ELSEVIER

Alzheimer's & Dementia 7 (2011) 386–395

Alzheimer's & Dementia

The Alzheimer's Association external quality control program for cerebrospinal fluid biomarkers

Niklas Mattsson[a,*], Ulf Andreasson[a], Staffan Persson[a], Hiroyuki Arai[b], Sat Dev Batish[c], Sergio Bernardini[d], Luisella Bocchio-Chiavetto[e], Marinus A. Blankenstein[f], Maria C. Carrillo[g], Sonia Chalbot[h], Els Coart[i], Davide Chiasserini[j], Neal Cutler[k], Gunilla Dahlfors[l], Stefan Duller[m], Anne M. Fagan[n], Orestes Forlenza[o], Giovanni B. Frisoni[e], Douglas Galasko[p], Daniela Galimberti[q], Harald Hampel[r], Aase Handberg[s], Michael T. Heneka[t], Adrianna Z. Herskovits[u], Sanna-Kaisa Herukka[v], David M. Holtzman[n], Christian Humpel[w], Bradley T. Hyman[u], Khalid Iqbal[h], Mathias Jucker[x], Stephan A. Kaeser[x], Elmar Kaiser[y], Elisabeth Kapaki[z], Daniel Kidd[aa], Peter Klivenyi[bb], Cindy S. Knudsen[s], Markus P. Kummer[t], James Lui[cc], Albert Lladó[dd], Piotr Lewczuk[ee], Qiao-Xin Li[ff], Ralph Martins[cc], Colin Masters[ff], John McAuliffe[c], Marc Mercken[gg], Abhay Moghekar[hh], José Luis Molinuevo[dd], Thomas J. Montine[ii], William Nowatzke[k], Richard O'Brien[hh], Markus Otto[jj], George P. Paraskevas[z], Lucilla Parnetti[j], Ronald C. Petersen[kk], David Prvulovic[r], Herman P. M. de Reus[ll,mm], Robert A. Rissman[p], Elio Scarpini[q], Alessandro Stefani[nn], Hilkka Soininen[v], Johannes Schröder[y], Leslie M. Shaw[oo], Anders Skinningsrud[pp], Brith Skrogstad[pp], Annette Spreer[qq], Leda Talib[o], Charlotte Teunissen[f], John Q. Trojanowski[oo], Hayrettin Tumani[jj], Robert M. Umek[rr], Bianca Van Broeck[gg], Hugo Vanderstichele[i], Laszlo Vecsei[bb], Marcel M. Verbeek[ll,mm], Manfred Windisch[m], Jing Zhang[ii], Henrik Zetterberg[a], Kaj Blennow[a]

[a]*Clinical Neurochemistry Laboratory, Institute of Neuroscience and Physiology, Department of Psychiatry and Neurochemistry, the Sahlgrenska Academy at the University of Gothenburg, Sahlgrenska University Hospital, Mölndal, Sweden*
[b]*Department of Geriatrics and Gerontology, Institute of Development, Aging and Cancer, Tohoku University, Sendai, Japan*
[c]*Athena Diagnostics Inc., Worchester, MA, USA*
[d]*Department Biochimica Clinica, Policlinico, University of Tor Vergata, Rome, Italy*
[e]*IRCCS Centro S. Giovanni di Dio—Fatebenefratelli, Brescia, Italy*
[f]*Department of Clinical Chemistry, VU University Medical Center, Amsterdam, The Netherlands*
[g]*The Alzheimer's Association, Chicago, IL, USA*
[h]*Department of Neurochemistry, New York State Institute for Basic Research in Developmental Disabilities, Staten Island, NY, USA*
[i]*Innogenetics, Ghent, Belgium*
[j]*Section of Neurology, Laboratory of Clinical Neurochemistry, Centre for Memory Disturbances, University of Perugia, Perugia, Italy*
[k]*Worldwide Clinical Trials, King of Prussia, PA, USA*
[l]*Department of Clinical Chemistry, Karolinska University Laboratory, Karolinska University Hospital, Stockholm, Sweden*
[m]*JSW Life Sciences GmbH, Grambach, Austria*
[n]*Department of Neurology, Alzheimer's Disease Research Center, Hope Center for Neurological Disorders, Washington University School of Medicine, St. Louis, MO, USA*
[o]*Laboratory of Neuroscience (LIM 27), Department and Institute of Psychiatry, Faculty of Medicine, University of Sao Paulo, Sao Paulo, Brazil*
[p]*Department of Neurosciences, UCSD School of Medicine, La Jolla, CA, USA*
[q]*Department of Neurological Sciences, University of Milan, Milan, Italy*
[r]*Department of Psychiatry, Psychosomatic Medicine and Psychotherapy, Goethe University, Frankfurt, Germany*
[s]*Department of Clinical Biochemistry, Aarhus Hospital, Aarhus University Hospital, Aarhus, Denmark*
[t]*Klinische Neurowissenschaften, Klinik und Poliklinik für Neurologie, University of Bonn, Bonn, Germany*

*Corresponding author. Tel.: +46-706-393-851; Fax: +46-313-432-426.
E-mail address: niklas.mattsson@neuro.gu.se

doi:10.1016/j.jalz.2011.05.2243

[u]MassGeneral Institute for Neurodegenerative Disease, Massachusetts General Hospital, Massachusetts Alzheimer Disease Research Center, Harvard Medical School, Charlestown, MA, USA
[v]Department of Neurology, University of Eastern Finland, Kuopio University Hospital, Kuopio, Finland
[w]Laboratory of Psychiatry and Experimental Alzheimers Research, Innsbruck Medical University, Innsbruck, Austria
[x]Department of Cellular Neurology, Hertie-Institute for Clinical Brain Research, University of Tübingen and DZNE—German Center for Neurodegenerative Diseases, Tübingen, Germany
[y]Section of Geriatric Psychiatry, University of Heidelberg, Heidelberg, Germany
[z]1st Department of Neurology, Eginition Hospital, National and Kapodistrian University of Athens, Athens, Greece
[aa]Janssen Alzheimer Immunotherapy Research and Development, South San Francisco, CA, USA
[bb]Department of Neurology, University of Szeged, Szeged, Hungary
[cc]Centre of Excellence for Alzheimer's Disease Research and Care, School of Exercise, Biomedical and Health Sciences, Edith Cowan University, Perth, Australia
[dd]Alzheimer's Disease and Other Cognitive Disorders Unit, Neurology Service, Hospital Clínic, Institut d'Investigació Biomédica August Pi i Sunyer (IDIBAPS), Barcelona, Spain
[ee]Department of Psychiatry and Psychotherapy, Universitätsklinikum Erlangen, Erlangen, Germany
[ff]Neuropathology Research Group, The Mental Health Research Institute, University of Melbourne, Melbourne, Australia
[gg]Janssen Pharmaceutica, J&J, Beerse, Belgium
[hh]Department of Neurology, Johns Hopkins School of Medicine, Baltimore, MD, USA
[ii]Department of Pathology, University of Washington, Seattle, WA, USA
[jj]Department of Neurology, University of Ulm, Ulm, Germany
[kk]Alzheimer's Disease Research Center, Mayo Clinic, Rochester, MN, USA
[ll]Department of Neurology, Radboud University Nijmegen Medical Centre, Donders Institute for Brain, Cognition and Behaviour, Alzheimer Centre Nijmegen, Nijmegen, The Netherlands
[mm]Department of Laboratory Medicine, Radboud University Nijmegen Medical Centre, Donders Institute for Brain, Cognition and Behaviour, Alzheimer Centre Nijmegen, Nijmegen, The Netherlands
[nn]IRCCS Fondazione S. Lucia, Rome, Italy
[oo]Department of Pathology and Laboratory Medicine, University of Pennsylvania Medical Center (ADNI Biomarker Core), Philadelphia, PA, USA
[pp]Department of Clinical Biochemistry, Center of Laboratory Medicine, Akershus University Hospital, Oslo, Norway
[qq]Department of Neurology, University of Göttingen, Göttingen, Germany
[rr]Meso Scale Discovery, Gaithersburg, MD, USA

