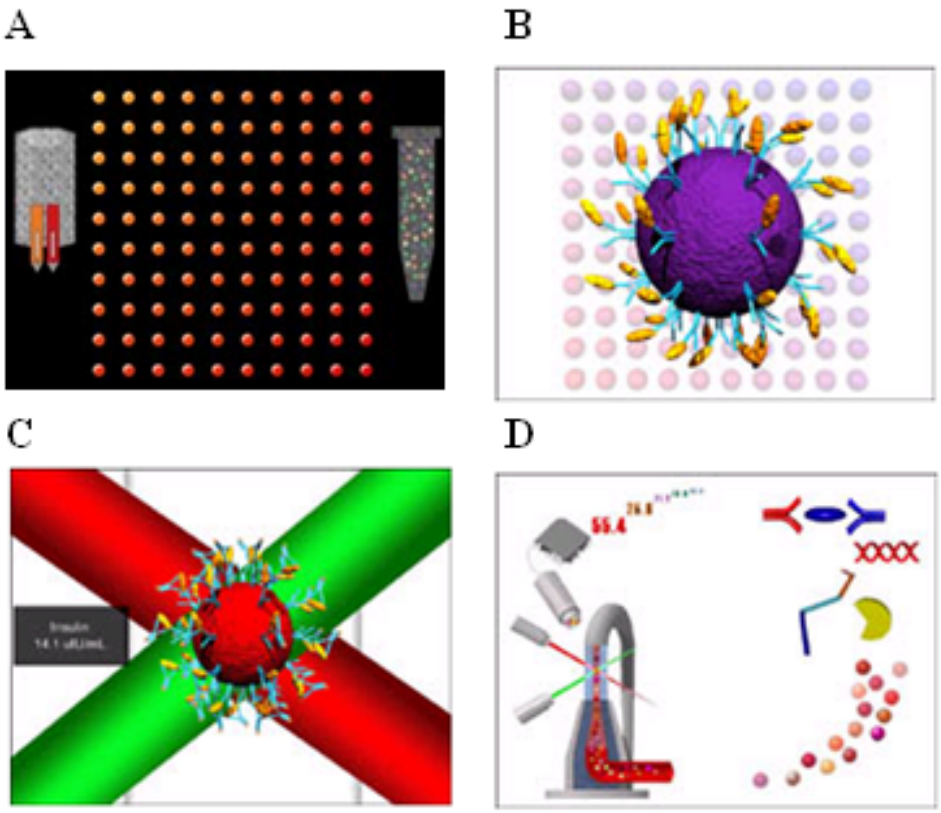
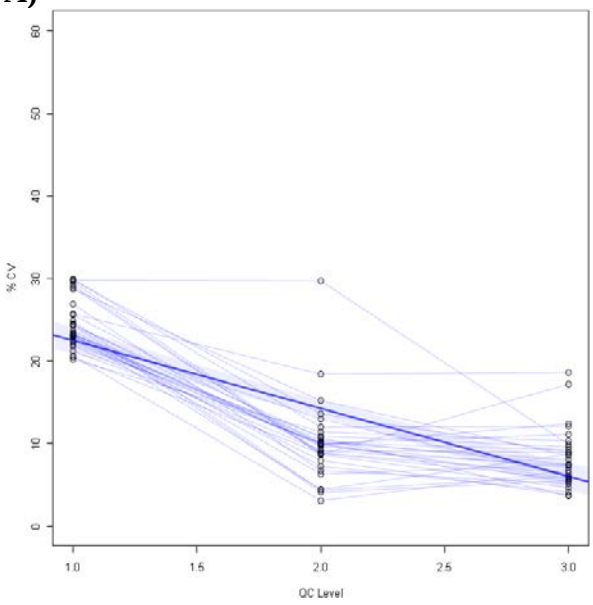


Figure 1: Luminex xMAP platform and basis of technology

A)



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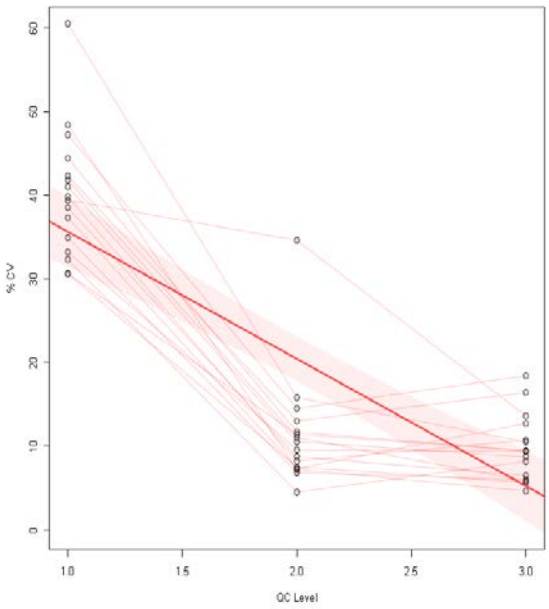

Glucagon-like Peptide 1, total (GLP-1 total)
Interleukin-1 beta (IL-1 beta)

Apolipoprotein C-III (Apo C-III)
Pancreatic Polypeptide (PPP)
E-Selectin
Fibroblast Growth Factor 4 (FGF-4)
Interleukin-1 receptor antagonist (IL-1ra)
Transforming Growth Factor beta-3 (TGF-beta-3)
Epidermal Growth Factor (EGF)
Interleukin-17 (IL-17)
Apolipoprotein D (Apo D)
Fibrinogen
AXL Receptor Tyrosine Kinase (AXL)
Alpha-Fetoprotein (AFP)
Fatty Acid-Binding Protein, heart (FABP, heart)
Interleukin-13 (IL-13)
Alpha-1-Microglobulin (A1Micro)
Thyroid-Stimulating Hormone (TSH)
Luteinizing Hormone (LH)
Pregnancy-Associated Plasma Protein A (PAPP-A)
Myeloid Progenitor Inhibitory Factor 1 (MPIF-1)
Prostate-Specific Antigen, Free (PSA-f)
Prolactin (PRL)
Matrix Metalloproteinase-9 (MMP-9)
Malondialdehyde-Modified Low-Density Lipoprotein (MDA-LDL)
Thrombopoietin
Sex Hormone-Binding Globulin (SHBG)
Agouti-Related Protein (AGRP)
Vascular Endothelial Growth Factor (VEGF)
Serum Glutamic Oxaloacetic Transaminase (SGOT)
Creatine Kinase-MB (CK-MB)
Pulmonary and Activation-Regulated Chemokine (PARC)
Adiponectin

Calcitonin
Hepatocyte Growth Factor (HGF)
Interleukin-25 (IL-25)
Intercellular Adhesion Molecule 1 (ICAM-1)
Cancer Antigen 19-9 (CA-19-9)
Immunoglobulin M (IGM)
Interleukin-6 (IL-6)
Tumor Necrosis Factor beta (TNF-beta)
Immunoglobulin E (IgE)
Matrix Metalloproteinase-2 (MMP-2)
CD40 Ligand (CD40-L)
Bone Morphogenetic Protein 6 (BMP-6)
Follicle-Stimulating Hormone (FSH)
Erythropoietin (EPO)

Figure 2: Summary of QC data for (A) the 28 analytes with QCs within the 20-30%CV range and (B) the 14 analytes with QCs >30%CV range

Table 1. Test/retest results for the 16 randomly selected CSF samples.

Table 1 CSF Test-Retest Stats

Analyte No.	Analytes	Units	Number of samples with non-missing data for test and retest	Mean response	Mean diff between test and retest	Mean pct diff (diff/mean response)	p-value for test that mean diff = 0	Mean absolute diff between test and retest	Mean pct absolute diff (absolute diff/mean response)	Intercept for Bland-Altman	p-value for Bland-Altman intercept=0	Slope for Bland-Altman	p-value for Bland-Altman slope=0
X.1	Alpha-1-Microglobulin (A1Micro)	ug/ml	16	0.0530	-0.0073	-10.6	0.050	0.0112	19.8	0.0004	0.949	-0.1458	0.167
X.2	Alpha-2-Macroglobulin (A2Macro)	mg/mL	16	0.0036	0.0012	31.5	0.000	0.0013	32.6	-0.0007	0.039	0.5391	0.000
X.3	Alpha-1-Antitrypsin (AAT)	mg/mL	16	0.0047	-0.0007	-15.8	0.005	0.0009	19.2	-0.0003	0.662	-0.0790	0.598
X.4	Angiotensin-Converting Enzyme (ACE)	ng/ml	16	2.1651	-0.1623	-6.3	0.047	0.2361	10.4	0.1600	0.451	-0.1489	0.119
X.5	Adiponectin	ug/mL	16	0.0046	-0.0003	-7.8	0.293	0.0011	27.0	0.0000	0.989	-0.0754	0.697
X.6	Alpha-Fetoprotein (AFP)	ng/mL	7	0.0465	-0.0042	-20.5	0.785	0.0322	68.7	-0.0626	0.200	1.2572	0.207
X.7	Agouti-Related Protein (AGRP)	pg/mL	16	52.7500	2.6000	-0.8	0.715	20.0375	40.5	-26.5985	0.201	0.5535	0.140
X.8	Angiopoietin-2 (ANG-2)	ng/mL	16	1.2611	-0.1944	-19.5	0.010	0.2582	23.6	-0.3593	0.067	0.1307	0.344
X.9	Apolipoprotein A-I (Apo A-I)	mg/mL	16	0.0008	0.0000	-6.5	0.317	0.0001	13.9	0.0000	0.677	-0.1133	0.322
X.10	Apolipoprotein C-III (Apo C-III)	ug/mL	16	0.0579	0.0077	12.4	0.082	0.0135	23.2	-0.0008	0.917	0.1484	0.234
X.11	Apolipoprotein D (Apo D)	ug/ml	16	5.3613	1.6738	28.7	0.006	1.9588	34.6	-0.8075	0.413	0.4628	0.012
X.12	Apolipoprotein E (Apo E)	ug/ml	16	7.0056	1.2925	18.2	0.009	1.6738	24.0	-0.1139	0.927	0.2008	0.237
X.13	Apolipoprotein H (Apo H)	ug/mL	16	0.8421	0.0241	3.6	0.631	0.1226	12.6	0.1026	0.279	-0.0932	0.324
X.14	Amphiregulin (AR)	pg/mL	1	119.0500	-53.9000	-45.3		53.9000	45.3	-53.9000		0.0000	
X.15	AXL Receptor Tyrosine Kinase (AXL)	ng/mL	16	4.2318	-0.5151	-14.5	0.005	0.6514	16.5	-0.6040	0.189	0.0210	0.830
X.16	Beta-2-Microglobulin (B2M)	ug/mL	16	1.5184	0.0529	3.4	0.454	0.2137	13.8	0.0365	0.832	0.0108	0.916
X.17	Brain-Derived Neurotrophic Factor (BDNF)	ng/mL	0										
X.18	B Lymphocyte Chemoattractant (BLC)	pg/ml	2	5.0850	-1.0300	-34.8	0.754	2.5300	58.4	-11.2000		2.0000	
X.19	Bone Morphogenetic Protein 6 (BMP-6)	ng/mL	6	0.1076	-0.0288	-32.3	0.293	0.0491	47.5	-0.0571	0.506	0.2632	0.720
X.20	Betacellulin (BTC)	pg/mL	7	40.5857	3.2286	-0.5	0.782	22.8857	55.0	-34.0077	0.365	0.9175	0.302
X.21	Complement C3 (C3)	mg/mL	16	0.0027	0.0001	1.2	0.473	0.0004	13.9	0.0001	0.797	0.0078	0.927
X.22	Cancer Antigen 125 (CA-125)	U/mL	0										
X.23	Cancer Antigen 19-9 (CA-19-9)	U/mL	15	1.0853	-0.0957	-5.5	0.493	0.3904	36.2	0.3918	0.383	-0.4492	0.258
X.24	Calcitonin	pg/mL	14	10.6450	0.9714	10.6	0.121	2.0100	23.5	-0.2317	0.834	0.1130	0.213
X.25	CD 40 antigen (CD40)	ng/mL	16	0.2272	-0.0029	-1.9	0.709	0.0243	10.8	-0.0211	0.532	0.0798	0.579
X.26	CD40 Ligand (CD40-L)	ng/mL	6	0.0101	-0.0052	-47.3	0.046	0.0063	65.8	-0.0001	0.972	-0.4951	0.225
X.27	Carcinoembryonic Antigen (CEA)	ng/mL	4	0.0429	-0.0127	-11.0	0.555	0.0237	44.0	0.0630	0.167	-1.7647	0.111
X.28	Chromogranin-A (CgA)	ng/mL	16	271.8000	6.8500	1.6	0.132	15.7750	6.2	-30.1198	0.131	0.1360	0.064
X.29	Creatine Kinase-MB (CK-MB)	ng/mL	0										
X.30	Clusterin (CLU)	ug/ml	16	30.3844	9.0063	27.3	0.001	10.7188	34.5	-2.0664	0.686	0.3644	0.031
X.31	Ciliary Neurotrophic Factor (CNTF)	pg/mL	12	9.6921	-3.0825	-26.3	0.149	5.2142	49.9	8.1642	0.192	-1.1604	0.071
X.32	Cortisol (Cortisol)	ng/ml	16	19.6028	-0.1531	-1.0	0.796	1.8406	9.5	-0.2364	0.908	0.0042	0.966
X.33	C-Reactive Protein (CRP)	ug/mL	16	0.0028	0.0008	12.6	0.140	0.0009	23.9	-0.0001	0.831	0.3283	0.011
X.34	Cystatin-C	ng/ml	16	2.9297	-0.1594	-7.4	0.149	0.3794	13.1	-0.7774	0.028	0.2110	0.061
X.35	Epidermal Growth Factor (EGF)	pg/mL	1	1.6550	-0.9100	-55.0		0.9100	55.0	-0.9100		0.0000	
X.36	Epithelial-Derived Neutrophil-Activating Prot	ng/mL	15	0.0122	-0.0005	-7.6	0.643	0.0031	28.2	-0.0024	0.098	0.1592	0.076
X.37	EN-RAGE	ng/mL	6	0.2856	0.0456	31.1	0.182	0.0577	41.4	0.0105	0.637	0.1229	0.033
X.38	Eotaxin-1	pg/mL	6	11.4300	-3.9200	-32.3	0.219	6.0033	52.0	-2.2477	0.869	-0.1463	0.899
X.39	Eotaxin-3	pg/mL	3	141.4667	29.6000	8.9	0.424	29.7333	9.2	-15.5700	0.000	0.3193	0.000

