

Plasma-based, combinatorial and multimodal biomarkers for Alzheimer's disease

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- **Introduction: Alzheimer's disease**
- **Global Economic Impact of Dementia**
- **Biomarker – definitions and background**
- **Blood-based protein biomarkers**
- **Candidate Biomarkers identified**
- **Qualification study: Preliminary results**

Why biomarkers?

Biomarkers may be used for a variety of purposes,

- **diagnosis**
- **patient stratification** and to monitor disease progression and establishing drug effects and safety.
- biomarkers could be used to ensure inclusion of subjects with AD-specific pathologies – therefore reducing the number of subjects required
- studies could use biomarkers to identify **biochemical drug effects** prior to moving on to large-scale Phase II/III trials – reducing expense of a clinical trial.

Biomarker Development Process

➤ **Discovery**

- Scientific expertise and appropriate technology platforms
- Collaboration among scientists, clinicians, statisticians
- Access to samples
- Discovery based on multiple technologies, access to samples, scientific/technical expertise, multi-disciplinary teams

➤ **Confirmation/validation**

- Technical validation
- Clinical validation

➤ **Assay development**

- Based on access to, or development of assay systems, reagents, technology platforms

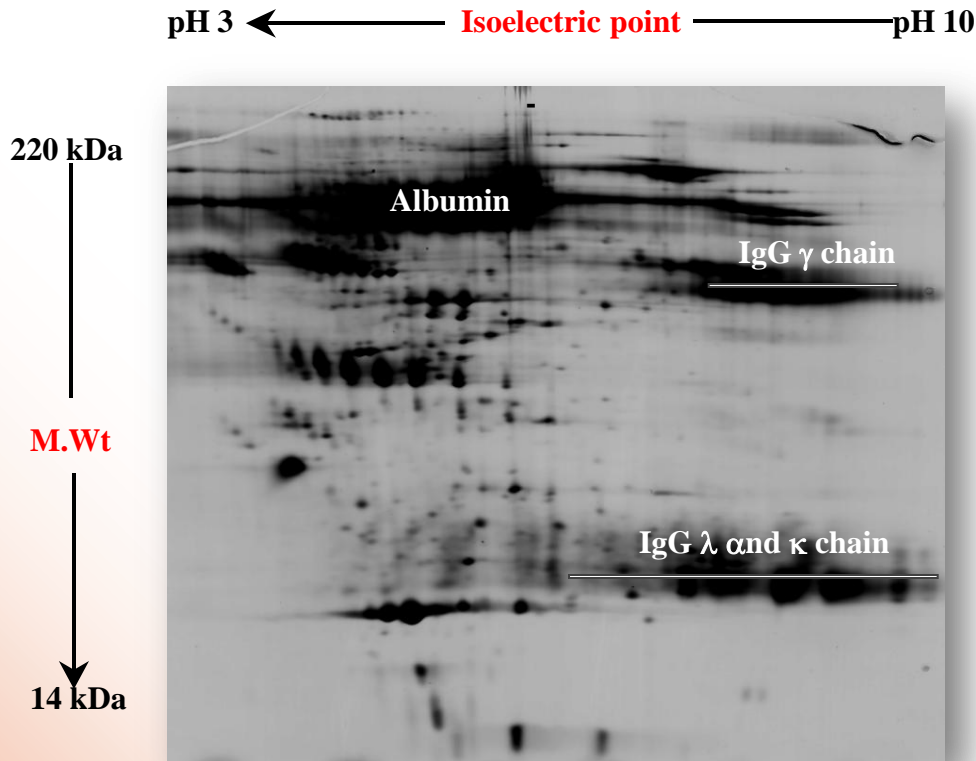
➤ **Regulatory approval**

➤ **Implementation/marketing**

- Clinical labs, commercial partners, strategic business decisions

Blood-based protein biomarkers

Gel based biomarker discovery in plasma



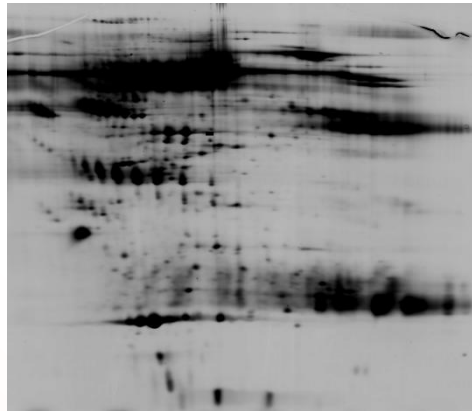
Case vs control study

Two dimensional gel electrophoresis (2-DGE)

A consideration with the use of plasma is the dynamic range more than 10 orders of magnitude in concentration separate highly abundant albumin and the lowest circulating proteins.