Abstract

Background: The cerebrospinal fluid (CSF) biomarkers amyloid β (Aβ)-42, total-tau (T-tau), and phosphorylated-tau (P-tau) demonstrate good diagnostic accuracy for Alzheimer's disease (AD). However, there are large variations in biomarker measurements between studies, and between and within laboratories. The Alzheimer's Association has initiated a global quality control program to estimate and monitor variability of measurements, quantify batch-to-batch assay variations, and identify sources of variability. In this article, we present the results from the first two rounds of the program.

Methods: The program is open for laboratories using commercially available kits for Aβ, T-tau, or P-tau. CSF samples (aliquots of pooled CSF) are sent for analysis several times a year from the Clinical Neurochemistry Laboratory at the Mölndal campus of the University of Gothenburg, Sweden. Each round consists of three quality control samples.

Results: Forty laboratories participated. Twenty-six used INNOTEST enzyme-linked immunosorbent assay kits, 14 used Luminex xMAP with the INNO-BIA AlzBio3 kit (both measure Aβ-(1-42), P-tau(181P), and T-tau), and 5 used Meso Scale Discovery with the Aβ triplex (AβN-42, AβN-40, and AβN-38) or T-tau kits. The total coefficients of variation between the laboratories were 13% to 36%. Five laboratories analyzed the samples six times on different occasions. Within-laboratory precisions differed considerably between biomarkers within individual laboratories.

Conclusions: Measurements of CSF AD biomarkers show large between-laboratory variability, likely caused by factors related to analytical procedures and the analytical kits. Standardization of laboratory procedures and efforts by kit vendors to increase kit performance might lower variability, and will likely increase the usefulness of CSF AD biomarkers.

Keywords: Alzheimer's disease; Cerebrospinal fluid; Biomarkers; External assurance; External control; Proficiency testing

1. Introduction

The three major brain hallmarks in Alzheimer's disease (AD) are extracellular amyloid plaques, axonal degeneration, and intraneuronal neurofibrillary tangles, which may be monitored with the cerebrospinal fluid (CSF) biomarkers amyloid β-42 (Aβ-42), total-tau (T-tau), and phosphorylated-tau (P-tau), respectively [1–4]. These three biomarkers have high diagnostic accuracy for established

AD [5]. They may also be used to identify AD before onset of dementia at the stage of mild cognitive impairment, as shown in both single-center [6–8] and large-scale heterogeneous multicenter studies [9–11], and to predict mild cognitive impairment/AD in those who are cognitively normal [12,13]. However, measured biomarker levels differ greatly between studies (Supplementary Fig. 1 and Supplementary Table 1), and the reported diagnostic accuracy of the biomarkers varies significantly [14,15]. These variations could be the result of preanalytical, analytical, or manufacturing processes that affect assay-related factors [16]. Preanalytical factors include selection of study participants, procedures of lumbar puncture, sample handling, and sample storage [16–20]. Possible analytical factors include various differences in laboratory procedures among centers and technicians [21]. Assay-related factors (between-lot) arise from manufacturing variations in the source material for components and reagents in the analytical kits and random variability of the production process. These issues are summarized in Table 1.

Table 1
Possible sources of variability between CSF studies

Source	Cause	Solution
Preanalytical	Subject selection	Harmonization of clinical procedures
	Diagnostic criteria	
	Intersubject variability	Knowledge of the issues
	Biologic	
	Circadian rhythms	
	CSF collection	
	Lumbar puncture	Standardization
	Binding to catheter tubing	Determine empirically
	Binding to collection tube	
	Binding to storage tube	
	Hemolysis	Evaluate hemolytic index
	Sample storage and shipment	Standardize and monitor
	Assay kit handling and storage	Follow Mfgr instructions
Analytical	Laboratory equipment	Perform maintenance
	Calibration	
	Detection instrument	
	Pipetting	
	Analyst	
	Competency	Train
	Familiarization with the method	
	Forward/reverse pipetting	Standardize
	Reagent handling	
Postanalytical	Data handling	Standardize
	Analyzing singlets/duplicates	
	Decisions for rejecting data	
	Type of curve fitting used	
	Software for data calculation	
Kit manufacturing	Documentation	
	Poor test procedure instructions	Standardize
	Minimal method optimization	Identify "Best Practices" and set standards
	Reagents	
	Source of reference standard	
	Buffer-based system	
	Lot-lot variability	
	Vendor-vendor variability	
	No quality controls	

Abbreviations: CSF, cerebrospinal fluid; Mfgr, manufacturer.

1.1. Interlaboratory variations

There are several commercially available assays for the determination of CSF Aβ-42, T-tau, and P-tau. Most laboratories in the program used the INNOTEST enzyme-linked immunosorbent assays (ELISAs) or the bead-based Luminex xMAP platform with the INNO-BIA AlzBio3 (both Innogenetics, Ghent, Belgium, www.innogenetics.com), which quantifies Aβ(1-42) (called Aβ-42 later in text), T-tau, and P-tau(181P) (called P-tau later in the text). Meso Scale Discovery (MSD, Gaithersburg, MD, www.mesoscale.com) technology was used by some laboratories for CSF AβN-42, AβN-40, AβN-38, and T-tau measurements. Although the observed biomarker concentrations may vary significantly between platforms, these techniques seem to have similar diagnostic accuracy for patients with AD versus controls [22]. The within-center coefficients of variation (CV) are low, generally within 10% to 15%, and the intra-assay CVs are generally within 5% to 10% [18,22–25]. However, two control surveys of CSF Aβ-42, T-tau, and P-tau reported interassay and interlaboratory CVs of approximately 20% to 35% [25,26]. These values are in agreement with the variability seen in the largest published multicenter trial of early-stage AD so far, which included measurements performed at several laboratories [9].

1.2. Introducing a new global quality control program

Novel biomarker measurements may initially present significant intercenter differences before quality control (QC) programs have been established. To facilitate the worldwide use of CSF biomarkers in clinical dementia investigations and in research, it was decided at the International Conference on Alzheimer's Disease (2009) in Vienna to initiate an international QC program for AD CSF biomarkers. The program is run by the Alzheimer's Association and administrated from the Clinical Neurochemistry Laboratory at the Mölndal campus of the University of Gothenburg, Sweden. The program consists of (1) a standardized operating procedure (SOP) for lumbar puncture and CSF sample handling procedures [5], and (2) an external comparison program of CSF analyses between laboratories. The program is open for any laboratory using a commercially available assay for CSF Aβ, T-tau, or P-tau. In-house assays and assays for which samples must be sent to kit vendors (e.g., P-tau231)

are not part of the program. The results of the first two rounds of the program, which were completed during the spring of 2010, are presented in this report.