Table 1 CSF Test-Retest Stats

Analyte No.	Analytes	Units	Number of samples with non-missing data for test and retest	Mean response	Mean diff between test and retest	Mean pct diff (diff/mean response)	p-value for test that mean diff = 0	Mean absolute diff between test and retest	Mean pct absolute diff (absolute diff/mean response)	Intercept for Bland-Altman	p-value for Bland-Altman intercept=0	Slope for Bland-Altman	p-value for Bland-Altman slope=0
X.40	Erythropoietin (EPO)	pg/mL	7	7.4000	-0.2543	-5.0	0.909	3.7400	48.0	1.0466	0.924	-0.1758	0.903
X.41	Epiregulin (EPR)	pg/mL	0										
X.42	E-Selectin	ng/mL	8	0.1469	-0.0253	-15.0	0.532	0.0628	36.8	0.0536	0.771	-0.5370	0.661
X.43	Endothelin-1 (ET-1)	pg/mL	16	13.3606	0.8125	2.8	0.522	3.9313	29.1	-7.4047	0.121	0.6150	0.079
X.44	Fatty Acid-Binding Protein, heart (FABP, hea	ng/mL	16	3.3781	-0.0888	-3.6	0.620	0.4638	13.4	-0.2371	0.591	0.0439	0.710
X.45	Factor VII	ng/mL	0										
X.46	FASLG Receptor (FAS)	ng/mL	12	1.5177	-0.4293	-18.8	0.065	0.6599	43.8	0.7449	0.148	-0.7736	0.024
X.47	Fas Ligand (FasL)	pg/mL	16	12.9272	-1.0181	0.0	0.524	4.2444	36.3	3.7687	0.096	-0.3703	0.013
X.48	Fibroblast Growth Factor 4 (FGF-4)	pg/mL	16	47.0469	-11.5563	-21.8	0.025	17.5313	40.0	4.3272	0.772	-0.3376	0.273
X.49	Fibroblast Growth Factor basic (FGF-basic)	pg/mL	4	58.2125	-6.8750	-4.3	0.746	25.0250	37.6	150.4566	0.139	-2.7027	0.126
X.50	Fibrinogen	mg/mL	16	0.0005	0.0001	9.4	0.047	0.0001	24.5	0.0000	0.443	0.2507	0.005
X.51	Ferritin (FRTN)	ng/mL	16	5.9369	1.5638	25.1	0.000	1.5638	25.1	-0.8447	0.424	0.4057	0.028
X.52	Follicle-Stimulating Hormone (FSH)	mIU/mL	14	1.2601	-0.1761	-9.3	0.142	0.2974	30.4	0.1244	0.439	-0.2385	0.030
X.53	Granulocyte Colony-Stimulating Factor (G-CS	pg/mL	5	1.6460	-0.0400	-0.7	0.923	0.6000	32.7	1.6171	0.596	-1.0067	0.583
X.54	Growth Hormone (GH)	ng/mL	5	0.0342	-0.0177	-42.7	0.115	0.0179	43.7	0.0187	0.459	-1.0662	0.179
X.55	Glucagon-like Peptide 1, total (GLP-1 total)	pg/ml	1	0.6370	-0.1920	-30.1		0.1920	30.1	-0.1920		0.0000	
X.56	Granulocyte-Macrophage Colony-Stimulatin	pg/mL	3	5.9933	0.4400	10.3	0.682	1.3800	24.9	0.9684	0.872	-0.0882	0.926
X.57	Growth-Regulated alpha protein (GRO-alpha	pg/mL	11	34.1227	-4.8636	-8.5	0.168	9.0455	31.7	1.3116	0.666	-0.1810	0.008
X.58	Haptoglobin	mg/mL	10	0.0006	0.0000	2.3	0.912	0.0000	6.6	0.0000	0.201	-0.0537	0.096
X.59	Heparin-Binding EGF-Like Growth Factor (HB	pg/mL	16	259.4063	-40.0625	-14.1	0.001	45.3125	16.9	41.9676	0.234	-0.3162	0.025
X.60	Chemokine CC-4 (HCC-4)	ng/mL	16	0.0480	-0.0044	-12.2	0.002	0.0053	14.0	-0.0026	0.197	-0.0382	0.255
X.61	Hepatocyte Growth Factor (HGF)	ng/mL	16	3.3003	-0.3844	-12.7	0.086	0.7031	23.2	-0.8909	0.434	0.1535	0.648
X.62	T Lymphocyte-Secreted Protein I-309 (I-309)	pg/mL	15	25.2730	-12.7340	-28.9	0.216	15.5607	41.2	34.1312	0.000	-1.8544	0.000
X.63	Intercellular Adhesion Molecule 1 (ICAM-1)	ng/mL	15	1.1018	0.1290	5.9	0.199	0.3207	32.3	-0.1212	0.517	0.2271	0.137
X.64	Interferon gamma (IFN-gamma)	pg/mL	1	0.5785	-0.1990	-34.4		0.1990	34.4	-0.1990		0.0000	
X.65	Immunoglobulin A (IgA)	mg/mL	16	0.0028	0.0002	5.9	0.279	0.0004	12.2	0.0000	0.935	0.0504	0.569
X.66	Immunoglobulin E (IgE)	U/mL	0										
X.67	Insulin-like Growth Factor-Binding Protein 2	ng/mL	16	108.3281	3.4313	2.4	0.354	11.1063	9.9	-22.6718	0.250	0.2410	0.181
X.68	Immunoglobulin M (IGM)	mg/mL	8	0.0003	0.0000	-4.3	0.821	0.0001	37.6	0.0000	0.724	0.1271	0.572
X.69	Interleukin-1 alpha (IL-1 alpha)	ng/mL	1	0.0005	-0.0001	-23.8		0.0001	23.8	-0.0001		0.0000	
X.70	Interleukin-1 beta (IL-1 beta)	pg/mL	1	0.2530	-0.1400	-55.3		0.1400	55.3	-0.1400		0.0000	
X.71	Interleukin-10 (IL-10)	pg/mL	2	20.3450	3.2900	10.5	0.492	3.2900	10.5	-0.2392		0.1735	
X.72	Interleukin-12 Subunit p40 (IL-12p40)	ng/mL	1	0.4965	0.0370	7.5		0.0370	7.5	0.0370		0.0000	
X.73	Interleukin-12 Subunit p70 (IL-12p70)	pg/mL	3	46.0100	1.7800	0.6	0.451	2.8667	19.2	-0.1233	0.958	0.0414	0.356
X.74	Interleukin-13 (IL-13)	pg/mL	9	3.4072	-0.2300	-17.5	0.702	1.2811	47.4	-0.9343	0.409	0.2067	0.452
X.75	Interleukin-15 (IL-15)	ng/mL	11	0.0561	-0.0277	-41.4	0.092	0.0412	65.3	0.0188	0.499	-0.8286	0.077
X.76	Interleukin-16 (IL-16)	pg/mL	15	13.8443	0.2380	-3.8	0.794	2.6073	23.5	-1.8274	0.102	0.1492	0.015
X.77	Interleukin-17 (IL-17)	pg/mL	10	1.1477	0.0159	3.9	0.938	0.5069	46.4	0.3240	0.626	-0.2685	0.624
X.78	Interleukin-18 (IL-18)	pg/mL	7	5.8214	-1.3343	-21.2	0.122	1.8886	32.9	1.9271	0.514	-0.5602	0.274

