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Assessing the commutability of reference material formats for the harmonization of amyloid-β measurements

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Abstract

Background: The cerebrospinal fluid (CSF) amyloid-β (Aβ42) peptide is an important biomarker for Alzheimer's disease (AD). Variability in measured Aβ42 concentrations at different laboratories may be overcome by standardization and establishing traceability to a reference system. Candidate certified reference materials (CRMs) are validated herein for this purpose.

Methods: Commutability of 16 candidate CRM formats was assessed across five CSF Aβ42 immunoassays and one mass spectrometry (MS) method in a set of 48 individual clinical CSF samples. Promising candidate CRM formats (neat CSF and CSF spiked with Aβ42) were identified and subjected to validation across eight (Elecsys, EUROIMMUN, IBL, INNO-BIA AlzBio3, INNOTEST, MSD, Simoa, and Saladax) immunoassays and the MS method in 32 individual CSF samples. Commutability was evaluated by Passing-Bablok regression and the candidate CRM termed commutable when found within the prediction interval (PI). The relative distance to the regression line was assessed.

Results: The neat CSF candidate CRM format was commutable for almost all method comparisons, except for the Simoa/MSD, Simoa/MS and MS/IBL where it was found just outside the 95% PI. However, the neat CSF was found within 5% relative distance to the regression line for MS/IBL, between 5% and 10% for Simoa/MS and between 10% and 15% for Simoa/MSD comparisons.

Conclusions: The neat CSF candidate CRM format was commutable for 33 of 36 method comparisons, only one comparison more than expected given the 95% PI acceptance limit. We conclude that the neat CSF candidate CRM can be used for value assignment of the kit calibrators for the different Aβ42 methods.

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Keywords: Alzheimer's disease; amyloid; biomarker; cerebrospinal fluid; commutability; reference material.

Introduction

The 42 amino acid-long amyloid-β (Aβ42) peptide found in cerebrospinal fluid (CSF) is an important biomarker for Alzheimer's disease (AD). It is used to aid in early clinical diagnosis, for enrichment purposes in clinical trials, to monitor the effect of therapeutics and for research purposes [1, 2]. CSF Aβ42 levels correlate inversely with neuropathological measures of plaque density in Alzheimer brains [3, 4] and show high concordance with amyloid positron emission tomography [5, 6]. Routine clinical use of CSF Aβ42 is part of the diagnostic process in an increasing number of countries and may be used as a surrogate for neuropathology to either support or rule out a diagnosis of AD in memory-impaired individuals [4, 6–8]. Together with other markers, Aβ42 is included in both the IWG-2 and the NIA-AA research criteria for AD [9–11]. To identify Aβ pathology early is becoming immensely important for the selection of patients in clinical trials on Aβ-targeting drug candidates and will have a central role in future medical treatment decisions based on knowledge about underlying pathology. The relevance of measuring Aβ42 in CSF is further supported by studies suggesting that it is altered very early in the disease course, when CSF tau levels are only marginally increased [12].

The high variability in Aβ42 concentrations obtained on the same set of samples in different laboratories even when using the same method, previously shown in multicenter studies [13, 14] and in the Alzheimer's Association quality control (QC) program for CSF biomarkers [15, 16], complicates the straightforward utility of Aβ42 as a biomarker. In addition, different method formats, e.g. ELISA and Luminex xMAP, give different values [17]. This variation precludes the introduction of generally applicable cut-off levels in routine clinical practice. In general, the discrepancy in observed CSF biomarker levels between centers is probably the result of differences in pre-analytical procedures (e.g. lumbar puncture procedure and CSF sample processing), analytical procedures [18], and batch-to-batch variation in the production of biomarker methods [19]. Effects of pre-analytical confounding factors may be reduced by standardization of procedures for lumbar puncture and sample handling [18, 20, 21]. However, the results from the Alzheimer's Association QC program, in which the same samples are analyzed at multiple sites with multiple lots over time, pinpoint a significant part of the variation to analytical procedures [15, 16]. This may be minimized by standardization of the analytical process at a laboratory, including the establishment of an internal control system and batch-bridging procedures [6], as well as the use of ready-to-use calibrators and transferring manual ELISA methods to automated pipetting robot systems [22, 23].