2. Materials and methods

2.1. CSF samples

CSF pools were constructed in Mölndal, Sweden, from a large number of fresh, de-identified samples from the clinical routine workflow. All samples tested negative for human immunodeficiency virus and hepatitis B and C. Samples with suspected Creutzfeldt–Jakob disease were excluded. The pools were prepared by experienced and certified laboratory technicians. The pools were thoroughly mixed and underwent one freeze–thaw cycle before aliquotation in 500-μL portions in polypropylene screw-cap tubes (Sarstedt Art. No. 72.692, 1.5 mL, Sarstedt AG & Co., Nümbrecht, Germany), were frozen at −80°C, and were distributed to the participating laboratories on dry ice by courier. All laboratories verified that the samples had arrived frozen. In total, the laboratories received six blinded QC samples, including one sample each from the pools 2009-1A and 2009-1B for the first round, and one sample each from the pools 2010-2A and 2010-2B for the second round. For each round, the laboratories also received one aliquot from the pool QC-L, which will be the same in the coming years, to evaluate longitudinal stability. The blinded challenge samples differed in their AD biomarker profiles. Samples 2009-1A, 2010-2A, and QC-L had levels of Aβ-42, T-tau, and P-tau essentially in the range for healthy subjects. Sample 2009-1B had a classical AD biomarker profile, with low Aβ-42 and high T-tau and P-tau. Sample 2010-2B had essentially normal levels of Aβ-42, combined with high T-tau and P-tau.

2.2. CSF analysis

Laboratories used assay lots that were available in their laboratories. Samples were analyzed in duplicate as part of the laboratories' ordinary activities. Five laboratories routinely processing a large number of samples assessed within-laboratory precision performance by analyzing the samples six times using different plates. These laboratories (Amsterdam, Mölndal, Erlangen, Ghent, and Pennsylvania) are called reference laboratories later in the text. All results were reported back to Mölndal for data analysis.

2.3. Data analysis

2.3.1. General statistics

Biomarker results were statistically analyzed and grouped by rounds, samples, and analytical techniques. Mean levels, standard deviations, and total CVs were calculated. For the reference laboratories, within-laboratory CVs were calculated. Correlations were assessed using Pearson correlation coefficient. GraphPad Prism 5 (GraphPad Software, La Jolla, CA, USA) was used for these analyses.

2.3.2. Variance component analysis

Analysis of variance was performed with the mixed procedure of SAS software version 9.2 (SAS Institute Inc., Cary, NC, USA) using Restricted Maximum Likelihood estimation of covariances. Analyses were performed in-line with International Organization for Standardization (ISO) standard ISO5725 and National Committee for Clinical Laboratory Standards (NCCLS) guideline Evaluation Of Precision Performance Of Quantitative Measurement Methods (EP5-A2). The estimated variance components were within-laboratory, between-laboratory, and between-lot variability. Following a widely accepted statistical convention, negative variance estimates were set to 0.

3. Results

3.1. Participants and analytical techniques

Forty laboratories participated (Supplementary Table 2). Two laboratories participated only in the first round, and three laboratories participated only in the second round. The laboratories used INNOTEST ELISAs (n = 26), Luminex xMAP with the INNO-BIA AlzBio3 kit (n = 14), and MSD with the Aβ triplex kit (n = 4 in the first round, n = 5 in the second round) or T-tau kit (MSD) (n = 1). Aβ triplex may be used with different Aβ detection antibodies. The 4G8 antibody binds to Aβ amino acid residues 18-22, and the 6E10 antibody binds to residues 3-8. Both these antibodies were used by laboratories in the program. Every sample volume was enough for duplicate analyses with ELISA (T-tau: 2 × 25 μL, Aβ-42: 2 × 25μL, and P-tau: 2 × 25 μL), xMAP (2 × 75 μL), and MSD (Aβ triplex: 2 × 25 μL and T-tau: 2 × 25 μL), or combinations of these. Several laboratories used multiple techniques.

3.2. Total variability

Results were grouped according to analytical techniques and samples. The total CVs among centers were 16% to 28% for ELISA (Fig. 1 A–C), 13% to 36% for xMAP (Fig. 1 D–F), and 16% to 36% for MSD (Fig. 1 G–I). CVs for MSD must be interpreted with caution, because they include both the 4G8 and 6E10 assays, and the 6E10 and the 4G8 antibodies bind to different epitopes on the Aβ peptide. Note that, given the study design of one reported mean value per sample and laboratory, this total variability includes both within- and between-center variability.

3.3. Correlations between A and B samples

For each round and analyte, correlations between results for the A and B samples were analyzed for ELISA, xMAP, and MSD (Supplementary Fig. 2). In the ideal

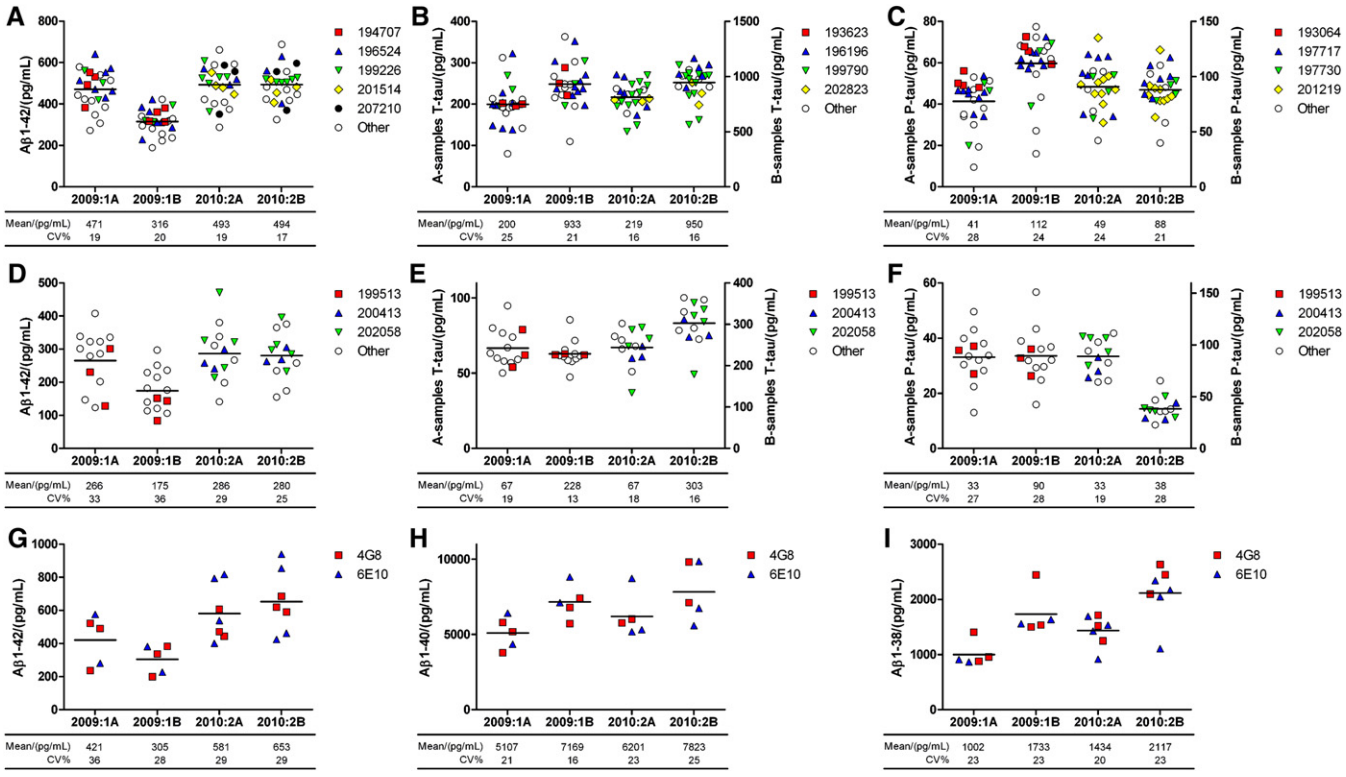

Fig. 1. Results for enzyme-linked immunosorbent assay (ELISA; A–C), xMAP (D–F), and Meso Scale Discovery (G–I) from rounds 1 (1A and 1B) and 2 (2A and 2B). Panels B, C, E, and F have secondary y-axes owing to large differences between samples. Different symbols indicate different kit batches for ELISA and xMAP, and different assays for Meso Scale Discovery (6E10, blue triangle; 4G8, red square).