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X.79	Interleukin-1 receptor antagonist (IL-1ra)	pg/mL	7	13.0993	-4.0643	-25.3	0.280	6.8986	49.5	16.8708	0.214	-1.5982	0.128
X.80	Interleukin-2 (IL-2)	pg/mL	0										
X.81	Interleukin-23 (IL-23)	ng/mL	3	0.3383	-0.0547	-11.2	0.540	0.0813	20.4	0.4375	0.241	-1.4545	0.212
X.82	Interleukin-25 (IL-25)	pg/mL	15	10.8567	-1.0547	-7.8	0.310	3.3880	35.5	2.2635	0.600	-0.3056	0.432
X.83	Interleukin-3 (IL-3)	ng/mL	16	0.0096	-0.0006	-11.6	0.296	0.0018	36.5	0.0001	0.859	-0.0803	0.193
X.84	Interleukin-4 (IL-4)	pg/mL	9	8.8683	-3.6944	-48.1	0.028	4.1700	52.4	-6.8975	0.195	0.3612	0.509
X.85	Interleukin-5 (IL-5)	pg/mL	12	1.3869	0.1213	7.7	0.664	0.8183	63.6	0.2865	0.641	-0.1191	0.759
X.86	Interleukin-6 (IL-6)	pg/mL	13	3.4208	-0.0285	-13.2	0.908	0.6472	29.0	-0.3859	0.120	0.1045	0.016
X.87	Interleukin-6 receptor (IL-6r)	ng/mL	16	1.0565	-0.0521	-3.3	0.152	0.1101	10.5	0.0609	0.534	-0.1070	0.227
X.88	Interleukin-7 (IL-7)	pg/mL	5	15.9810	-3.7260	-21.0	0.043	4.0740	25.0	0.7778	0.803	-0.2818	0.189
X.89	Interleukin-8 (IL-8)	pg/mL	16	64.9125	5.2375	6.2	0.122	9.6250	16.5	-3.0176	0.300	0.1272	0.001
X.90	Insulin	uIU/mL	2	0.0499	-0.0067	0.3	0.674	0.0119	23.9	0.0142		-0.4176	
X.91	Interferon gamma Induced Protein 10 (IP-10	pg/ml	16	760.7688	-97.0875	-15.6	0.003	113.0875	19.0	-33.5154	0.270	-0.0836	0.006
X.92	Leptin	ng/mL	16	0.1201	0.0113	11.6	0.126	0.0232	22.2	-0.0082	0.586	0.1628	0.160
X.93	Luteinizing Hormone (LH)	mIU/mL	13	0.4311	-0.2035	-41.8	0.001	0.2205	50.3	0.0690	0.572	-0.6321	0.033
X.94	Lectin-Like Oxidized LDL Receptor 1 (LOX-1)	ng/mL	16	6.6303	-0.4969	-8.2	0.060	0.8769	13.5	-0.2127	0.753	-0.0429	0.650
X.95	Apolipoprotein(a) (Lp(a))	ug/mL	16	0.0200	0.0027	12.2	0.042	0.0035	19.2	-0.0004	0.807	0.1561	0.016
X.96	Lymphotactin	ng/mL	0										
X.97	Monocyte Chemotactic Protein 1 (MCP-1)	pg/mL	16	530.2813	19.6875	4.3	0.301	62.5625	12.0	45.0174	0.489	-0.0478	0.682
X.98	Monocyte Chemotactic Protein 2 (MCP-2)	pg/ml	14	9.4850	0.4700	24.4	0.435	1.5871	33.7	1.2598	0.007	-0.0833	0.000
X.99	Monocyte Chemotactic Protein 3 (MCP-3)	pg/mL	1	0.7175	-0.0130	-1.8		0.0130	1.8	-0.0130		0.0000	
X.100	Monocyte Chemotactic Protein 4 (MCP-4)	pg/ml	2	69.4750	3.0500	10.7	0.764	7.8500	15.6	22.4412		-0.2791	
X.101	Macrophage Colony-Stimulating Factor 1 (M	ng/mL	16	0.5839	-0.0496	-7.9	0.013	0.0691	12.2	0.0222	0.714	-0.1230	0.227
X.102	Malondialdehyde-Modified Low-Density Lipc	ng/mL	6	91.0917	25.3833	19.2	0.253	35.0833	33.6	-156.8000	0.000	2.0000	0.000
X.103	Macrophage-Derived Chemokine (MDC)	pg/mL	5	59.0750	5.8660	15.4	0.351	6.6460	40.3	0.0540	0.951	0.0984	0.001
X.104	Macrophage Migration Inhibitory Factor (MI	ng/mL	16	0.2821	0.0123	6.1	0.563	0.0611	24.4	0.0131	0.729	-0.0027	0.980
X.105	Monokine Induced by Gamma Interferon (M	pg/ml	15	402.7867	-66.7067	-21.0	0.000	66.7067	21.0	-59.7579	0.022	-0.0173	0.695
X.106	Macrophage Inflammatory Protein-1 alpha (	pg/mL	11	16.0468	0.2791	-2.4	0.693	1.6300	17.3	-0.4346	0.555	0.0445	0.083
X.107	Macrophage Inflammatory Protein-1 beta (M	pg/mL	16	34.1713	1.2800	-1.5	0.495	4.3700	16.0	-2.5942	0.037	0.1134	0.000
X.108	Macrophage Inflammatory Protein-3 alpha (	pg/ml	3	12.5033	-9.5933	-77.1	0.015	9.5933	77.1	-4.7260	0.801	-0.3893	0.795
X.109	Matrix Metalloproteinase-2 (MMP-2)	ng/mL	16	11.4016	-5.2369	-43.4	0.000	5.6544	48.5	0.6815	0.788	-0.5191	0.023
X.110	Matrix Metalloproteinase-3 (MMP-3)	ng/mL	16	0.3441	0.0048	-2.1	0.755	0.0443	12.4	-0.0865	0.006	0.2651	0.002
X.111	Matrix Metalloproteinase-9 (MMP-9)	ng/mL	4	2.5450	-0.4200	-19.3	0.605	1.1700	43.6	-1.7902	0.728	0.5384	0.784
X.112	Myeloid Progenitor Inhibitory Factor 1 (MPIF	ng/mL	5	0.0800	-0.0584	-87.6	0.001	0.0584	87.6	-0.0653	0.022	0.0859	0.645
X.113	Myeloperoxidase (MPO)	ng/mL	3	39.0250	8.6767	2.1	0.442	9.5367	27.3	-1.1226	0.477	0.2511	0.041
X.114	Myoglobin	ng/mL	16	0.3569	0.0684	19.6	0.001	0.0707	20.4	0.0113	0.729	0.1600	0.060
X.115	Neutrophil Gelatinase-Associated Lipocalin (	ng/ml	16	1.9938	-0.1848	-10.2	0.021	0.2598	14.1	-0.0220	0.901	-0.0816	0.322
X.116	Nerve Growth Factor beta (NGF-beta)	ng/mL	14	0.0261	-0.0077	-34.1	0.071	0.0134	55.0	-0.0119	0.378	0.1614	0.739
X.117	Neuronal Cell Adhesion Molecule (Nr-CAM)	ng/mL	5	80.4500	-5.5400	-4.2	0.391	9.3400	9.6	13.0047	0.412	-0.2305	0.240

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Analyte No.	Analytes	Units	Number of samples with non-missing data for test and retest	Mean response	Mean diff between test and retest	Mean pct diff (diff/mean response)	p-value for test that mean diff = 0	Mean absolute diff between test and retest	Mean pct absolute diff (absolute diff/mean response)	Intercept for Bland-Altman	p-value for Bland-Altman intercept=0	Slope for Bland-Altman	p-value for Bland-Altman slope=0
X.118	N-terminal prohormone of brain natriuretic	pg/ml	16	170.4625	-27.7000	-17.0	0.000	29.9500	18.5	-12.7169	0.555	-0.0879	0.470
X.119	Osteopontin	ng/ml	16	34.3000	1.3875	4.4	0.141	2.7375	10.0	1.4144	0.628	-0.0008	0.992
X.120	Plasminogen Activator Inhibitor 1 (PAI-1)	ng/mL	16	0.9596	0.1819	19.1	0.000	0.1906	19.8	0.0422	0.756	0.1455	0.291
X.121	Prostatic Acid Phosphatase (PAP)	ng/mL	16	0.2279	-0.0205	-18.1	0.309	0.0628	29.2	-0.0998	0.002	0.3480	0.003
X.122	Pregnancy-Associated Plasma Protein A (PAF	mIU/mL	16	0.0107	-0.0001	-4.5	0.946	0.0030	27.9	-0.0048	0.182	0.4406	0.171
X.123	Pulmonary and Activation-Regulated Chemo	ng/mL	0										
X.124	Platelet-Derived Growth Factor BB (PDGF-BB	pg/ml	2	20.0750	-1.0500	-10.7	0.897	6.4500	33.8	-41.2000		2.0000	
X.125	Placenta Growth Factor (PLGF)	pg/ml	16	63.4156	-7.2688	-13.0	0.057	12.6688	21.3	-2.0677	0.798	-0.0820	0.473
X.126	Pancreatic Polypeptide (PPP)	pg/ml	16	3.5822	-0.7968	-26.9	0.000	0.7968	26.9	-0.2844	0.150	-0.1430	0.004
X.127	Prolactin (PRL)	ng/ml	16	1.7340	-0.0482	-4.2	0.410	0.1769	10.4	-0.3804	0.116	0.1916	0.154
X.128	Progesterone	ng/ml	0										
X.129	Prostate-Specific Antigen, Free (PSA-f)	ng/mL	8	0.0084	-0.0013	-26.3	0.289	0.0023	47.6	-0.0015	0.397	0.0355	0.816
X.130	Receptor for advanced glycosylation end prc	ng/mL	1	0.0559	-0.0183	-32.8		0.0183	32.8	-0.0183		0.0000	
X.131	T-Cell-Specific Protein RANTES (RANTES)	ng/mL	16	0.0031	0.0004	14.4	0.061	0.0007	24.9	0.0002	0.512	0.0544	0.552
X.132	Resistin	ng/ml	16	0.0737	-0.0232	-34.6	0.000	0.0251	41.5	-0.0149	0.028	-0.1127	0.115
X.133	S100 calcium-binding protein B (S100-B)	ng/mL	16	2.6475	0.1188	2.7	0.300	0.3175	11.6	-0.4455	0.163	0.2131	0.068
X.134	Serum Amyloid P-Component (SAP)	ug/mL	16	0.0034	-0.0003	-8.7	0.038	0.0005	14.5	0.0001	0.673	-0.1243	0.005
X.135	Stem Cell Factor (SCF)	pg/mL	16	51.0000	-16.0625	-28.6	0.000	17.6250	33.5	20.3427	0.051	-0.7138	0.001
X.136	Serum Glutamic Oxaloacetic Transaminase (S	ug/mL	16	4.5413	0.4463	6.5	0.266	1.1375	23.2	-6.5480	0.001	1.5402	0.001
X.137	Sex Hormone-Binding Globulin (SHBG)	nmol/L	16	0.1277	0.0046	4.6	0.320	0.0133	11.0	0.0046	0.699	-0.0005	0.995
X.138	Superoxide Dismutase 1, Soluble (SOD-1)	ng/mL	7	87.6000	-10.1143	-11.4	0.039	12.6000	14.1	4.2962	0.847	-0.1645	0.518
X.139	Sortilin	ng/mL	16	6.5453	0.1706	1.8	0.495	0.7731	11.7	-0.6047	0.533	0.1184	0.411
X.140	Thyroxine-Binding Globulin (TBG)	ug/mL	16	0.2611	0.0920	29.7	0.004	0.1034	36.7	-0.0401	0.187	0.5056	0.000
X.141	Testosterone, Total	ng/ml	13	0.1248	0.0565	49.6	0.011	0.0591	52.4	-0.0087	0.612	0.5227	0.000
X.142	Tissue Factor (TF)	ng/mL	16	3.9103	-0.3032	-6.3	0.082	0.4906	14.0	0.0244	0.953	-0.0838	0.397
X.143	Trefoil Factor 3 (TFF3)	ug/ml	16	0.0175	0.0005	0.4	0.394	0.0018	10.0	-0.0028	0.075	0.1865	0.028
X.144	Transforming Growth Factor alpha (TGF-alph	pg/mL	10	11.8185	1.6310	16.1	0.275	3.5650	33.2	4.0372	0.585	-0.2036	0.738
X.145	Transforming Growth Factor beta-3 (TGF-bet	pg/mL	0										
X.146	Tamm-Horsfall Urinary Glycoprotein (THP)	ug/ml	9	0.0001	0.0000	31.1	0.017	0.0000	31.2	0.0000	0.908	0.3685	0.292
X.147	Thrombospondin-1	ng/mL	1	2.8850	1.5700	54.4		1.5700	54.4	1.5700		0.0000	
X.148	Tissue Inhibitor of Metalloproteinases 1 (TIM	ng/mL	16	39.7406	1.7688	3.2	0.364	5.2438	12.7	-3.5430	0.553	0.1337	0.352
X.149	Thrombomodulin (TM)	ng/ml	16	0.1460	-0.0160	-11.6	0.351	0.0461	29.8	-0.0117	0.815	-0.0295	0.927
X.150	Tenascin-C (TN-C)	ng/mL	0										
X.151	Tumor Necrosis Factor alpha (TNF-alpha)	pg/mL	9	2.5517	0.2767	19.1	0.487	0.9478	46.6	0.6470	0.335	-0.1451	0.473
X.152	Tumor Necrosis Factor beta (TNF-beta)	pg/mL	3	2.1317	-1.4433	-63.6	0.135	1.4433	63.6	2.1373	0.538	-1.6797	0.372
X.153	Tumor Necrosis Factor Receptor 2 (TNFR2)	ng/mL	16	0.7806	0.1578	19.7	0.001	0.1579	19.8	0.0449	0.643	0.1446	0.214
X.154	Thrombopoietin	ng/mL	8	0.2695	-0.0201	-3.6	0.806	0.1736	66.6	0.1296	0.645	-0.5554	0.577
X.155	TNF-Related Apoptosis-Inducing Ligand Rece	ng/mL	16	0.7563	-0.2176	-26.7	0.001	0.2436	31.0	0.1495	0.224	-0.4854	0.005
X.156	Thyroid-Stimulating Hormone (TSH)	uIU/mL	12	0.0213	-0.0062	-30.8	0.003	0.0065	31.9	-0.0055	0.408	-0.0345	0.907

Table 1 CSF Test-Retest Stats

Analyte No.	Analytes	Units	Number of samples with non-missing data for test and retest	Mean response	Mean diff between test and retest	Mean pct diff (diff/mean response)	p-value for test that mean diff = 0	Mean absolute diff between test and retest	Mean pct absolute diff (absolute diff/mean response)	Intercept for Bland-Altman	p-value for Bland-Altman intercept=0	Slope for Bland-Altman	p-value for Bland-Altman slope=0
X.157	Vascular Cell Adhesion Molecule-1 (VCAM-1)	ng/mL	16	19.7191	1.2969	11.3	0.185	2.7256	14.3	3.6986	0.002	-0.1218	0.003
X.158	Vascular Endothelial Growth Factor (VEGF)	pg/mL	16	482.2500	33.2500	6.2	0.037	53.7500	11.3	-22.2371	0.632	0.1151	0.219
X.159	von Willebrand Factor (vWF)	ug/mL	16	0.0349	-0.0018	-4.2	0.162	0.0039	12.4	0.0020	0.604	-0.1082	0.300

Table 2: List of analytes, units, LDD, dynamic range in the plasma of healthy volunteers and summary QC statistics for each analyte.