Abdul Hye, Madhav Thambisetty, Latha Velayudhan

Validation of CFH & A2M as a marker



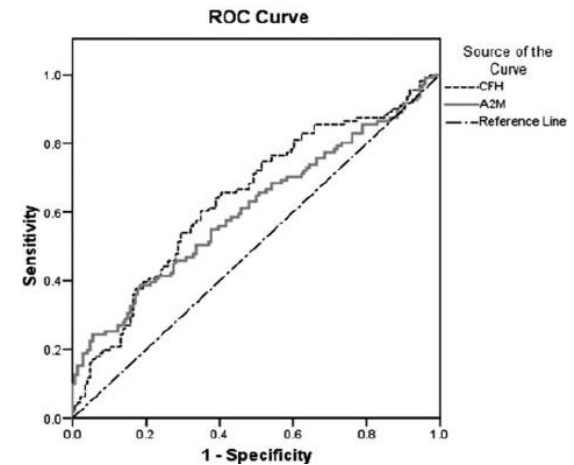
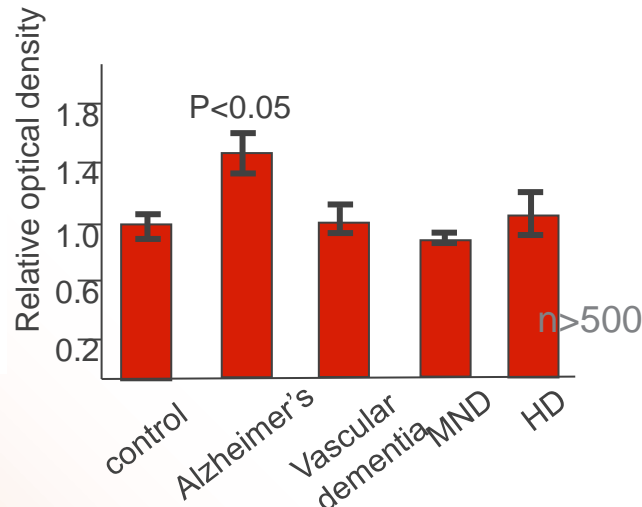
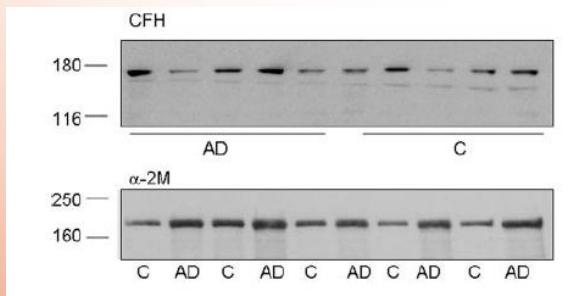
doi:10.1093/brain/awl279

Brain (2006) 129, 3042–3050

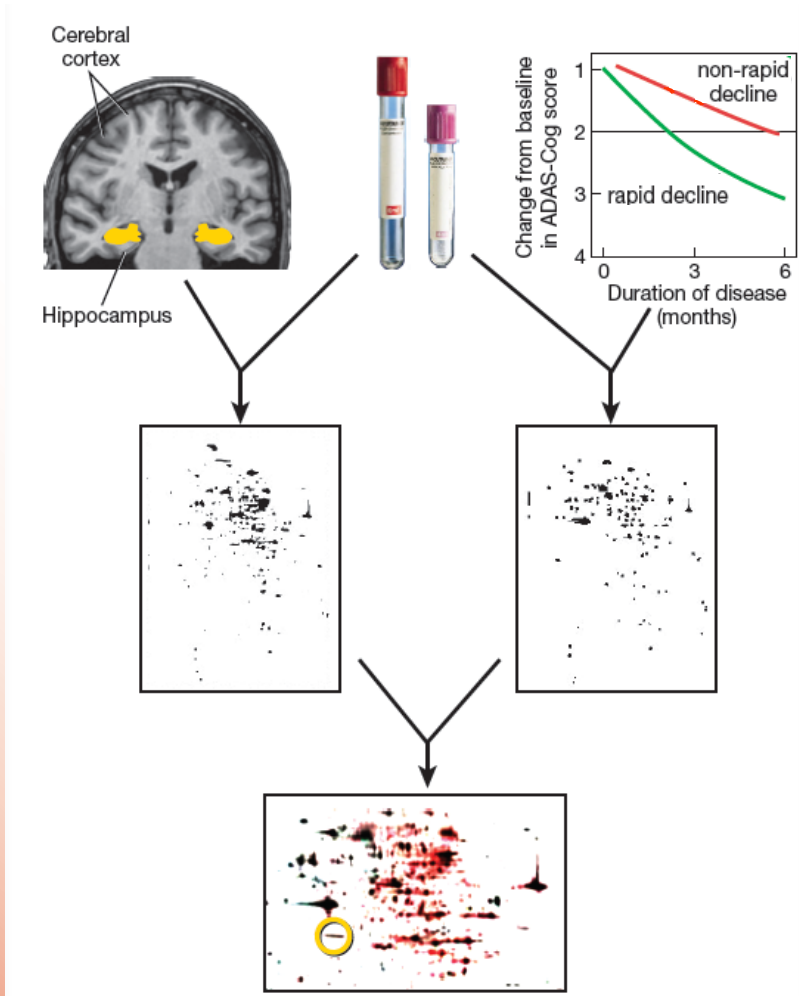
Proteome-based plasma biomarkers for Alzheimer's disease