situation, the measured concentration range is small, and the correlation is then of secondary interest. However, when the range is wide, as in the present results, a high correlation indicates differences between laboratories but consistency within laboratories, whereas a low correlation may indicate inconsistency within laboratories combined with other variation.

3.4. Within-laboratory precision

Within-laboratory CVs were examined at the reference laboratories for ELISA and xMAP in the first (Figs. 2 and 3) and the second round (Figs. 4 and 5). CVs were 3.2% to 24% for ELISA and 2.3% to 26% for xMAP, but differed between analytes within individual laboratories, indicating assay-dependent variations. For example, in xMAP runs for sample 2009-1A, reference laboratory 5 had low variations for Aβ-42 and T-tau but high variation for P-tau (Fig. 3 A–C). For the same analyte and platform, important differences in within-center variability could be noticed among reference laboratories. Most striking are the consistently low CVs for Aβ-42 measured with xMAP by reference laboratory 5. Also, a platform-dependent variation was observed with larger differences in mean levels between laboratories for the xMAP format as compared with ELISA.

3.5. Longitudinal evaluation

The QC-L sample was analyzed in both rounds. Mean levels and total CVs among the laboratories are presented in Supplementary Fig. 3. There were no major changes in total CVs over time, except a decrease in variation for T-tau measured by ELISA. We also calculated within-laboratory CVs between the two rounds. For ELISA, the means of these between-round CVs were 14%, 10%, and 11% for Aβ-42, T-tau, and P-tau, respectively. For xMAP, the means were 14%, 9%, and 11%, respectively.

3.6. Differences in absolute values

The analytical techniques reported different absolute values for the biomarkers. ELISA values were higher than xMAP values, especially for Aβ-42 and T-tau. MSD values for Aβ-42 were intermediate to ELISA and xMAP in the first round and higher than ELISA values in the second round (Fig. 1).

3.7. Contributions of between-laboratory, within-laboratory, and between-lot variability to the total variability

Contributions of between-laboratory, within-laboratory, and between-lot variability to the total variability were

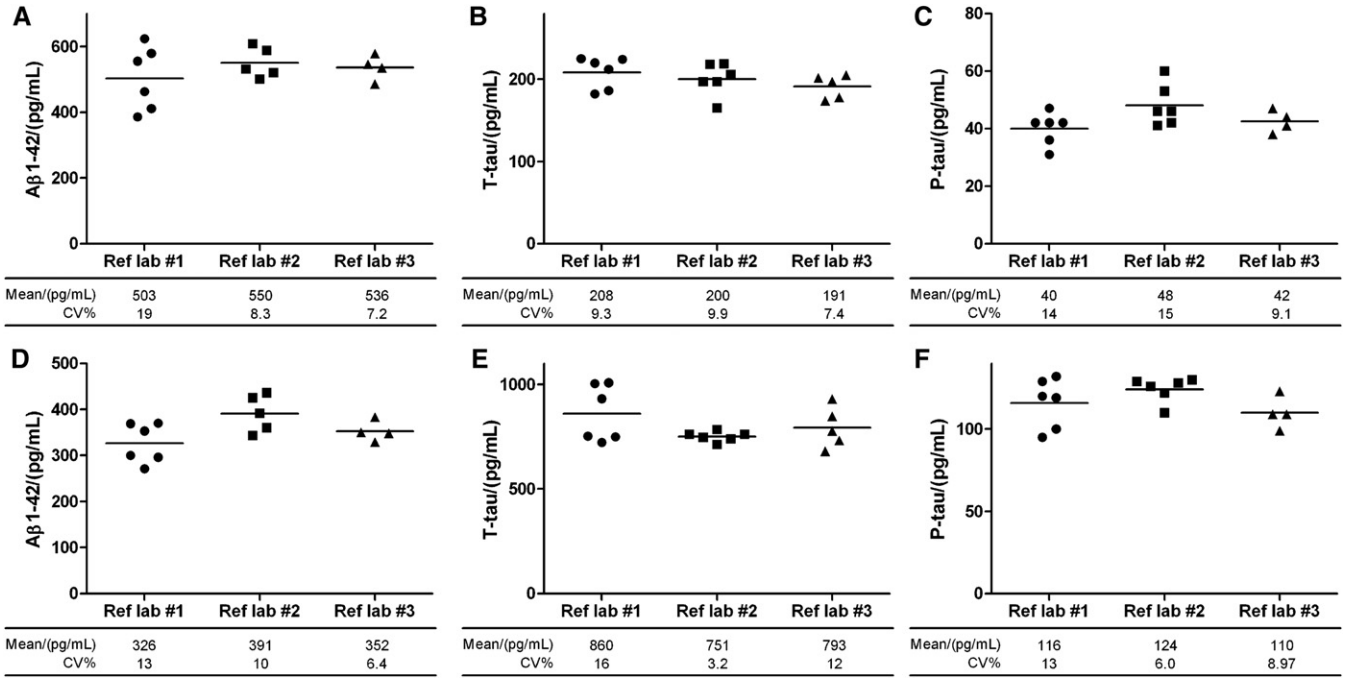

Fig. 2. ELISA results from the reference laboratories from the first round. Results for sample 2009-1A are shown in panels A–C, and results for sample 2009-1B are shown in panels D–F.

estimated using variance component analysis for ELISA and Luminex measurements. Samples from lots that were used in a minimum of 10 repeats were included. Estimates for the within-laboratory components were based only on data pertaining to the QC-L sample that were repeated in round 1 and round 2. Because of the unbalanced design and limited information per assay lot, variance components were estimated with large uncertainties. Therefore, we decided to limit interpretation of analysis of variance to the rankings of the different factors in contribution to overall variability. The rankings of the contributing factors differed among techniques and analytes (Supplementary Table 3).

4. Discussion

This is the first data report from the Alzheimer's Association QC program for AD CSF biomarkers. The total CVs between laboratories ranged from 13% to 36%, which is comparable with what has been seen in earlier smaller investigations [25,26]. No major differences in CVs were seen between the two rounds, which was as expected because there were no active interventions between the rounds. As the QC program continues, the most likely causes for the variations can be identified and addressed. For example, if a laboratory consistently reports low-rank data, the divergence is probably because of analytical factors. Moreover, oscillations between low- and high-rank results suggest that the origin of the inconsistency may be either analytical or assay-related factors, or a combination of both. Well-established routine CSF parameters, such as albumin and immunoglobulin levels, often have between-laboratory CVs of less than 10% to 15% in external control assurance programs. Biomarker scientists and manufacturers should strive to achieve this level of reproducibility for CSF AD markers. Such a goal is already within reach for some of the markers.