The immunoassays used for the measurements of Aβ42 are very sensitive and selective. They are useful in a clinical setting since they allow for a fast assessment of the analyte in a large number of samples. All available commercial methods utilize the sandwich antibody technique to increase specificity. The level of analytical sensitivity may depend on the applied detection method [24]. A number of factors contribute to the result of the analysis such as antibody specificity, antigen epitope availability, the antibody antigen reaction kinetics and equilibrium, and the influence of differences in the matrix between calibrators and samples. Moreover, in order to obtain results that are comparable over time and between kits, these techniques depend on a calibrator (recombinant peptide or protein) with a value that is correctly assigned. Due to the lack of a readily available certified reference material (CRM), the value assignment differs among different vendors, which may result in systematic bias of measured concentrations across different kits for Aβ42. Batch-to-batch variation of the calibrator value is a potential source to the variability, which can also be affected by the oligomeric state of Aβ42 in the calibration vials included in the commercial kit and differences in method formats that would affect its degree of aggregation during the analytical procedure. Despite all confounding factors, promising results have been obtained suggesting that it may be possible to harmonize results by the use of calibrators prepared in CSF-like materials [25], but the long-term solution will be the introduction of a CRM.

Comparability of results over time and across formats and platforms can be achieved by standardizing pre-analytical and analytical measurements and establishing traceability to a reference system. CRMs are key components of such reference systems and for establishing traceability (https://ec.europa.eu/jrc/en/research-topic/certified-reference-materials). Commutability of CRMs is a critical property to ensure that they are fit for the intended use. Commutability is defined as the ability of a CRM to show interassay properties that are equivalent to those of representative clinical samples of healthy and diseased individuals respectively. In the present study, we assess the commutability of candidate CRM formats across a broad range of CSF Aβ42 methods, including a selected reaction monitoring (SRM) liquid chromatography-mass

spectrometry (LC-MS)-based method for the analyte. The most promising candidate CRM formats were identified and subjected to validation in a new set of CSF samples.

Materials and methods

Method comparisons commutability I

In the first commutability study, five different immunoassays were evaluated at the Clinical Neurochemistry Laboratory of the Sahlgrenska University Hospital, Mölndal, Sweden: 1) MSD® 96-Well MULTI-ARRAY® Human (4G8) Abeta42 Ultra-Sensitive Kit (Meso Scale Discovery, Gaithersburg, MD, USA), 2) Human β Amyloid(1-42) ELISA Kit Wako High-Sensitive (Wako Pure Chemical Industries, Ltd., Osaka, Japan), 3) Human Amyloid β (1-42) (N) assay kit – IBL (Immuno-Biological Laboratories Co., Ltd., Fujioka, Japan, distributed by IBL International GmbH), 4) INNOTEST® β-AMYLOID (1-42) and 5) INNO-BIA AlzBio3 (Fujirebio-Europe, Inc., Ghent, Belgium). Commutability between the immunoassay Aβ42 measurement results and Aβ42 quantification by SRM performed on a triple quadrupole MS (TSQ Vantage, Thermo Scientific, Waltham, MA, USA) was also assessed.