A. Hye,¹ S. Lynham,¹ M. Thambisetty,¹ M. Causevic,¹ J. Campbell,³ H. L. Byers,³ C. Hooper,¹ F. Rijdsdijk,² S. J. Tabrizi,⁴ S. Banner,¹ C. E. Shaw,¹ C. Foy,¹ M. Poppe,¹ N. Archer,¹ G. Hamilton,¹ J. Powell,¹ R. G. Brown,¹ P. Sham,² M. Ward³ and S. Lovestone¹

Complement Factor H (CFH)



Using imaging to search for biomarkers



- **Correlation with cortical atrophy**
- **Correlation with cognition (MMSE)**
- **Correlation with speed of decline**

Validation – correlation with imaging, cognition and progression

Imaging: entorhinal atrophy

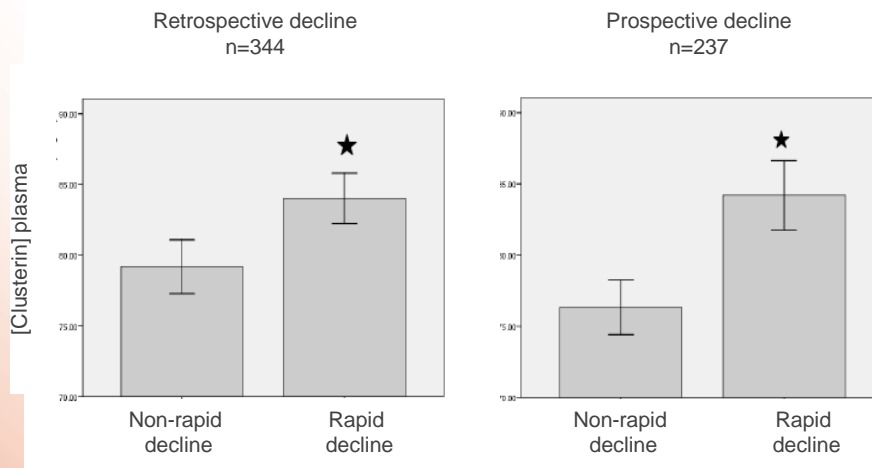
AD and MCI: n=220, R=-0.14 and p=0.04

AD only : n=113, R= -0.31 and p=0.001

Cognition : MMSE

AD only : n=576, r=-0.22; p<0.001

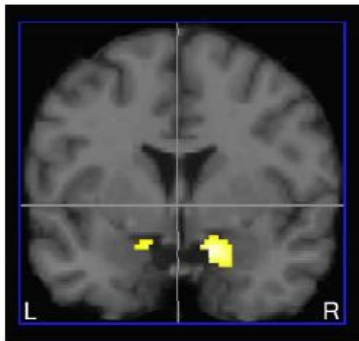
Progression: before *and* after sample point



Andreas Guentert, Abdul Hye,
Anna Kinsey

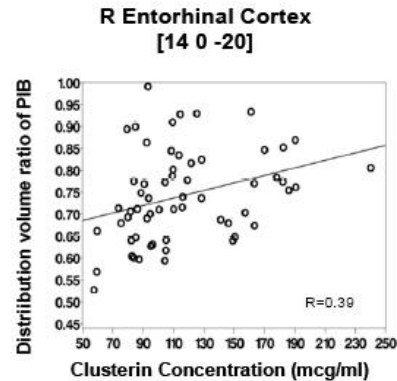
In man....