Table 2 Plasma QC Statistics

Analyte					QC 1			QC 2			QC 3			Summary CV			Missing data	
Name	Unit	Least Detectable Dose	RBM Low Serum Range	RBM High Serum Range	Mean	SD	CV	Mean	SD	CV	Mean	SD	CV	Low	High	Average	LOW	HIGH
Insulin-like Growth Factor-Binding Protein 2 (IGFBP-2)	ng/mL	0.464	25.9	247	18.4	0.83	4.5	64.7	2.94	4.54	180	8.18	4.55	4.5	4.55	4.530	0	8
Sortilin	ng/mL	0.0728	7.38	27.1	2.92	0.19	6.51	9.65	0.52	5.39	21.6	1.42	6.56	5.39	6.56	6.153		
Platelet-Derived Growth Factor BB (PDGF-BB)	pg/ml	55.6	6770	27400	1156	57	4.93	8880	482	5.43	37570	2546	6.78	4.93	6.78	5.713	26	
Angiotensin-Converting Enzyme (ACE)	ng/ml	0.08	13	191.1122697	0.726	0.0494	6.8	12.7	0.64	5.04	101	6.39	6.31	5.04	6.8	6.050		
Chemokine CC-4 (HCC-4)	ng/mL	0.00968	1.41	7.89	0.155	0.0112	7.21	0.61	0.0378	6.19	2.65	0.171	6.44	6.19	7.21	6.613		
Myoglobin	ng/mL	0.0196	4.47	56.8	3.77	0.242	6.41	14.5	1.06	7.29	48.4	3.45	7.14	6.41	7.29	6.947		
Monocyte Chemotactic Protein 2 (MCP-2)	pg/ml	3.368	14.8	92	35.2	1.96	5.57	542	29.5	5.45	2730	207	7.59	5.45	7.59	6.203	22	
Cystatin-C	ng/ml	0.04	476	1250	8.67	0.661	7.62	38.8	2.59	6.69	163	9.78	5.98	5.98	7.62	6.763		
Superoxide Dismutase 1, Soluble (SOD-1)	ng/mL	0.2632	12.9	115	6.23	0.491	7.87	20.1	1.26	6.29	57.8	3.8	6.57	6.29	7.87	6.910	0	89
Nerve Growth Factor beta (NGF-beta)	ng/mL	0.02252	<LOW>	Not Detected	1.14	0.0898	7.88	3.54	0.228	6.43	8.24	0.376	4.57	4.57	7.88	6.293	45	
Osteopontin	ng/ml	0.6	0.485	12.4	73.3	5.97	8.14	543	19.8	3.65	3126	209	6.69	3.65	8.14	6.160		
Macrophage-Derived Chemokine (MDC)	pg/mL	4.6	153	867	63	5.15	8.16	432	29.1	6.75	2162	109	5.03	5.03	8.16	6.647	21	
Cortisol (Cortisol)	ng/ml	0.4	51	249	131	7.85	5.99	355	24.3	6.85	1047	88.8	8.48	5.99	8.48	7.107	6	
Testosterone, Total	ng/ml	0.034	0.1	8.8	3.22	0.281	8.73	10	0.697	6.97	36.4	2.87	7.88	6.97	8.73	7.860	86	
Thrombomodulin (TM)	ng/ml	0.0404	2.98	8	0.927	0.0815	8.79	4.04	0.262	6.47	34.3	1.13	3.3	3.3	8.79	6.187	1	
Interleukin-5 (IL-5)	pg/mL	1.6	<LOW>	34.2	30.2	2.67	8.85	212	13.7	6.45	961	69.3	7.21	6.45	8.85	7.503	76	
Interleukin-23 (IL-23)	ng/mL	0.3272	<LOW>	5.9	6.52	0.585	8.98	24.3	1.76	7.26	102	4.48	4.39	4.39	8.98	6.877	22	
Complement C3 (C3)	mg/mL	5.7875E-06	0.893	2.46	0.107	0.00983	9.19	0.328	0.0257	7.84	0.939	0.0534	5.69	5.69	9.19	7.573		
Tumor Necrosis Factor Receptor 2 (TNFR2)	ng/mL	0.00218	3.6	12	5.45	0.511	9.39	15.1	1.07	7.05	42.4	3.14	7.4	7.05	9.39	7.947		
Apolipoprotein E (Apo E)	ug/ml	0.00128	12.2	94	7.26	0.683	9.4	13.6	1.09	7.97	35.3	3	8.48	7.97	9.4	8.617		
Interleukin-6 receptor (IL-6r)	ng/mL	0.00592	11.5	40	0.206	0.0194	9.44	3.88	0.242	6.23	18.7	0.78	4.17	4.17	9.44	6.613		
Tissue Inhibitor of Metalloproteinases 1 (TIMP-1)	ng/mL	0.0405	71.1	322	10	0.954	9.5	20.3	1.11	5.46	48.4	3.54	7.31	5.46	9.5	7.423		
Thyroxine-Binding Globulin (TBG)	ug/mL	0.0001324	41.7	115	2.49	0.233	9.35	7.32	0.683	9.33	24.5	2.34	9.56	9.33	9.56	9.413		
Tenascin-C (TN-C)	ng/mL	5.66	246	1340				262	25.4	9.68	1311	69.4	5.29	5.29	9.68	7.485	327	
Neuronal Cell Adhesion Molecule (Nr-CAM)	ng/mL	0.026	0.564	3.54	3.95	0.383	9.7	11.5	0.672	5.82	22.7	2.21	9.71	5.82	9.71	8.410	0	242
Progesterone	ng/ml	0.248275862	1.75862069	31.48890614	52.2	4.59	8.8	188	14.7	7.79	788	76.9	9.76	7.79	9.76	8.783	293	
Interleukin-8 (IL-8)	pg/mL	1.516	<LOW>	236	46.5	4.56	9.81	289	22	7.61	1420	74.5	5.25	5.25	9.81	7.557		
Monocyte Chemotactic Protein 4 (MCP-4)	pg/ml	80.8	105	797	753	73.9	9.81	4827	308	6.39	43490	1968	4.53	4.53	9.81	6.910	22	
Macrophage Colony-Stimulating Factor 1 (M-CSF)	ng/mL	0.04	<LOW>	Not Detected	6.67	0.674	10.1	116	5.05	4.35	1179	58.6	4.97	4.35	10.1	6.473		
Eotaxin-3	pg/mL	27.96	<LOW>	230	4366	384	8.8	22690	1207	5.32	104050	10831	10.4	5.32	10.4	8.173	212	
N-terminal prohormone of brain natriuretic peptide (NT proBNP)	pg/ml	40.8	<LOW>	411	207	21.6	10.4	1196	51.5	4.3	5372	451	8.39	4.3	10.4	7.697		
Immunoglobulin A (IgA)	mg/mL	0.0000535	0.575	5.83	0.256	0.0264	10.3	0.696	0.0738	10.6	1.9	0.154	8.11	8.11	10.6	9.670	1	
Macrophage Inflammatory Protein-1 beta (MIP-1 beta)	pg/mL	8.52	14.8	719	132	14.1	10.7	924	64.1	6.94	4567	247	5.41	5.41	10.7	7.683		
Receptor for advanced glycosylation end products (RAGE)	ng/mL	0.04	1.35	7.76	1	0.108	10.7	3.73	0.317	8.51	16.3	0.997	6.11	6.11	10.7	8.440	293	
Serum Amyloid P-Component (SAP)	ug/mL	0.0000644	15.3	48.2	1.05	0.112	10.6	3.24	0.237	7.31	9.46	1.02	10.8	7.31	10.8	9.570		
Myeloperoxidase (MPO)	ng/mL	1.296	67	2410	872	94.9	10.9	2311	175	7.59	5915	433	7.31	7.31	10.9	8.600	259	
S100 calcium-binding protein B (S100-B)	ng/mL	0.1212	<LOW>	0.314	6.09	0.401	6.6	24.1	1.19	4.96	102	11.1	10.9	4.96	10.9	7.487		
Interleukin-10 (IL-10)	pg/mL	2.528	<LOW>	20.3	15.1	1.66	11	213	15.7	7.41	994	47.9	4.82	4.82	11	7.743	81	
Interleukin-3 (IL-3)	ng/mL	0.01944	<LOW>	1.08	0.0272	0.00299	11	0.3	0.0297	9.89	1.45	0.0645	4.44	4.44	11	8.443	8	
Interleukin-7 (IL-7)	pg/mL	23.68	<LOW>	60.3	117	13.1	11.2	966	51.4	5.32	4963	250	5.04	5.04	11.2	7.187	22	
Interleukin-1 alpha (IL-1 alpha)	ng/mL	0.002844	<LOW>	0.272	0.00917	0.00104	11.4	0.219	0.0111	5.06	1.14	0.0645	5.68	5.06	11.4	7.380	183	
Apolipoprotein A-I (Apo A-I)	mg/mL	0.000006875	0.288	1.27	0.0524	0.00603	11.5	0.184	0.0166	9.01	0.609	0.07	11.5	9.01	11.5	10.670		
Apolipoprotein H (Apo H)	ug/mL	0.003225	127	437	44.9	4.12	9.17	77.2	8.45	10.9	158	18.1	11.5	9.17	11.5	10.523		
Interferon gamma Induced Protein 10 (IP-10)	pg/ml	12.92	130	1350	222	25.5	11.5	2808	161	5.72	22300	1737	7.79	5.72	11.5	8.337		
Cancer Antigen 125 (CA-125)	U/mL	2.602985075	<LOW>	12.46268657	31.9	3.54	11.1	317	25.5	8.04	2423	284	11.7	8.04	11.7	10.280	298	
Interferon gamma (IFN-gamma)	pg/mL	1.276	<LOW>	18	19.6	2.35	11.9	384	36.2	9.42	2095	119	5.7	5.7	11.9	9.007	282	
Chromogranin-A (CgA)	ng/mL	4.08	<LOW>	778	61	7.26	11.9	264	20.6	7.82	779	93.5	12	7.82	12	10.573		
Granulocyte-Macrophage Colony-Stimulating Factor (GM-CSF)	pg/mL	15.6	<LOW>	38.5	96.6	11.6	12	1211	83.3	6.88	5953	440	7.39	6.88	12	8.757	168	
Stem Cell Factor (SCF)	pg/mL	17.28	<LOW>	276	285	34.4	12	1660	195	11.8	5968	458	7.67	7.67	12	10.490		
Epithelial-Derived Neutrophil-Activating Protein 78 (ENA-78)	ng/mL	0.0132	<LOW>	5	0.165	0.0199	12.1	2.01	0.0904	4.5	10.8	0.547	5.06	4.5	12.1	7.220	36	
Interleukin-12 Subunit p70 (IL-12p70)	pg/mL	10.24	<LOW>	36.8	163	19.7	12.1	1734	126	7.24	8759	467	5.33	5.33	12.1	8.223	272	
Vascular Cell Adhesion Molecule-1 (VCAM-1)	ng/mL	0.0148	386	865	30.9	2.5	8.1	101	5.41	5.34	296	36.8	12.4	5.34	12.4	8.613		
Brain-Derived Neurotrophic Factor (BDNF)	ng/mL	0.0092	0.266	31.4	0.167	0.0209	12.5	2.21	0.122	5.52	15.2	0.956	6.27	5.52	12.5	8.097	325	
Interleukin-2 (IL-2)	pg/mL	7.56	<LOW>	Not Detected	94.3	11.8	12.5	582	49.4	8.48	2898	154	5.33	5.33	12.5	8.770	243	
Monocyte Chemotactic Protein 3 (MCP-3)	pg/mL	0.41	<LOW>	1.71	5.54	0.625	11.3	148	15.2	10.2	924	117	12.6	10.2	12.6	11.367	283	
T-Cell-Specific Protein RANTES (RANTES)	ng/mL	0.000327	0.943	63.3	0.947	0.117	12.4	3.06	0.292	9.55	9.55	1.24	12.9	9.55	12.9	11.617		
Angiopoietin-2 (ANG-2)	ng/mL	0.1268	2.06	12.8	3.72	0.485	13	16.3	0.673	4.12	48	1.6	3.33	3.33	13	6.817		
Growth Hormone (GH)	ng/mL	0.0368	<LOW>	7.56	0.433	0.0562	13	2.74	0.194	7.07	17.7	1.4	7.87	7.07	13	9.313	167	
Interleukin-12 Subunit p40 (IL-12p40)	ng/mL	0.0904	<LOW>	1.1	1.27	0.166	13	7.03	0.653	9.28	34.9	2.84	8.13	8.13	13	10.137	261	
Tumor Necrosis Factor alpha (TNF-alpha)	pg/mL	2.8	<LOW>	34	39.6	5.2	13.1	396	44.5	11.2	2472	176	7.1	7.1	13.1	10.467	115	
Alpha-1-Antitrypsin (AAT)	mg/mL	9.02462E-06	0.863636364	2.0569	0.096	0.0127	13.2	0.296	0.0277	9.36	0.861	0.0914	10.6	9.36	13.2	11.053		
Beta-2-Microglobulin (B2M)	ug/mL	0.000665	1.29	4.42	0.186	0.0247	13.2	0.353	0.0336	9.51	0.867	0.0838	9.67	9.51	13.2	10.793		
Clusterin (CLU)	ug/ml	0.00131	70	224	71.7	6.03	8.41	253	18.4	7.29	890	117	13.2	7.29	13.2	9.633		
Placenta Growth Factor (PLGF)	pg/ml	3.7	21.4	69	112	14.7	13.2	1942	232	11.9	7464	600	8.03	8.03	13.2	11.043		
Monocyte Chemotactic Protein 1 (MCP-1)	pg/mL	16.08	35.9	815	75.3	10.2	13.6	540	33.5	6.21	2915	128	4.39	4.39	13.6	8.067		
FASLG Receptor (FAS)	ng/mL	0.488	3.52	12.3	8.83	1.21	13.7	36.6	2.28	6.21	77	7.58	9.85	6.21	13.7	9.920	6	
Interleukin-4 (IL-4)	pg/mL	13.44	<LOW>	32.2	231	31.9	13.8	1106	54.8	4.96	5460	286	5.23	4.96	13.8	7.997	157	
B Lymphocyte Chemoattractant (BLC)	pg/ml	22.04	3.62	54.3	477	55	11.5	708	77.1	10.9	2476	345	13.9	10.9	13.9	12.100	115	2
Plasminogen Activator Inhibitor 1 (PAI-1)	ng/mL	0.0112	8.33	148	7.81	1.08	13.9	24.8	2.14	8.64	77	10.7	13.9	8.64	13.9	12.147		
Apolipoprotein(a) (Lp(a))	ug/mL	0.00133064	9.393939394	2316.498316	9.69	1.35	14	32.8	4.1	12.5	105	11.4	10.9	10.9	14	12.467		