Method comparisons commutability II

The second commutability study assessed eight different immunoassays: 1) MSD® 96-Well MULTI-SPOT® Human Aβ42 V-PLEX Kit (Meso Scale Discovery, Gaithersburg, MD, USA), 2) Amyloid-beta (1-42) CSF ELISA (IBL International GmbH, Hamburg, Germany), 3) VITROS® Immunodiagnostic Amyloid Beta 42 Assay (AB-42) (Saladax Biomedical, Bethlehem, PA, USA), 4) Elecsys® β-Amyloid (1-42) immunoassay (Roche Diagnostics, Penzberg, Germany), 5) EUROIMMUN Beta-Amyloid (1-42) (ADx NeuroSciences NV, Gent, Belgium), 6) INNO-BIA AlzBio3 (Fujirebio-Europe, Ghent, Belgium), 7) INNOTEST® β-AMYLOID (1-42) (with ready-to-use calibrators, Fujirebio-Europe), and 8) Simoa Human Aβ42 (Quanterix Corporation, Lexington, MA, USA). The first five methods were run in the facilities of the corresponding manufacturers, the INNO-BIA was analyzed in the Biomarker Research laboratory at Perelman School of Medicine, University of Pennsylvania, PA, USA, and the last two immunoassays and the SRM performed on a triple quadrupole MS (TSQ Vantage, Thermo Scientific, Waltham, MW, USA) were evaluated in the Clinical Neurochemistry laboratory at Sahlgrenska University Hospital, Mölndal, Sweden.

Material and measurement procedure commutability I and II

The immunoassay analyses were performed according to each manufacturer's protocol. The sample preparation and MS quantification procedure is described elsewhere [26].

For commutability study I, a total of 48 individual CSF samples were selected to cover the clinical spectrum (patient and control sample concentration range) of Aβ42 values. Duplicate samples were analyzed on two plates (plate 1 with samples 1–24 and plate 2 with samples 25–48), each plate also contained duplicates of 16 non-individual samples that were assessed as candidate CRM formats (see Table 1). Both plates were measured at the same time point. Identical samples were measured sequentially by SRM. Briefly, the non-individual samples consisted of neat and detergent diluted (Tween® 20 0.05%, Sigma-Aldrich®, St. Louis, MO, USA) decoded CSF pools with low and high concentration of Aβ42 as determined by INNOTEST® ELISA. One set of the neat and detergent diluted pools were spiked with Aβ42 (rPeptide, Bogat, GA, USA) starting at a concentration of 2000 ng/L and reaching the final concentration of 250 ng/L by serial dilution (factor 1:2). The concentration of the Aβ42 peptide was determined by SRM analysis using the heavy ^{15}N-labeled Aβ42 peptide calibrator. The calibrator concentration was determined by amino acid analysis [26]. Furthermore, artificial CSF (aCSF; pH 7.3) and phosphate buffered saline (PBS) (neat and detergent diluted) were used as a matrices to spike 1000 ng/L of Aβ42 into. The aCSF was prepared according Alzet® Osmotic Pumps protocol (http://www.alzet.com/products/guide_to_use/cfs_preparation.html) with minor modifications, i.e. addition of bovine serum albumin and glucose at a final concentration of 4.5 nM and 4.5 mM, respectively.

For commutability study II, 32 individual CSF samples selected to cover the clinical spectrum of Aβ42 values were analyzed in duplicates (with the exception of Elecsys due to limited availability of samples). For each method also quadruplicates of four pooled (non-individual) samples that were considered as candidate CRM formats were measured. The first non-individual sample was a pool

Table 1: Non-individual samples assessed as candidate CRM formats in the first commutability study.

No.	Symbols	Non individual samples	Spiked Aβ42 concentration, ng/L
	●	Individual CSF samples	0
1	●	CSF pool low Aβ42	0
2		CSF pool high Aβ42	0
3	■	aCSF	1000
4		PBS	1000
5	◆	CSF pool low Aβ42	2000
6		CSF pool low Aβ42	1000
7		CSF pool low Aβ42	500
8		CSF pool low Aβ42	250
9	●	CSF pool low Aβ42+0.05% Tween	0
10		CSF pool high Aβ42+0.05% Tween	0
11	■	aCSF + 0.05% Tween	1000
12		PBS + 0.05% Tween	1000
13	◆	CSF pool low Aβ42+0.05% Tween	2000
			1000
14		CSF pool low Aβ42+0.05% Tween	
15		CSF pool low Aβ42+0.05% Tween	500
16		CSF pool low Aβ42+0.05% Tween	250

of 24 neat CSF samples with a final Aβ42 concentration of approximately 760 ng/L as determined with INNOTEST. The neat CSF pool was spiked with an Aβ42 calibrator from JRC-IRMM (indicative concentration of stock solution: 86 mg/L) to prepare the other three non-individual samples with the following Aβ42 spiking concentration: 300 ng/L, 800 ng/L, and 1300 ng/L. The SRM method was performed on a triple quadrupole MS as previously described [26], except that calibration was performed using the surrogate analyte approach [27].