4.1. What causes the variability?

The key question is what causes the total variability described. Because pooled QC samples prepared in bulk at a single site were used in this study, preanalytical confounding factors related to the sample preparation were eliminated. Detected variations must have been caused by differences in other preanalytical procedures (e.g., handling/storage of QC samples or commercial kits at individual sites), analytical procedures, or variations related to the commercial assays themselves. With only two program rounds analyzed and many different assay lots used, the estimates of the contributions from between-laboratory, within-laboratory, and between-lot components to the total variability could only be interpreted as rankings instead of quantitative CVs. In general, different kit batches were rather evenly spread among the reported results, indicating that the total variations were not mainly caused by batch-to-batch variability. Intrabatch variability will contribute to the observed variations but cannot be singled out in this study. It may be noted that variations between laboratories were less for the reference laboratories than for all participating laboratories. Because the reference laboratories routinely process large

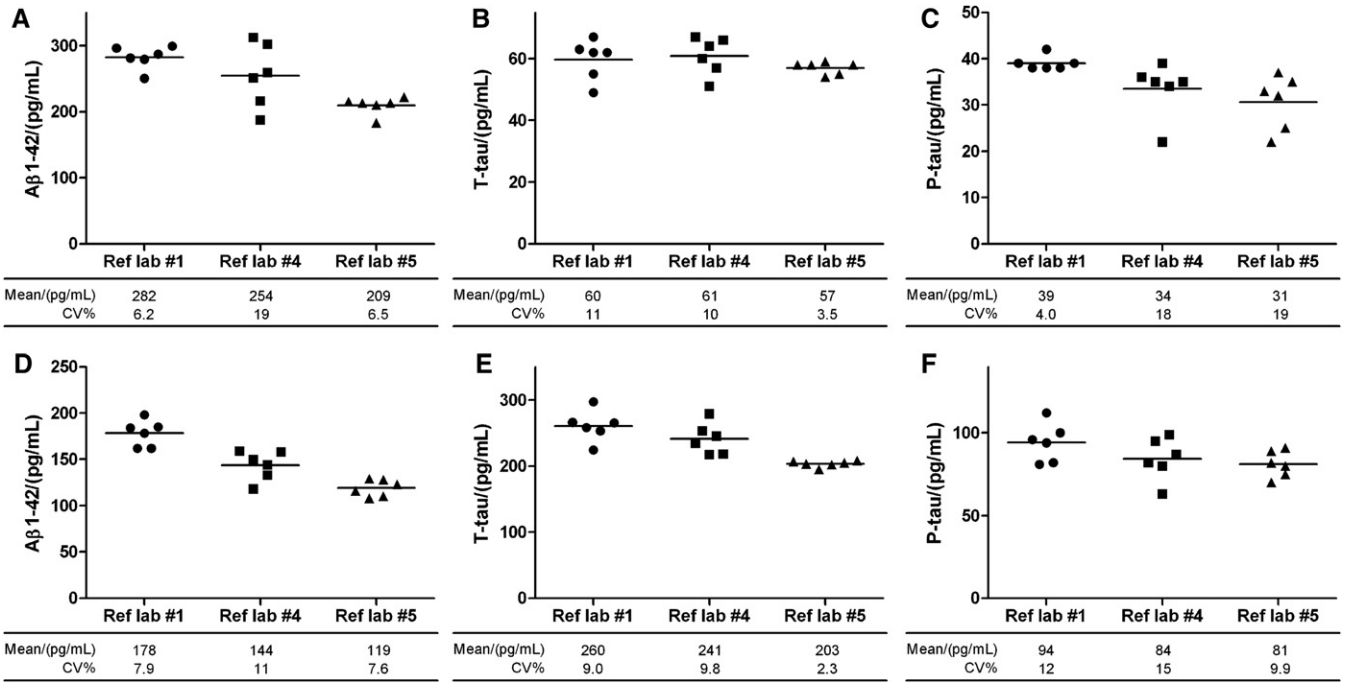

Fig. 3. xMAP results from the reference laboratories from the first round. Results for sample 2009-1A are shown in panels A–C, and results for sample 2009-1B are shown in panels D–F. Data points missing in panels B and E are because of experimental error related to high background in the tau assay.

amounts of samples, this highlights the importance of experience to decrease variations.

Differences in within-laboratory CVs among the biomarkers within individual reference laboratories suggest that assay-related factors are important. For example, for the xMAP analyses of sample 2009-1A, reference laboratory 1 had low CV for P-tau and high for Aβ-42 and T-tau, whereas reference laboratory 5 had high CV for P-tau and low for Aβ-42 and T-tau (Fig. 3 A–C). Because all analytes are measured simultaneously with the xMAP system, such

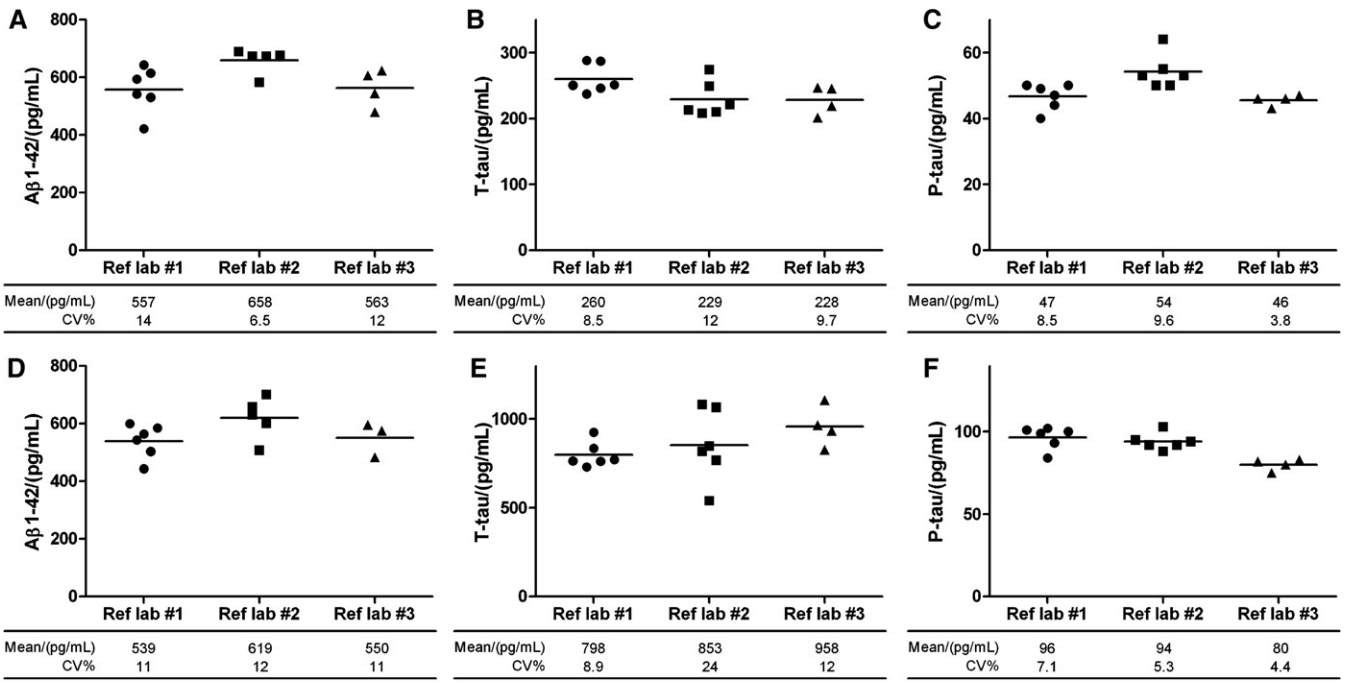

Fig. 4. ELISA results from the reference laboratories from the second round. Results for sample 2010-2A are shown in panels A–C, and results for sample 2010-2B are shown in panels D–F.