Table 2 Plasma QC Statistics

Analyte					QC 1			QC 2			QC 3			Summary CV			Missing data	
Name	Unit	Least Detectable Dose	RBM Low Serum Range	RBM High Serum Range	Mean	SD	CV	Mean	SD	CV	Mean	SD	CV	Low	High	Average	LOW	HIGH
Haptoglobin	mg/mL	2.43881E-05	0.23006993	3.488613019	0.0857	0.0121	14.1	0.259	0.03	11.6	0.734	0.0844	11.5	11.5	14.1	12.400	99	
Macrophage Migration Inhibitory Factor (MIF)	ng/mL	0.00664	0.11	1.2	0.627	0.0568	9.06	3.65	0.269	7.37	9.49	1.34	14.1	7.37	14.1	10.177		
Amphiregulin (AR)	pg/mL	67.2	<LOW>	464	2129	176	8.28	16060	1677	10.4	61740	8746	14.2	8.28	14.2	10.960	191	
Heparin-Binding EGF-Like Growth Factor (HB-EGF)	pg/mL	5.2	<LOW>	104	190	27.1	14.3	1660	182	11	6218	890	14.3	11	14.3	13.200		
Ciliary Neurotrophic Factor (CNTF)	pg/mL	5.2	<LOW>	12.1	27.8	4.03	14.5	759	73.1	9.63	5188	621	12	9.63	14.5	12.043	67	
Ferritin (FRTN)	ng/mL	0.0153	8.75	631	35.2	4.26	12.1	93.8	8.44	9	257	37.2	14.5	9	14.5	11.867		
Macrophage Inflammatory Protein-3 alpha (MIP-3 alpha)	pg/ml	11.6	<LOW>	76	110	15.9	14.5	1890	87.4	4.63	8483	376	4.43	4.43	14.5	7.853	21	
von Willebrand Factor (vWF)	ug/mL	0.001108	1.84	42.2	28.6	4.14	14.5	87.3	7.68	8.8	256	25.2	9.84	8.8	14.5	11.047		
Neutrophil Gelatinase-Associated Lipocalin (NGAL)	ng/ml	0.084	102	822	12.1	1.77	14.6	85.8	5.73	6.67	490	30.9	6.29	6.29	14.6	9.187		
EN-RAGE	ng/mL	0.0092	13	316	12.8	1.31	10.2	28.8	4.21	14.7	54.9	7.16	13	10.2	14.7	12.633	14	
Monokine Induced by Gamma Interferon (MIG)	pg/ml	53.2	877	9260	918	135	14.7	8600	454	5.28	168800	19775	11.7	5.28	14.7	10.560	5	
Epiregulin (EPR)	pg/mL	6.98	<LOW>	41	219	22.7	10.3	1213	180	14.8	4900	643	13.1	10.3	14.8	12.733	327	
Factor VII	ng/mL	0.228	129	682	8.54	1.29	15.1	125	6.65	5.31	825	61.1	7.41	5.31	15.1	9.273	313	
TNF-Related Apoptosis-Inducing Ligand Receptor 3 (TRAIL-R3)	ng/mL	0.0948	3.72	18.4	2.49	0.376	15.1	10.3	0.741	7.18	39.9	2.49	6.25	6.25	15.1	9.510		
Macrophage Inflammatory Protein-1 alpha (MIP-1 alpha)	pg/mL	7.76	<LOW>	60.75	50.7	7.71	15.2	1082	70.5	6.52	6647	479	7.2	6.52	15.2	9.640	76	
Interleukin-15 (IL-15)	ng/mL	0.2312	<LOW>	2.72	0.97	0.149	15.3	4.29	0.3	6.99	25.4	1.09	4.29	4.29	15.3	8.860	78	
C-Reactive Protein (CRP)	ug/mL	2.5844E-06	0.128368794	48.93617021	0.201	0.0299	14.9	0.745	0.0576	7.73	2.26	0.349	15.4	7.73	15.4	12.677	0	1
Matrix Metalloproteinase-3 (MMP-3)	ng/mL	0.02632	2.17	51	0.635	0.0798	12.6	9.12	0.425	4.66	55	8.58	15.6	4.66	15.6	10.953		
Fas Ligand (FasL)	pg/mL	4.28	5.88	34.7	90.1	14.1	15.7	1199	97	8.09	10043	884	8.8	8.09	15.7	10.863	8	
Interleukin-16 (IL-16)	pg/mL	6.56	240	1580	62.6	9.96	15.9	831	66.2	7.96	4514	218	4.82	4.82	15.9	9.560	7	
Endothelin-1 (ET-1)	pg/mL	5.76	<LOW>	5.43	45.5	7.32	16.1	2074	127	6.12	9860	533	5.41	5.41	16.1	9.210	7	
Leptin	ng/mL	0.0468	0.465	41.8	0.253	0.0407	16.1	9.05	0.563	6.23	43.4	2.41	5.56	5.56	16.1	9.297	11	
Thrombospondin-1	ng/mL	2.28	20700	112000	1114	172	15.5	3388	537	15.8	7729	1241	16.1	15.5	16.1	15.800	291	
Tamm-Horsfall Urinary Glycoprotein (THP)	ug/ml	0.00014	0.00791	0.0531	3.21	0.519	16.2	13.9	2.01	14.5	71.9	3.32	4.62	4.62	16.2	11.773	75	
Transforming Growth Factor alpha (TGF-alpha)	pg/mL	2.68	8	31	84.7	13.7	16.2	1837	147	8.01	7868	571	7.25	7.25	16.2	10.487	15	
T Lymphocyte-Secreted Protein I-309 (I-309)	pg/mL	17.2	<LOW>	2200	261	42.9	16.4	2872	409	14.2	13810	2041	14.8	14.2	16.4	15.133	7	
Trefoil Factor 3 (TFF3)	ug/ml	0.0001	0.0205	0.173	0.367	0.0502	13.7	2.05	0.338	16.5	11.3	1.2	10.6	10.6	16.5	13.600		
Betacellulin (BTC)	pg/mL	58.4	<LOW>	446	229	39	17	2721	225	8.28	17170	1721	10	8.28	17	11.760	177	
Carcinoembryonic Antigen (CEA)	ng/mL	0.0756	<LOW>	5.72	0.88	0.149	17	25.5	1.48	5.8	176	6.85	3.88	3.88	17	8.893	216	3
Growth-Regulated alpha protein (GRO-alpha)	pg/mL	17.24	272	1560	74.1	12.6	17.1	804	86	10.7	5046	616	12.2	10.7	17.1	13.333	6	
Alpha-2-Macroglobulin (A2Macro)	mg/mL	0.000131	0.404	7.93	0.323	0.0334	10.3	0.839	0.0532	6.35	3.46	0.593	17.2	6.35	17.2	11.283		
Lymphotactin	ng/mL	0.0904	<LOW>	0.127	0.647	0.111	17.2	3.07	0.109	3.54	15	0.886	5.92	3.54	17.2	8.887	322	
Tissue Factor (TF)	ng/mL	0.1256	<LOW>	4.68	3.71	0.636	17.2	31.2	2.55	8.17	261	21.4	8.21	8.17	17.2	11.193		
Granulocyte Colony-Stimulating Factor (G-CSF)	pg/mL	1.36	0.72	121	9.04	1.57	17.3	500	44.2	8.85	2995	207	6.91	6.91	17.3	11.020	162	
Lectin-Like Oxidized LDL Receptor 1 (LOX-1)	ng/mL	0.3292	<LOW>	3	8.21	1.43	17.4	35.2	4.44	12.6	146	13.2	9.07	9.07	17.4	13.023		
Resistin	ng/ml	0.012	0.059	3.6	0.203	0.0354	17.4	1.81	0.179	9.89	8.53	0.808	9.47	9.47	17.4	12.253	4	
CD 40 antigen (CD40)	ng/mL	0.00872	0.17	6.1	0.0421	0.00752	17.9	2.11	0.169	8	10	0.861	8.59	8	17.9	11.497		
Insulin	uIU/mL	0.2648	<LOW>	49.3	0.251	0.0453	18.1	3.65	0.28	7.68	22.1	1.07	4.84	4.84	18.1	10.207	39	
Fibroblast Growth Factor basic (FGF-basic)	pg/mL	99.6	<LOW>	264	396	72.2	18.2	21000	1355	6.45	128600	2914	2.27	2.27	18.2	8.973	152	
Interleukin-18 (IL-18)	pg/mL	10.12	106	651	77.8	14.6	18.7	1649	96.2	5.83	7964	389	4.89	4.89	18.7	9.807	116	
Prostatic Acid Phosphatase (PAP)	ng/mL	0.008461539	0.099038462	2.019230769	0.426	0.0798	18.7	9.53	0.678	7.11	50.4	6.63	13.2	7.11	18.7	13.003		
Eotaxin-1	pg/mL	7.52	<LOW>	218	52.5	10.1	19.2	623	90.5	14.5	4428	512	11.6	11.6	19.2	15.100	12	
Apolipoprotein C-III (Apo C-III)	ug/mL	0.0040625	35.1	253	15.1	3.05	20.2	43.2	4.92	11.4	118	12.2	10.3	10.3	20.2	13.967		
Pancreatic Polypeptide (PPP)	pg/ml	2	2.67	337.6	4.47	0.916	20.5	76.5	6.74	8.82	616	35	5.69	5.69	20.5	11.670	8	
E-Selectin	ng/mL	0.2052	4.3	21	0.867	0.179	20.6	18.1	0.554	3.07	84.1	5.67	6.75	3.07	20.6	10.140	126	
Fibroblast Growth Factor 4 (FGF-4)	pg/mL	36.08	<LOW>	104	123	26.2	21.2	1813	182	10.1	8085	750	9.27	9.27	21.2	13.523	1	
Interleukin-1 receptor antagonist (IL-1ra)	pg/mL	17.52	6.1	1310	96	20.9	21.8	4032	368	9.12	21230	1572	7.4	7.4	21.8	12.773	17	
Transforming Growth Factor beta-3 (TGF-beta-3)	pg/mL	6.72	<LOW>	12.4	86.2	19	22	1642	178	10.9	14420	1389	9.63	9.63	22	14.177	292	
Epidermal Growth Factor (EGF)	pg/mL	1.196	<LOW>	493	5.85	1.31	22.3	546	54.1	9.9	2641	153	5.8	5.8	22.3	12.667	262	
Interleukin-17 (IL-17)	pg/mL	2.632	<LOW>	24.3	12.7	2.85	22.5	43.9	4.32	9.84	136	16.8	12.4	9.84	22.5	14.913	123	
Apolipoprotein D (Apo D)	ug/ml	0.0258	58.5	169	50	11.4	22.9	132	13.1	9.95	397	44	11.1	9.95	22.9	14.650		
Fibrinogen	mg/mL	0.0000175	<LOW>	0.0303	0.0736	0.0168	22.9	0.241	0.025	10.4	0.701	0.0523	7.46	7.46	22.9	13.587	1	
AXL Receptor Tyrosine Kinase (AXL)	ng/mL	0.0202	4.09	14.4	0.161	0.0371	23	1.85	0.147	7.95	17.2	0.643	3.74	3.74	23	11.563		
Alpha-Fetoprotein (AFP)	ng/mL	0.051908397	0.5	4.427480916	0.764	0.177	23.1	22.7	2.97	13	146	8.79	6.01	6.01	23.1	14.037	127	
Fatty Acid-Binding Protein, heart (FABP, heart)	ng/mL	0.2824	<LOW>	10.3	2.76	0.641	23.2	42.7	1.9	4.44	281	24.4	8.69	4.44	23.2	12.110		
Interleukin-13 (IL-13)	pg/mL	6.36	<LOW>	100	9.02	2.09	23.2	36.7	3.7	10.1	123	9.86	8.01	8.01	23.2	13.770	45	
Alpha-1-Microglobulin (A1Micro)	ug/ml	0.000097	5.7	17.1	0.941	0.219	23.3	1.48	0.144	9.68	3.7	0.263	7.12	7.12	23.3	13.367		
Thyroid-Stimulating Hormone (TSH)	uIU/mL	0.0082	0.444	5.11	0.0943	0.0221	23.4	4.51	0.283	6.29	24.9	1.85	7.4	6.29	23.4	12.363	66	
Luteinizing Hormone (LH)	mIU/mL	0.321543408	<LOW>	41.80064309	2.29	0.544	23.7	25.2	1.82	7.23	206	8.47	4.12	4.12	23.7	11.683	35	
Pregnancy-Associated Plasma Protein A (PAPP-A)	mIU/mL	0.0044	0.0131	0.0783	0.0139	0.00337	24.3	0.825	0.0885	10.7	3.22	0.118	3.66	3.66	24.3	12.887	1	
Myeloid Progenitor Inhibitory Factor 1 (MPIF-1)	ng/mL	0.0624	1	3	0.167	0.0408	24.4	3.43	0.14	4.09	19.2	1.06	5.53	4.09	24.4	11.340	11	
Prostate-Specific Antigen, Free (PSA-f)	ng/mL	0.005882353	<LOW>	0.572192513	0.0235	0.00576	24.5	0.243	0.0223	9.18	1.05	0.0933	8.93	8.93	24.5	14.203	131	
Prolactin (PRL)	ng/ml	0.2	0.103	6.79962514	0.144	0.0358	24.8	4.54	0.197	4.34	38.7	2.34	6.03	4.34	24.8	11.723		
Matrix Metalloproteinase-9 (MMP-9)	ng/mL	4.08	<LOW>	318	14.9	3.81	25.6	160	16	9.99	1922	104	5.39	5.39	25.6	13.660	217	
Malondialdehyde-Modified Low-Density Lipoprotein (MDA-LDL)	ng/mL	21.92	<LOW>	32	647	166	25.7	7999	1476	18.4	26630	4946	18.6	18.4	25.7	20.900	165	
Thrombopoietin	ng/mL	1.756	<LOW>	4.44	0.788	0.212	26.9	8.86	0.597	6.73	58.8	3.02	5.14	5.14	26.9	12.923	1	
Sex Hormone-Binding Globulin (SHBG)	nmol/L	0.000936	18	114	2.81	0.808	28.7	7.32	1.11	15.2	25.6	2.18	8.52	8.52	28.7	17.473		
Agouti-Related Protein (AGRP)	pg/mL	66	<LOW>	136	178	51.6	28.9	1250	109	8.71	12807	2201	17.2	8.71	28.9	18.270	7	
Vascular Endothelial Growth Factor (VEGF)	pg/mL	6.8	107	1010	51.7	15.1	29.2	2727	236	8.65	14000	650	4.64	4.64	29.2	14.163		
Serum Glutamic Oxaloacetic Transaminase (SGOT)	ug/mL	1	<LOW>	11.3	1.75	0.518	29.6	7.97	0.707	8.86	38.2	2.44	6.39	6.39	29.6	14.950		