PIB Retention

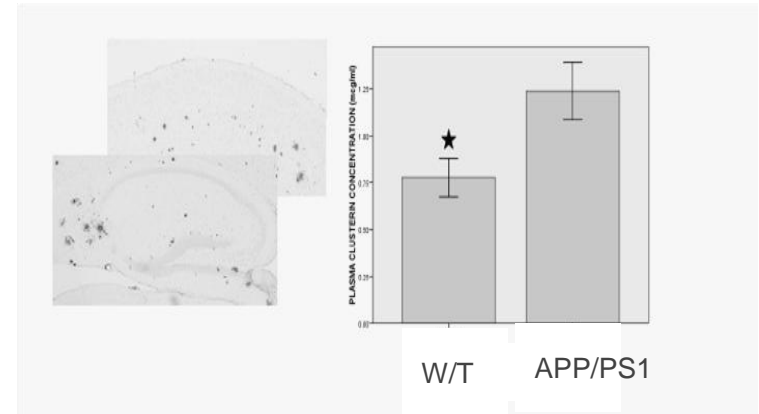


PET scan

Pittsburgh Compound B (PIB)



In mouse....



In brain....

Plasma clusterin *in life* correlates with brain clusterin in superior temporal gyrus

R=0.47 ; p = 0.027 ; N=22

Susan Resnick, Madhav Thambisetty and the BLSA study team

David Howlett, Paul Francis, Andreas Guentert

Muzamil Saleem, Andreas Guentert

Association of Plasma Clusterin Concentration With Severity, Pathology, and Progression in Alzheimer Disease

Madhav Thambisetty, MD, PhD; Andrew Simmons, PhD; Latha Velayudhan, DNB (Psychiatry); Abdul Hye, PhD; James Campbell, PhD; Yi Zhang, MD; Lars-Olof Wahlund, MD; Eric Westman, PhD; Anna Kinsey, PhD; Andreas Güntert, PhD; Petroula Proitsi, PhD; John Powell, PhD; Mirsada Causevic, PhD; Richard Killick, PhD; Katie Lunnon, PhD; Steven Lynham, MSc; Martin Broadstock, PhD; Fahd Choudhry, PhD; David R. Howlett, PhD; Robert J. Williams, PhD; Sally I. Sharp, PhD; Cathy Mitchelmore, PhD; Catherine Tunnard, BSc; Rufina Leung, BSc; Catherine Foy, PhD; Darragh O'Brien, MSc; Gerome Breen, PhD; Simon J. Furney, PhD; Malcolm Ward, MSc; Iwona Kloszewska, MD; Patrizia Mecocci, MD; Hilikka Soininen, MD; Magda Tsolaki, MD; Bruno Vellas, MD; Angela Hodges, PhD; Declan G. M. Murphy, MB BS, FRCPsych; Sue Parkins, PhD; Jill C. Richardson, PhD; Susan M. Resnick, PhD; Luigi Ferrucci, MD, PhD; Dean F. Wong, MD, PhD; Yun Zhou, PhD; Sebastian Muehlboeck, MSc; Alan Evans, PhD; Paul T. Francis, PhD; Christian Spenger, PhD; Simon Lovestone, MRC Psych, PhD

Context: Blood-based analytes may be indicators of pathological processes in Alzheimer disease (AD).

Objective: To identify plasma proteins associated with AD pathology using a combined proteomic and neuroimaging approach.

Design: Discovery-phase proteomics to identify plasma proteins associated with correlates of AD pathology. Confirmation and validation using immunodetection in a replication set and an animal model.

Setting: A multicenter European study (AddNeuroMed) and the Baltimore Longitudinal Study of Aging.

Participants: Patients with AD, subjects with mild cognitive impairment, and healthy controls with standardized clinical assessments and structural neuroimaging.

Main Outcome Measures: Association of plasma proteins with brain atrophy, disease severity, and rate of clinical progression. Extension studies in humans and trans-

genic mice tested the association between plasma proteins and brain amyloid.