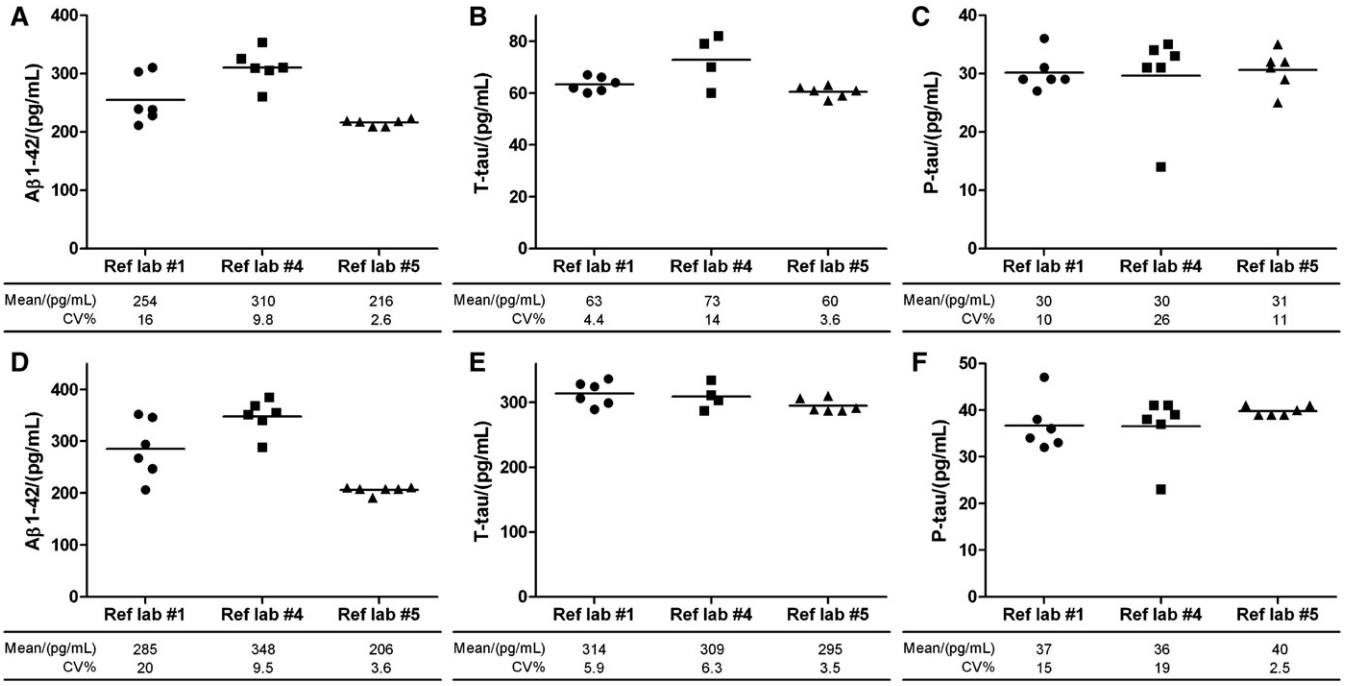

Fig. 5. xMAP results from the reference laboratories from the second round. Results for sample 2010-2A are shown in panels A–C, and results for sample 2010-2B are shown in panels D–F.

discrepancies are difficult to explain by variations in laboratory procedures and more likely caused by variations inherent to the kit itself. However, it cannot be ruled out that individual analytes in a multiplexed assay might be more or less sensitive to certain laboratory procedures. Possible assay-related factors are variations in antibody purification, coating of plates and beads, and preparation and stability of standards. Such sources of variation need to be decreased to a minimum, which requires increased efforts by kit manufacturers. The ideal approach is a collaborative effort between commercial kit vendors, instrument platform manufacturers, reference standardization programs, and laboratories using these methods.

4.2. Lack of certified reference materials

Mean levels of biomarkers differ between the analytical techniques ELISA, xMAP, and MSD. This is ultimately caused by the lack of certified reference materials (CRMs) and calibrators for CSF Aβ-42, T-tau, and P-tau. CRMs (also called standard reference materials) are developed by metrology institutes, such as the United States Pharmacopeia and the National Institute of Standards and Technology (NIST) in the United States, and the International Federation of Clinical Chemistry and Laboratory Medicine (IFCC), World Health Organization, and the National Institute for Biological Standards and Control [27]. The reference materials include primary CRMs, produced with a certified value of purity, and secondary CRMs, which often are samples of human body fluids evaluated against primary CRMs. It is relatively easy to determine the purity of small molecules, such as glucose or cholesterol, which allow measurements in SI units in a defined matrix (e.g., serum or CSF). However, it is more difficult to establish the purity of proteins because of heterogeneities caused by post-translational modifications or contaminations. This makes it difficult to reach full SI traceability for proteins, and standardization is sometimes done with "artifact standards," traced to the World Health Organization reference preparations, reporting concentrations in International Units (IUs) instead of SI units. One recent example of the complexity of establishing a protein CRM is the development of the troponin standard SRM 2921 (human cardiac troponin complex) [28]. The development of CRMs for CSF AD biomarkers would be a major challenge for the AD biomarker community. Such a complicated task would require devotion and orchestrated efforts by researchers, industry, and metrology institutes. If successful, it would allow full global traceability and comparability of biomarker results, also among analytical techniques and centers.

4.3. Standardization among clinical studies

The QC program was recently extended with a standardization program for clinical studies, called *University of Gothenburg CSF 2010* (UGOT CSF 2010). For this, a CSF pool of 2000 mL was constructed and aliquoted in 500-μL portions. Multiple aliquots have been analyzed in Gothenburg to determine biomarker concentrations with high precision in this center. These aliquots may be requested by contacting the QC program coordinator at neurochem@neuro.gu.se. When including UGOT CSF 2010 biomarker

measurements in publications, researchers enable normalization of their data or comparison with other studies. Authors may, for example, report their measured concentrations in their publications and conclude that "The UGOT CSF 2010 samples were within mean ±2 SD for Aβ-42, T-tau and P-tau."

4.4. Future prospects and conclusions

The QC program will continue with multiple test rounds each year. The program is still open for enrollment, and inquiries regarding participation can be made to the coordinator at neurochem@neuro.gu.se. The next rounds will include checklists for each analytical technique, in an attempt to identify analytical factors differing between laboratories. These checklists include information on instrument calibration, use of manual or automated techniques, sample handling and storage, handling of assay reagents and calibrators, use of internal control samples, assay conditions during preincubation and incubation, settings for data analysis, and criteria for run acceptance (for more information and checklists, see the program homepage http://neurochem.gu.se/TheAlzAssQCProgram). The aim is that this information will serve as a basis to identify factors that influence within- and between-laboratory variations. The participating laboratories may use the summary data to alter their procedures to harmonize their measurements. The QC program can be used to monitor the progress of these efforts.