Table 2 Plasma QC Statistics

Analyte					QC 1			QC 2			QC 3			Summary CV			Missing data	
Name	Unit	Least Detectable Dose	RBM Low Serum Range	RBM High Serum Range	Mean	SD	CV	Mean	SD	CV	Mean	SD	CV	Low	High	Average	LOW	HIGH
Creatine Kinase-MB (CK-MB)	ng/mL	0.364705882	<LOW>	19.76470588	0.524	0.156	29.8	5.32	0.724	13.6	42.2	3.78	8.95	8.95	29.8	17.450	33	
Pulmonary and Activation-Regulated Chemokine (PARC)	ng/mL	0.028	11.3	42.6	0.0566	0.0169	29.8	0.0905	0.0269	29.7	0.176	0.0175	9.89	9.89	29.8	23.130	327	
Adiponectin	ug/mL	0.000282	1.82	11.2	0.197	0.0589	29.9	0.623	0.0749	12	2.04	0.246	12.1	12	29.9	18.000		
Glucagon-like Peptide 1, total (GLP-1 total)	pg/ml	3	<LOW>	131.7780685	6.74	2.06	30.6	118	13.4	11.4	810	76.5	9.45	9.45	30.6	17.150	258	
Interleukin-1 beta (IL-1 beta)	pg/mL	0.456	<LOW>	7.81	1.78	0.545	30.6	90.6	6.78	7.48	451	25.5	5.65	5.65	30.6	14.577	262	
Calcitonin	pg/mL	2.898203593	<LOW>	8.443113773	11.4	3.66	32.3	181	8.14	4.5	926	75.7	8.18	4.5	32.3	14.993	12	
Hepatocyte Growth Factor (HGF)	ng/mL	0.0812	1.51	2.69	2.7	0.896	33.2	8.95	0.855	9.55	42.7	4	9.36	9.36	33.2	17.370		
Interleukin-25 (IL-25)	pg/mL	5.24	<LOW>	78.4	26.8	9.34	34.9	1886	128	6.81	9178	550	5.99	5.99	34.9	15.900	6	
Intercellular Adhesion Molecule 1 (ICAM-1)	ng/mL	0.84	63.8	272	1.54	0.573	37.3	40.1	2.93	7.31	180	22.8	12.7	7.31	37.3	19.103	15	
Cancer Antigen 19-9 (CA-19-9)	U/mL	0.648	<LOW>	52	2.74	1.06	38.5	76.5	6.67	8.73	416	26.9	6.48	6.48	38.5	17.903	12	
Immunoglobulin M (IGM)	mg/mL	0.000100125	0.304	3.32	0.187	0.0737	39.4	0.573	0.198	34.6	1.73	0.236	13.6	13.6	39.4	29.200	54	2
Interleukin-6 (IL-6)	pg/mL	0.72	<LOW>	42.6	5.92	2.36	39.8	300	24.6	8.2	1757	188	10.7	8.2	39.8	19.567	39	
Tumor Necrosis Factor beta (TNF-beta)	pg/mL	7.32	<LOW>	27.5	23.8	9.77	41	207	21.6	10.5	948	82.5	8.7	8.7	41	20.067	175	
Immunoglobulin E (IgE)	U/mL	2.54	<LOW>	606	9.36	3.92	41.8	224	24.6	11	1396	81.3	5.82	5.82	41.8	19.540	277	
Matrix Metalloproteinase-2 (MMP-2)	ng/mL	5.96	<LOW>	151	25.8	10.9	42.3	370	48.1	13	2216	364	16.4	13	42.3	23.900	2	
CD40 Ligand (CD40-L)	ng/mL	0.00504	0.14	4.9	0.0208	0.00924	44.4	1.31	0.153	11.7	7.1	0.669	9.42	9.42	44.4	21.840	124	
Bone Morphogenetic Protein 6 (BMP-6)	ng/mL	0.1468	<LOW>	Not Detected	0.331	0.157	47.2	18.5	2.69	14.5	25.5	4.68	18.4	14.5	47.2	26.700	69	
Follicle-Stimulating Hormone (FSH)	mIU/mL	1.074380166	0.088016529	10.87130643	1.56	0.757	48.4	29.1	2.1	7.21	280	13	4.63	4.63	48.4	20.080	19	
Erythropoietin (EPO)	pg/mL	5.6	<LOW>	Not Detected	21.8	13.2	60.5	380	60.2	15.8	1839	194	10.5	10.5	60.5	28.933	87	

Table 3: Demographics of the CSF MyriadRBM multiplex biomarker cohort

	Control	MCI	AD
N baseline	92	149	69
Age	76 (62-90)	75 (57-89)	75 (56-88)
Gender M/F (baseline)	46/46	103/47	39/30
ApoE4% (baseline)	24%	54%	71%
MMSE (range)	29.1 (25-30)	27.0 (23-30)	23.5 (20-27)

Table 4: Column header definitions in the ADNI CSF Myriad**RBM Multiplex Raw Data**

This dataset structure is one record per sample per analyte and contains both the raw value obtained directly from RBM and the analysis value, which may be transformed or imputed.