Results: Clusterin/apolipoprotein J was associated with atrophy of the entorhinal cortex, baseline disease severity, and rapid clinical progression in AD. Increased plasma concentration of clusterin was predictive of greater fibrillar amyloid- β burden in the medial temporal lobe. Subjects with AD had increased clusterin messenger RNA in blood, but there was no effect of single-nucleotide polymorphisms in the gene encoding clusterin with gene or protein expression. *APP/PS1* transgenic mice showed increased plasma clusterin, age-dependent increase in brain clusterin, as well as amyloid and clusterin colocalization in plaques.

Conclusions: These results demonstrate an important role of clusterin in the pathogenesis of AD and suggest that alterations in amyloid chaperone proteins may be a biologically relevant peripheral signature of AD.

Arch Gen Psychiatry. 2010;67(7):739-748

N=14,000

Genome-wide association study identifies variants at *CLU* and *CR1* associated with Alzheimer's disease

Jean-Charles Lambert¹⁻³, Simon Heath⁴, Gael Even^{1,2}, Dominique Campion⁵, Kristel Slegers^{6,7}, Mikko Hiltunen⁸, Onofre Combarros⁹, Diana Zelenika⁴, Maria J Bullido¹⁰, Béatrice Tavernier¹¹, Luc Letenneur¹², Karolien Bettens^{6,7}, Claudine Berr¹³, Florence Pasquier^{3,14}, Nathalie Fiévet^{1,2}, Pascale Barberger-Gateau¹², Sebastiaan Engelborghs^{7,15}, Peter De Deyn^{7,15}, Ignacio Mateo⁹, Ana Franck¹⁶, Seppo Helisalmi⁸, Elisa Porcellini¹⁷, Olivier Hanon¹⁸, the European Alzheimer's Disease Initiative Investigators¹⁹, Marian M de Pancorbo²⁰, Corinne Lendon²¹, Carole Dufouil^{22,23}, Céline Jaillard²⁴, Thierry Leveillard²⁴, Victoria Alvarez²⁵, Paolo Bosco²⁶, Michelangelo Mancuso²⁷, Francesco Panza²⁸, Benedetta Nacmias²⁹, Paola Bossù³⁰, Paola Piccardi³¹, Giorgio Annoni³², Davide Seripa³³, Daniela Galimberti³⁴, Didier Hannequin⁵, Federico Licastro¹⁷, Hilikka Soinenen⁸, Karen Ritchie¹³, Hélène Blanche³⁵, Jean-François Dartigues¹², Christophe Tzourio^{22,23}, Ivo Gut⁴, Christine Van Broeckhoven^{6,7}, Annick Alperovitch^{22,23}, Mark Lathrop^{4,35} & Philippe Amouyel^{1-3,14}

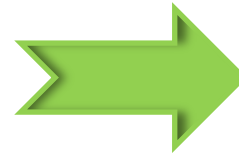
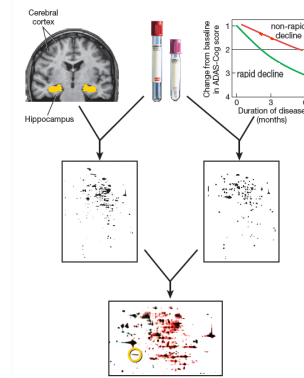
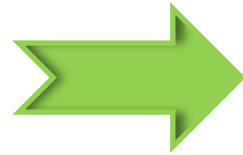
N=16,000

Genome-wide association study identifies variants at *CLU* and *PICALM* associated with Alzheimer's disease