This initiative should be viewed in the larger context of the development of SOPs for the measurement of diagnostic markers for the early detection of AD. This is needed for all biomarker modalities, including biochemical markers, magnetic resonance imaging markers, and positron emission tomography imaging markers using fluorodeoxyglucose or amyloid ligands [29]. An effort similar to the QC program described in this article is the development of SOPs for magnetic resonance imaging measurements of hippocampal atrophy, which is being carried out by an international workgroup [30]. The development of SOPs for biochemical and imaging markers will be a mandatory step for the introduction of new revised diagnostic criteria for AD that include biomarker information.

It should be noted that the data presented in this article do not hinder the implementation of CSF biomarkers for research or clinical use, but they highlight the present difficulties in establishing universal cutoff levels for the biomarkers. The variations put great demands on each laboratory to develop routines to ensure longitudinal stability in the values they report, for example, by testing multiple incoming kit lots and selecting the ones that best reproduce values in internal controls. Each laboratory must develop their own reference limits or check their method agreements against laboratories who have published such data. These efforts will increase the availability of AD CSF biomarkers as tools for researchers and clinicians.

Acknowledgments

The authors thank Åsa Källén, Monica Christiansson, Sara Hullberg, and Dzemila Secic for excellent technical assistance.

K.B., H.Z., N.M., and U.A. designed the study. N.M. and U.A. performed general statistical analyses, and E.C. performed the variance component analysis. N.M. drafted the manuscript. S.P. was the study coordinator. All authors participated in interpretation of data, revised the manuscript for intellectual content, and approved the final version.

A generous grant from the Alzheimer's Association supported this study.

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Supplementary Table 1
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7	(Schoonenboom, Pijnenburg et al. 2004)
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9	(Rosler, Wichart et al. 2001)
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15	(Kapaki, Kilidireas et al. 2001)
16	(Sjogren, Minthon et al. 2000)
17	(Mollenhauer, Cepek et al. 2005)
18	(Sjogren, Davidsson et al. 2001)
19	(Riemenschneider, Wagenpfeil et al. 2002)
20	(Stefani, Bernardini et al. 2005)
21	(Maruyama, Arai et al. 2001)
22	(Reijn, Rikkert et al. 2007)
23	(Mollenhauer, Bibl et al. 2006)
24	(Sjogren, Davidsson et al. 2002)
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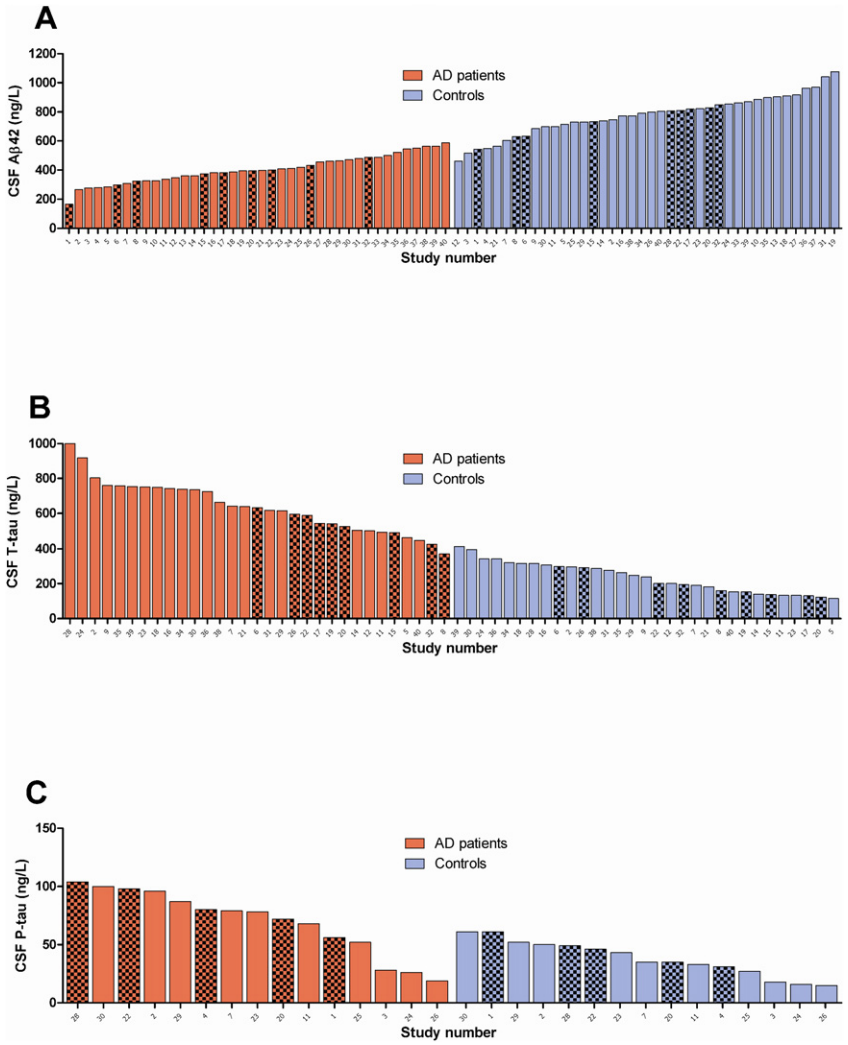
Supplementary Table 2
Participating laboratories

City	Country	Lab type (Clinical/ Research/Industry)	Principal investigator
Aarhus	Denmark	Clinical	Aase Handberg
Amsterdam	Netherlands	Clinical/Research	Marinus A. Blankenstein
Athens	Greece	Clinical/Research	Elisabeth Kapaki
Austin	USA	Industry	William Nowatzke
Baltimore	USA	Clinical/Research	Marilyn Albert
Barcelona	Spain	Clinical/Research	Albert Lladó José Luis Molinuevo
Beerse	Belgium	Industry	Marc Mercken
Bonn	Germany	Clinical/Research	Michael Heneka
Brescia	Italy	Clinical/Research	Giovanni B. Frisoni
Charlestown	USA	Research	Bradley T. Hyman
Erlangen	Germany	Clinical/Research	Piotr Lewczuk
Frankfurt	Germany	Clinical/Research	Harald Hampel
Gent	Belgium	Industry	Hugo Vanderstichele Els Coart
Grambach	Austria	Research/Industry	Manfred Windisch
Gothenburg/ Mölndal	Sweden	Clinical/Research	Kaj Blennow
Göttingen	Germany	Clinical/Research	Annette Spreer
Heidelberg	Germany	Clinical/Research	Johannes Schröder
Innsbruck	Austria	Clinical/Research	Christian Humpel
Kuopio	Finland	Clinical/Research	Hilkka Soininen
La Jolla	USA	Research	Robert Rissman Douglas Galasko
Melbourne	Australia	Clinical/Research	Colin Masters
Milan	Italy	Clinical/Research	Daniela Galimberti
Nijmegen	Netherlands	Clinical/Research	Marcel Verbeek
Oslo	Norway	Clinical/Research	Anders Skinningsrud
Perth	Australia	Clinical/Research	Ralph Martins
Perugia	Italy	Clinical/Research	Lucilla Parnetti
Philadelphia	USA	Clinical/Research	Leslie M. Shaw John Q Trojanowski
Rochester	USA	Research	Ronald C. Petersen
Rome	Italy	Clinical/Research	Alessandro Stefani
South San Fransisco	USA	Industry	Daniel Kidd
Sao Paolo	Brazil	Clinical/Research	Wagner Gattaz
Seattle	USA	Research	Thomas Montine
Sendai	Japan	Clinical	Hiroyuki Arai
St. Louis	USA	Clinical/Research	Anne M. Fagan David M. Holtzman
Staten Island	USA	Research	Khalid Iqbal
Stockholm	Sweden	Clinical/Research	Gunilla Dahlfors
Szeged	Hungary	Clinical/Research	Laszlo Vecsei
Tübingen	Germany	Clinical/Research	Mathias Jucker
Ulm	Germany	Clinical/Research	Markus Otto
Worcester	USA	Industry	Dev Batish