Variable Name	Description and Coding
ID	record ID
RID	ADNI subject ID
sampleID	ID of CSF sample
Plate	ID of Plate used
Visit_Code	Visit Designator (bl = baseline)
analyte	Name of Analyte with Units
LDD	Least Detectable Dose (see above for details)
avalue	Recorded Value
analval	Numeric Value after possible imputation/transformation (see above and SAP for details)
belowLDD	Is analval < LDD? Note: this flag pertains to both recorded value and imputed value (0=no ; 1=yes)
readLOW	Is recorded value <LOW> or numeric? (0=numeric; 1=<LOW> - see primer for details)
ReadHIGH	Is recorded value HIGH (> limit) or actual? (0=actual value; 1=HIGH – see primer for details
Logtrans	Is analval log transformed? (1=yes; 0=no)
Outlier	Is recorded value an outlier? (0=no; 1=yes) - outliers imputed to 5SD from mean

Table 5. Column header definitions in the ADNI CSF Myriad**RBM QC Multiplex data**

This is the value-added analysis dataset, structured as one record per sample.

Variable Name	Description
ID	record ID
RID	ADNI subject ID
sampleID	Sample ID from UPenn
Sample_Recieved_date	Date sample received at UPenn
Visit_Code	Visit Designator (bl = baseline;

The remainder of the columns denote 159 analytes measured by RBM, populated with numeric, possibly imputed values (see above for details)

APPENDIX III
Biomarkers Consortium Project
Use of Targeted Multiplex Proteomic Strategies to Identify CSF-Based Biomarkers in Alzheimer's Disease
Statistical Analysis Plan

Biomarkers Consortium Project
Use of Targeted Multiplex Proteomic Strategies to Identify CSF-Based Biomarkers in Alzheimer's Disease (AD)

Statistical Analysis Plan

Introduction

The Analysis Plan described within this document represents the work of the Biomarkers Consortium Project "**Use of Targeted Multiplex Proteomic Strategies to Identify CSF-Based Biomarkers in Alzheimer's Disease"**. This project was submitted to the Biomarkers Consortium Neuroscience Steering Committee by a subgroup of the Alzheimer's Disease Neuroimaging Initiative (ADNI) Industry Private Partner Scientific Board (PPSB) for execution and was managed by a Biomarkers Consortium Project Team that includes members from academia, government and the pharmaceutical industry. Funding for this project was provided by the Alzheimer's Drug Discovery Foundation, Eisai, Lilly, Merck, Pfizer, and Takeda. This project is the second part of a multi-phased effort seeking to utilize samples collected by ADNI to qualify multiplex panels in both plasma and cerebrospinal fluid (CSF) to diagnose patients with Alzheimer's Disease (AD) and monitor disease progression. An earlier phase of the program focused on analysis of data from ADNI plasma samples run on a multiplex panel (Soares et al, in prep, data available on the ADNI website, www.adni.loni.edu).

Biomarker tools for early diagnosis and disease progression in Alzheimer's disease (AD) remain key issues in AD drug development. Identification and validation of cost-effective methods to identify early AD and to monitor treatment effects in mild-moderate AD patients could revolutionize current clinical trial practice. Treatment prior to the onset of dementia may also ensure intervention occurs before irreversible neuropathology.

The aim of the project is to determine the ability of a multiplex CSF based immunoassay panel to discriminate among disease states and to monitor disease progression over a one year period. The multiplex panel is based upon luminex immunoassay technology and has been developed by Rules Based Medicine (RBM) to measure a range of inflammatory, metabolic, lipid and other disease relevant endpoints. Prior studies using an older version of the RBM panel (an 89 analyte version) suggested some analytes on the panel differed between AD and controls. The panel has been expanded to include analytes from a recent article describing plasma based biomarkers of AD. For this project, a 159-analyte version of the panel focused on analytes believed to be relevant to AD will be used.

The analyses described in this statistical analysis plan should be regarded as exploratory and meant for hypothesis and model generation, rather than for hypothesis confirmation and model validation. Results from this study will be compared with those from other studies on CSF proteins in AD, and findings will need to be confirmed and expanded upon in subsequent studies using other, independent data sets.

Study Design and Objectives

Study Design

A total of 327 CSF samples from the baseline ADNI sample set will be assessed (N= 92 Controls, 69 AD, 149 for amnestic mild cognitive impairment (MCI) and 1 unknown diagnosis, plus 16 technical replicates). These baseline CSF samples have matching aliquots from year 1, which may be assayed at a future date. Of the 149 MCI subjects, 38 subjects had progressed to dementia as of March 2010. This statistical analysis plan addresses the analysis of data from these samples.

Previously, 1062 ADNI plasma samples were run on the RBM 190 analyte panel. Data from the plasma study have already been analyzed (Soares et al, in preparation). The 327 subjects with CSF samples are a partial subset of the subjects in the plasma study. Therefore, findings in plasma can be used in evaluating the results of the CSF study.

Study Objectives

- To determine whether baseline levels for individual analytes are associated with patient demographics (age, gender) or disease status.
- To determine whether baseline levels for a combination of analytes will provide a panel with distinctly different profiles for the ADNI normal controls (NC), MCI or AD.
- To determine whether baseline levels for a combination of analytes derived from either a biological pre-selection based method and/or from a statistically based/machine learning approach will provide a panel that discriminates pre-demented subjects who will progress to dementia in up to 4 years.
- To compare analyte associations and discrimination models in CSF with those found in plasma.

Univariate Analysis

Univariate analyses will be performed first. The results of the univariate analyses may be used to inform and select analytes to be used in the pathway analyses and multivariate predictive model-building. Results from the univariate and multivariate sets of analyses will be compared for overlap and a final panel selected based on optimal overlap.

Classification Endpoints

Clinical diagnosis at time of enrollment/collection will be used to classify AD, MCI and control groups. Clinical diagnosis of amnestic MCI followed by diagnosis of AD will be used to classify pre-demented progressors.

Data Quality Control (QC)

Up to 159 analytes may be measured in the CSF RBM panel. CSF data will be analyzed separately and compared for each analyte dependent upon sample availability. The data will be prepared for all analysis as follows:

- Review of the ADNI CSF test/re-test QC samples data to determine the precision performance for each analyte. The specific precision parameters examined for the 16 pairs of CSF sample aliquots include: mean analyte concentration for the replicate samples; mean difference between the test CSF sample and the retested replicate CSF sample aliquot; mean % difference for the test/retest samples; *p* value for testing for difference from 0; mean of the absolute concentration values for each pair of CSF samples; mean % difference of the absolute concentration values for each of CSF samples; intercept and slope values for Bland-Altman analyses and respective *p* values for testing for difference from 0.
- Review of the quality control samples data for each run to determine the variability characteristics of the spiked plasma (or serum) QC samples. Characteristics examined for the LOW, MEDIUM and HIGH QC samples for each biomarker will include mean, standard deviation (SD) and the percent coefficient of variation (%CV) for each analyte to determine not only the variability at each concentration but whether or not there is a major change in variability across the concentration range for each analyte.
- Analytes with more than 10% missing data will not be analyzed further. Missing data are generally indicated by "QNS" (quantity not sufficient for analysis) by RBM.
- Analytes with more than 10% recorded as "LOW" or ">value" will not be included in the multivariate analysis. These analytes will be assessed to compare the proportion of measurable samples in each disease status category. If proportions differ substantially among disease status categories for some analytes, alternative approaches may be explored for incorporating such analytes in the multivariate analyses described below.
- For each analyte, the distribution of measured values within each diagnostic group will be examined. If the distributions are not normal, the team will seek appropriate transformations (e.g., Box-Cox transformations **(Box and Cox, 1964)** so the transformed markers approximate normality. All subsequent data preparation and analyses will then be conducted on the transformed values.
- Analytes with less than 10% missing/"LOW"/">value" values will have the non-numeric values imputed as follows:
    - Values recorded as "LOW" will be imputed to LLD/2
    - Values recorded as ">value" will be imputed to 2 times the maximum non-missing value for that analyte.
    - Missing values will be imputed to be the mean of the non-missing values for that analyte.
    - Samples with imputed values for more than 25% of the analytes will be excluded from the analysis
- Multidimensional scaling and/or Mahalanobis distances will be used to detect sample outliers and misclassified subjects.
- For univariate analysis, outliers that are more than 5 STD from mean will be assigned the value of the nearest non-outlier point. For outliers apparent in multivariate reviews, outliers will be imputed using a nearest neighbor or other appropriate algorithm.

The imputation and outlier definition strategy defined above is only one of many possible strategies that could be used. If resources permit a limited number of alternative strategies may be used to assess the robustness of the analytical conclusions obtained using the strategy defined above.

As part of data QC, patient, visit, and sample identifiers will be checked for uniqueness and logical consistency. Graphical displays will be used to check for systematic patterns related to batch, run date, sample quality measures, and QC sample characteristics.

Cleaning, outlier detection, and distribution displays of all samples will be performed prior to merging phenotype data with the biomarker data. Misclassification assessment will be performed prior to statistical analysis.

For each sample with technical replicates, one replicate will be selected at random for use in any analysis that includes samples that did not have technical replicates.

General approach

Analysis of variance (ANOVA) and analysis of covariance (ANCOVA) models will be used to compare mean analyte levels among groups of interest. These ANOVA/ANCOVA models will include the diagnosis/disease status group and other covariates including age, gender and apoE4 genotype/status, as well as possible interactions among these factors. The interactive effect between group and other covariates will be tested. Depending on the outcome of these tests, the differences between groups will be tested either by the main effect of diagnosis or the effect of diagnosis at a fixed level of other covariates (i.e., apoE4 status) or through the adjusted least square means.

A major analytic concern in these tests is the control of overall type I error rate due to the relatively large number of CSF proteins tested in this project. The team will address this concern using false discovery rate (FDR) methodology.

Hypotheses to Be Tested

The following univariate hypotheses will be addressed for each analyte:

HO1i: Analyte i is not associated with age [age treated as a continuous variable]
HO2i: Analyte i is not associated with gender
HO3i: Analyte i is not associated with ApoE status

HO4i: Analyte i is not associated with disease status or change in disease status (adjusted for age, gender, and/or ApoE status as necessary)

An initial set of analyses will look at whether the mean baseline level of each individual marker differs among disease groups (normal, MCI, AD) via an ANOVA or ANCOVA and t-test

analysis. "Disease status" will be based on the clinical calls recorded at baseline in the ADNI database. Additional analyses may be conducted using disease status defined using one or more alternative definitions based on cognitive and/or functional tests. Change in disease status will be based on the same data cut used for the plasma data (March 2010). A second status change analysis using an updated current status may also be performed.

Positive false discovery (pFDR) corrections (Storey, 2003) will be applied to p-values and will be reported along with raw p-values.

A second set of analyses will be performed using data only from MCI subjects. ANOVA/ANCOVAs similar to the above will be run to assess whether mean baseline levels of the analytes differ among MCI non-converters and converters.

A third set of analyses will be run to determine whether any of the analytes correlate with significant changes in Clinical Dementia Rating Scale-Sum of the Boxes (CDR-SB) or Auditory-Verbal Learning Test (AVLT).

A fourth set of analyses will determine whether levels of any of the analytes are associated with low CSF abeta/high tau, high amyloid brain burden and significant brain atrophy.

Analyses to examine relationships between analyte levels and use of acetyl cholinesterase inhibitors or other medications by subjects may also be performed.

4 Pathway Analysis of Biomarkers

Although statistical machine learning-based approaches can generate a short list of discriminatory proteins, such analyses reveal little about biological relevance. In addition to machine learning approaches, the current proposal will use a systems biology approach to better understand pathway relationships between identified proteins. The Project Team will use pathway mining tools, such as those offered by Ingenuity and Pathway studio, to find the functional connections between the markers from plasma samples. This will provide direct evidence to support key hypotheses. To further increase the biological relevance of the protein markers in the predictive models, biomarkers will be selected based on their presence in distinct biological pathways.