Denise Harold^{1,45*}, Richard Abraham^{1,45}, Paul Hollingworth^{1,45}, Rebecca Sims¹, Amy Gerrish¹, Marian L Hamshere¹, Jaspreet Singh Pahwa¹, Valentina Moskvina¹, Kimberley Dowzell¹, Amy Williams¹, Nicola Jones¹, Charlene Thomas¹, Alexandra Stretton¹, Angharad R Morgan¹, Simon Lovestone², John Powell³, Petroula Proitsi³, Michelle K Lupton³, Carol Brayne⁴, David C Rubinsztein⁵, Michael Gill⁶, Brian Lawlor⁶, Aoibhinn Lynch⁶, Kevin Morgan⁷, Kristelle S Brown⁷, Peter A Passmore⁸, David Craig⁸, Bernadette McGuinness⁸, Stephen Todd⁸, Clive Holmes⁹, David Mann¹⁰, A David Smith¹¹, Seth Love¹², Patrick G Kehoe¹², John Hardy¹³, Simon Mead¹⁴, Nick Fox¹⁵, Martin Rossor¹⁵, John Collinge¹⁴, Wolfgang Maier¹⁶, Frank Jessen¹⁶, Britta Schürmann¹⁶, Hendrik van den Bussche¹⁷, Isabella Heuser¹⁸, Johannes Kornhuber¹⁹, Jens Wiltfang²⁰, Martin Dichgans^{21,22}, Lutz Frölich²³, Harald Hampel^{24,25}, Michael Hüll²⁶, Dan Rujescu²⁵, Alison M Goate²⁷, John S K Kauwe²⁸, Carlos Cruchaga²⁷, Petra Nowotny²⁷, John C Morris²⁷, Kevin Mayo²⁷, Kristel Slegers^{29,30}, Karolien Bettens^{29,30}, Sebastiaan Engelborghs^{30,31}, Peter P De Deyn^{30,31}, Christine Van Broeckhoven^{29,30}, Gill Livingston³², Nicholas J Bass³², Hugh Gurling³², Andrew McQuillin³², Rhian Gwilliam³³, Panagiotis Deloukas³³, Ammar Al-Chalabi³⁴, Christopher E Shaw³⁴, Magda Tsolaki³⁵, Andrew B Singleton³⁶, Rita Guerreiro³⁶, Thomas W Mühleisen^{37,38}, Markus M Nöthen^{37,38}, Susanne Moebus³⁹, Karl-Heinz Jöckel³⁹, Norman Klopp⁴⁰, H-Erich Wichmann⁴⁰⁻⁴², Minerva M Carrasquillo⁴³, V Shane Pankratz⁴⁴, Steven G Younkin⁴³, Peter A Holmans¹, Michael O'Donovan¹, Michael J Owen¹ & Julie Williams¹

Biomarker Development Process

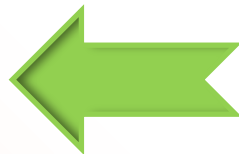
Discovery



Candidate proteins



Multiplexing proteins



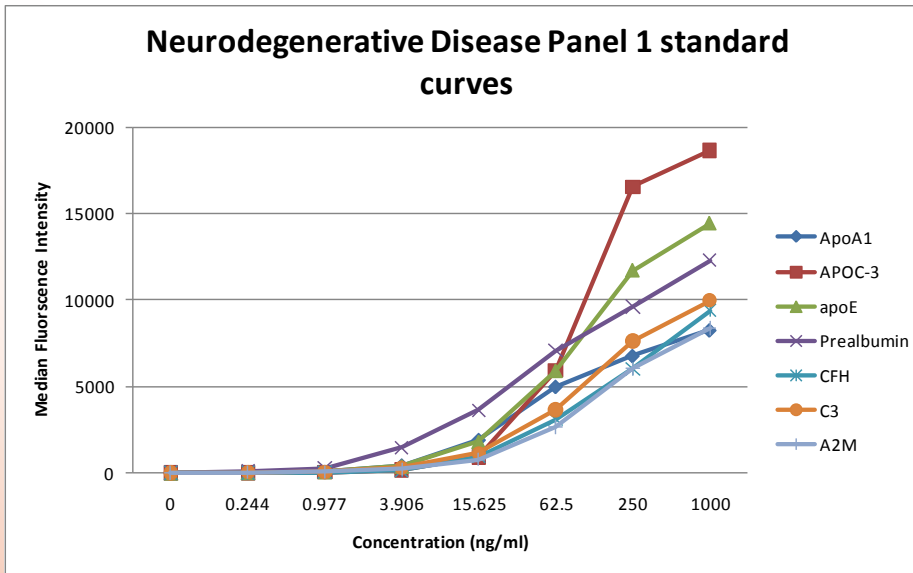
Assay development

Development of assay systems, reagents, technology platforms



MILLIPLEX® MAP Human Neurodegenerative Disease Panel 1 Assay Performance & Precision

The inter-assay precision is calculated as the coefficient of variation (%) for each analyte.



Apo A1	14%
Apo CIII	29%
Apo E	12%
Prealbumin	18%
Complement Factor H	12%
Complement C3	14%
Aα2-macroglobulin	24%

Combinatorial biomarkers - summary

- **Discovery novel biomarkers in plasma by combining imaging and cognitive assessments to identify novel markers**
- **Qualification study in progress to validate candidate biomarkers using Luminex Technology and 7 different MILLIPLEX® MAP assays**
- **The most promising use of the multiplex panel measurements would be a combinatorial approach, whereby we integrate our plasma protein data with**
 - Brain imaging
 - Genomics
 - Transcriptomics
 - Cognitive assessments

To achieve an accurate multimodal biomarker panel