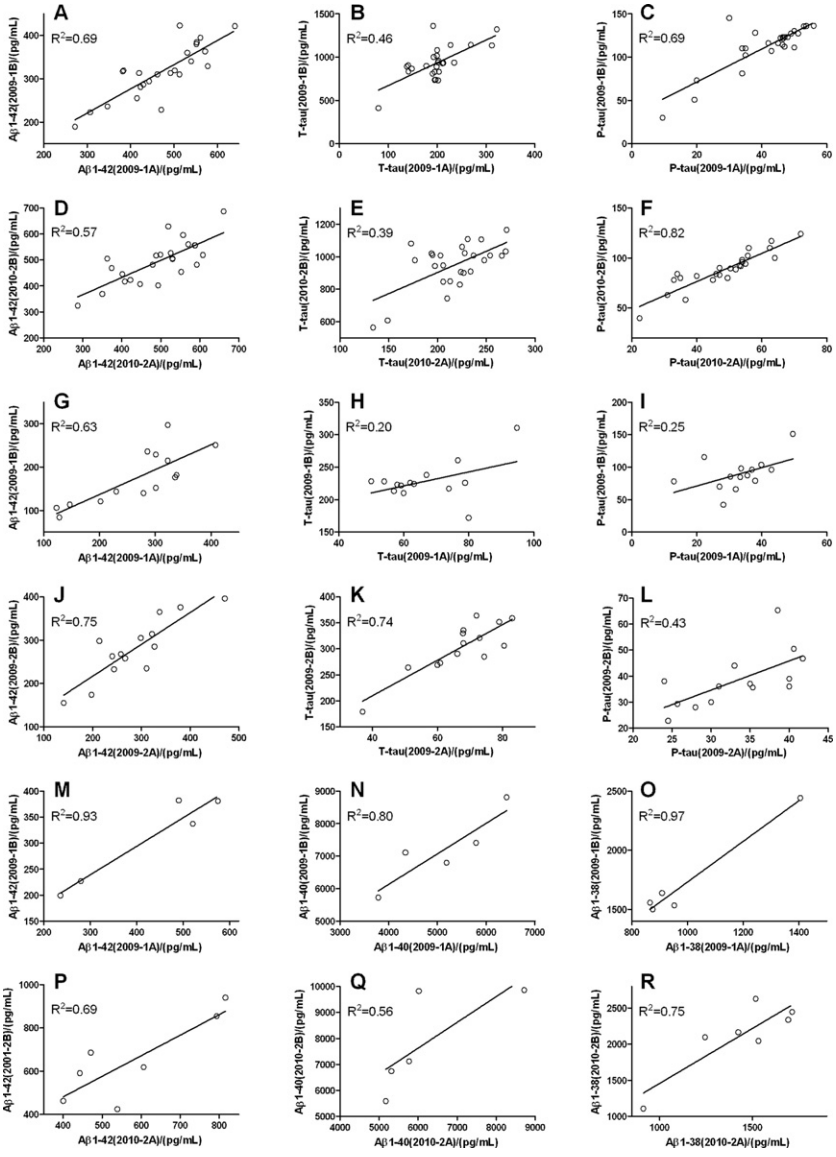
Supplementary Table 3
Factors contributing to the total variability[a]

Technology	Biomarker	Ranking of factors contributing to the total variability[b]
ELISA	Aβ42	1. Between-laboratory and within-laboratory (equal contributions)
		2. Between-lot
	T-tau	1. Within-laboratory
		2. Between-laboratory
		3. Between-lot
	P-tau	1. Between-laboratory
		2. Within-laboratory
		3. Between-lot
Luminex	Aβ42	1. Between-laboratory
		2. Between-lot
		3. Within-laboratory
	T-tau	1. Within-laboratory
		2. Between-laboratory
		3. Between-lot
	P-tau	1. Within-laboratory
		2. Between-laboratory
		3. Between-lot

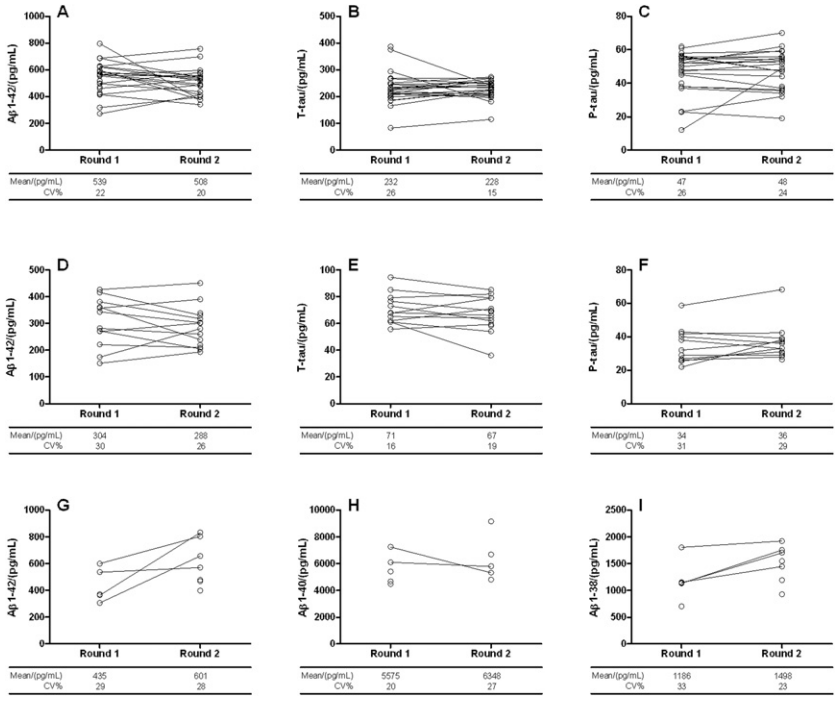
[a] Variability estimated using variance component analysis.
[b] 1 indicates the most contribution and 3 the least contribution to the total variability. The ranking should be interpreted with caution due to few data points in the analysis.



Supplementary Fig. 1. Mean and median levels of CSF A(1-42) (A), CSF T-tau (B) and CSF-P-tau181 (C) in 40 studies using the INNOTEST® ELISA (Innogenetics, Ghent, Belgium). The studies included approximately 2700 AD patients and 1400 controls. Studies reporting median levels are indicated by checked boxes. See supplementary table 1 for references to studies. The aim of this figure is to show the variation between studies, not to give a complete review of all CSF AD biomarker studies.



Supplementary Fig. 2. Correlations between sample A and B for ELISA (round 1, A–C; round 2, D–F), xMAP® (round 1, G–I; round 2, J–L) and MSD® (round 1, M–O; round 2, P–R).



Supplementary Fig. 3. Results for QC-L samples measured at the first and the second round. ELISA results in panels A–C, xMAP® results in panels D–F and MSD® results in panels G–I.