In addition empirical characterizations of marker data such as pair-wise correlations or higher-order relations (e.g. principal components analysis (PCA)) will be used. This analysis will derive an initial short list that will then be analyzed using multivariate and machine learning language approaches.

5 Multiple Marker Models

Multivariate statistical methods and multiple machine learning approaches will be used to identify optimal combinations of groups of proteins for two different prediction problems:

1) To discriminate among diagnosis groups at baseline
2) To discriminate between MCI progressors and non-progressors.

The problem of classification and prediction has received a great deal of attention in mining "-omics" data. In the case of this project, the task will be to classify and predict the diagnostic category or progressor/non-progressor status of a sample on the basis of protein quantitative profiles. The main type of statistical problem is the identification of "marker" genes that characterize the difference between groups (e.g. AD, MCI) – the so called "variable/feature selection" problem. One challenge is to find the optimal combination of uncorrelated proteins. This factor not only is very important to improve prediction accuracy but also contributes to the merits of a good classifier: the simplicity and insight gained into the predictive structure of the data.

In all multivariate model building, feature selection will be done using data only from the training set. Feature selection based on a completely independent data set is not feasible for this project due to sample size and the fact that this is the first CSF study to use this version of the RBM panel.

Multiple marker analysis will be used to build relationships to the disease groups. The candidate models include: logistic regression, linear discriminant analysis, nearest shrunken centroid, random forests, support vector machines and partial least squares. The technique of **Xiong et al. (2004)** may be applied to search for the linear combination of informative proteins that optimally discriminates between the diagnostic groups. Models generated by the various methods will be compared and the "best" model will be chosen based on model fit, robustness, and parsimony considerations.

Models will be fit with two sets of covariates, 1) assay results only and 2) assays results plus additional patient information including gender, age, and ApoE4 allele status. Other biomarkers such as amyloid PIB load, hippocampal atrophy, baseline mini-mental state examination (MMSE), and/or baseline Alzheimer's Disease Assessment Scale-Cognitive Subscale 11 (ADAS-cog 11), and tau and Abeta levels determined by luminex assays may also be used. For a specific model, differences in performance between models fit using the two classes of predictors variables should be characterized to understand the predictive ability of the assays beyond that of routine clinical information on the patients. If possible, formal inference should be made regarding the statistical significance of including the assay variables above and beyond that of the clinical data. Analysis will focus on the following:

- good characterizations of error rates; poor fitting models should not be interpreted.
- any feature selection routines should be extensively cross-validated **(see Ambroise and McLachlan, 2002)**
- measures of marker importance should be biased towards those that use uncertainty (e.g. logistic regression slope tests) as opposed to those that do not (e.g. random forest variable importance, etc).

The multivariate results will be compared to the single marker analysis and (especially) biological relevance.

5.1 Analyte Filtering

Several approaches to filtering and feature selection may be examined. Results of the univariate analyses described above may be used to define a starting set of markers for the analysis. Results of the pathway analysis may also be used to define a starting set. In addition, pre-filtering of markers in an unsupervised fashion prior to building models based on empirical measures may also be applied.

5.2 Model Building Approach

For each type of model, predictive model building will be based on an iterative resampling approach.

For each of the K resampling iterations, the steps will include:

- Splitting the data into training and test sets
- Applying an unsupervised filter on the predictors based on data in the training set only.
- Building and tuning the predictive model on the current training set
- Predicting the current test set
- Calculating and saving the performance (classification accuracy, Kappa)
- End resampling iteration
- Assess performance of the model over the K sets of performance metrics

In the above algorithm, the resampling schemes can include cross-validation, the bootstrap and repeated training/test set splits. Methods for unsupervised feature selection can include filters on variance of individual predictors, high pair-wise predictor correlations, etc.

6 Power Calculations

The sample size for this project and resulting analyses are based upon and limited by the availability of ADNI samples. Additional post-hoc analysis will be completed based upon variability characteristics of the current study to understand power requirements for subsequent analysis of future datasets, in discussion with the Project Team.

7 References

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8 Appendix I

RBM 159 Analyte Panel for CSF Proteomics Project

1 Alpha-1-Microglobulin (A1Micro)
2 Alpha-2-Macroglobulin (A2Macro)
3 Alpha-1-Antitrypsin (AAT)
4 Angiotensin-Converting Enzyme (ACE)
5 Adiponectin
6 Alpha-Fetoprotein (AFP)
7 Agouti-Related Protein (AGRP)
8 Angiopoietin-2 (ANG-2)
9 Apolipoprotein A-I (Apo A-I)
10 Apolipoprotein C-III (Apo C-III)
11 Apolipoprotein D (Apo D)
12 Apolipoprotein E (Apo E)
13 Apolipoprotein H (Apo H)
14 Amphiregulin (AR)
15 AXL Receptor Tyrosine Kinase (AXL)
16 Beta-2-Microglobulin (B2M)
17 Brain-Derived Neurotrophic Factor (BDNF)
18 B Lymphocyte Chemoattractant (BLC)
19 Bone Morphogenetic Protein 6 (BMP-6)
20 Betacellulin (BTC)
21 Complement C3 (C3)
22 Cancer Antigen 125 (CA-125)
23 Cancer Antigen 19-9 (CA-19-9)
24 Calcitonin
25 CD 40 antigen (CD40)
26 CD40 Ligand (CD40-L)
27 Carcinoembryonic Antigen (CEA)
28 Chromogranin-A (CgA)
29 Creatine Kinase-MB (CK-MB)
30 Clusterin (CLU)
31 Ciliary Neurotrophic Factor (CNTF)
32 Cortisol (Cortisol)
33 C-Reactive Protein (CRP)
34 Cystatin-C
35 Epidermal Growth Factor (EGF)
36 Epithelial-Derived Neutrophil-Activating Protein 78 (ENA-78)
37 EN-RAGE
38 Eotaxin-1

39 Eotaxin-3
40 Erythropoietin (EPO)
41 Epiregulin (EPR)
42 E-Selectin
43 Endothelin-1 (ET-1)
44 Fatty Acid-Binding Protein, heart (FABP, heart)
45 Factor VII
46 FASLG Receptor (FAS)
47 Fas Ligand (FasL)
48 Fibroblast Growth Factor 4 (FGF-4)
49 Fibroblast Growth Factor basic (FGF-basic)
50 Fibrinogen
51 Ferritin (FRTN)
52 Follicle-Stimulating Hormone (FSH)
53 Granulocyte Colony-Stimulating Factor (G-CSF)
54 Growth Hormone (GH)
55 Glucagon-like Peptide 1, total (GLP-1 total)
56 Granulocyte-Macrophage Colony-Stimulating Factor (GM-CSF)
57 Growth-Regulated alpha protein (GRO-alpha)
58 Haptoglobin
59 Heparin-Binding EGF-Like Growth Factor (HB-EGF)
60 Chemokine CC-4 (HCC-4)
61 Hepatocyte Growth Factor (HGF)
62 T Lymphocyte-Secreted Protein I-309 (I-309)
63 Intercellular Adhesion Molecule 1 (ICAM-1)
64 Interferon gamma (IFN-gamma)
65 Immunoglobulin A (IgA)
66 Immunoglobulin E (IgE)
67 Insulin-like Growth Factor-Binding Protein 2 (IGFBP-2)
68 Immunoglobulin M (IGM)
69 Interleukin-1 alpha (IL-1 alpha)
70 Interleukin-1 beta (IL-1 beta)
71 Interleukin-10 (IL-10)
72 Interleukin-12 Subunit p40 (IL-12p40)
73 Interleukin-12 Subunit p70 (IL-12p70)
74 Interleukin-13 (IL-13)
75 Interleukin-15 (IL-15)
76 Interleukin-16 (IL-16)
77 Interleukin-17 (IL-17)
78 Interleukin-18 (IL-18)
79 Interleukin-1 receptor antagonist (IL-1ra)
80 Interleukin-2 (IL-2)

81	Interleukin-23 (IL-23)
82	Interleukin-25 (IL-25)
83	Interleukin-3 (IL-3)
84	Interleukin-4 (IL-4)
85	Interleukin-5 (IL-5)
86	Interleukin-6 (IL-6)
87	Interleukin-6 receptor (IL-6r)
88	Interleukin-7 (IL-7)
89	Interleukin-8 (IL-8)
90	Insulin
91	Interferon gamma Induced Protein 10 (IP-10)
92	Leptin
93	Luteinizing Hormone (LH)
94	Lectin-Like Oxidized LDL Receptor 1 (LOX-1)
95	Apolipoprotein(a) (Lp(a))
96	Lymphotactin
97	Monocyte Chemotactic Protein 1 (MCP-1)
98	Monocyte Chemotactic Protein 2 (MCP-2)
99	Monocyte Chemotactic Protein 3 (MCP-3)
100	Monocyte Chemotactic Protein 4 (MCP-4)
101	Macrophage Colony-Stimulating Factor 1 (M-CSF)
102	Malondialdehyde-Modified Low-Density Lipoprotein (MDA-LDL)
103	Macrophage-Derived Chemokine (MDC)
104	Macrophage Migration Inhibitory Factor (MIF)
105	Monokine Induced by Gamma Interferon (MIG)
106	Macrophage Inflammatory Protein-1 alpha (MIP-1 alpha)
107	Macrophage Inflammatory Protein-1 beta (MIP-1 beta)
108	Macrophage Inflammatory Protein-3 alpha (MIP-3 alpha)
109	Matrix Metalloproteinase-2 (MMP-2)
110	Matrix Metalloproteinase-3 (MMP-3)
111	Matrix Metalloproteinase-9 (MMP-9)
112	Myeloid Progenitor Inhibitory Factor 1 (MPIF-1)
113	Myeloperoxidase (MPO)
114	Myoglobin
115	Neutrophil Gelatinase-Associated Lipocalin (NGAL)
116	Nerve Growth Factor beta (NGF-beta)
117	Neuronal Cell Adhesion Molecule (Nr-CAM)
118	N-terminal prohormone of brain natriuretic peptide (NT proBNP)
119	Osteopontin

120 Plasminogen Activator Inhibitor 1 (PAI-1)
121 Prostatic Acid Phosphatase (PAP)
122 Pregnancy-Associated Plasma Protein A (PAPP-A)
123 Pulmonary and Activation-Regulated Chemokine (PARC)
124 Platelet-Derived Growth Factor BB (PDGF-BB)
125 Placenta Growth Factor (PLGF)
126 Pancreatic Polypeptide (PPP)
127 Prolactin (PRL)
128 Progesterone
129 Prostate-Specific Antigen, Free (PSA-f)
130 Receptor for advanced glycosylation end products (RAGE)
131 T-Cell-Specific Protein RANTES (RANTES)
132 Resistin
133 S100 calcium-binding protein B (S100-B)
134 Serum Amyloid P-Component (SAP)
135 Stem Cell Factor (SCF)
136 Serum Glutamic Oxaloacetic Transaminase (SGOT)
137 Sex Hormone-Binding Globulin (SHBG)
138 Superoxide Dismutase 1, Soluble (SOD-1)
139 Sortilin
140 Thyroxine-Binding Globulin (TBG)
141 Testosterone, Total
142 Tissue Factor (TF)
143 Trefoil Factor 3 (TFF3)
144 Transforming Growth Factor alpha (TGF-alpha)
145 Transforming Growth Factor beta-3 (TGF-beta-3)
146 Tamm-Horsfall Urinary Glycoprotein (THP)
147 Thrombospondin-1
148 Tissue Inhibitor of Metalloproteinases 1 (TIMP-1)
149 Thrombomodulin (TM)
150 Tenascin-C (TN-C)
151 Tumor Necrosis Factor alpha (TNF-alpha)
152 Tumor Necrosis Factor beta (TNF-beta)
153 Tumor Necrosis Factor Receptor 2 (TNFR2)
154 Thrombopoietin
155 TNF-Related Apoptosis-Inducing Ligand Receptor 3 (TRAIL-R3)
156 Thyroid-Stimulating Hormone (TSH)
157 Vascular Cell Adhesion Molecule-1 (VCAM-1)
158 Vascular Endothelial Growth Factor (VEGF)
159 von Willebrand Factor (vWF)