All CSF samples were left-over samples from the clinical routine at the Clinical Neurochemistry Laboratory, Sahlgrenska University Hospital, Mölndal, Sweden. The samples were thawed once, CSF pools were immediately prepared and individual and non-individual pooled samples were aliquoted into equal proportions into polypropylene tubes and frozen at −80 °C pending analyses. The samples were de-identified and coded. This procedure follows the Swedish Biobank law and is approved by the Ethical Review Board at University of Gothenburg.

Statistical analysis of commutability

In commutability study I, pair-wise comparisons of the mean values of the individual samples using linear regression was used for method comparisons; the goodness of fit for each method comparison is presented by the coefficient of determination (R^2). The calculation of a 95% prediction interval (PI) was done in order to conclude whether the assessed CRM formats were commutable with the clinical individual samples based on the position of its values with respect to the PI.

In commutability study II, Analyze-it® for Microsoft Excel (version 2.30; Leeds, UK) was used for linear and Passing-Bablok regression [28] analyses of mean values of individual samples (only single measurements were available for Elecsys and patient samples 17 and 23 analyzed with Saladax) for method comparisons. The mean values for quadruplicate measurements were used for the candidate CRM formats, with the exception of Elecsys where only duplicate measurements were done. Additionally, the results of the spiked level 3 analyzed with the EUROIMMUN method were out of range for two of the four measurements. Thus only duplicate measurements were used for this method. A commutability software (ACOMED Statistik, Leipzig, Germany) that runs on Microsoft Excel and R software (version 3.0.2) [29] was used in to generate the 95% PI for the Passing-Bablok regression lines.

Results

Commutability study I

Method comparisons

For the immunoassays, the mean coefficient of variation (CV) of the individual CSF duplicate samples varied between 2.5% and 4.4%, while the mean CVs for the CSF non-individual samples distributed on two plates were between 3.2% and 8.5%. For the MS method the mean CV for the CSF individual samples was 8.9%, while the mean CV for the pooled CSF was 10.7%.

Table 2: Linear regression of the mean values for the commutability I individual samples.

Linear regression	R^2 and number of individual samples				
	MSD	Alzbio3	IBL	WAKO	SRM
INNOTEST	0.96	0.91	0.93	0.96	0.83
	48	45	48	48	48
MSD	x	0.85	0.98	0.94	0.88
		45	48	48	48
Alzbio3		x	0.82	0.84	0.77
			45	45	45
IBL			x	0.92	0.89
				48	48
WAKO				x	0.81
					48

Values represent correlation coefficient. R^2 and number of individual samples included in analyses. All correlations were significant ($p<0.0001$).

The R^2 values varied between 0.82 and 0.98 for the immunoassay comparisons of the individual CSF samples; for most of the comparisons the coefficients of determination were above 0.90. The R^2 values for the comparisons between the SRM and immunoassays were between 0.77 and 0.89 (see Table 2). These results indicate that the different methods correlate well ($p<0.0001$). However, the slopes between the different method comparisons varied substantially, from 0.39 to 2.41 (Supplemental Data, Table 1), which is also reflected by the discrepant values for the same CSF samples measured by the different methods.

Commutability of candidate CRM formats

Duplicates of 48 individual CSF samples were measured together with quadruplicates of 16 different candidate CRM formats (see Table 1). The neat CSF pools with low or high intrinsic Aβ42 concentration (individual samples combined to form low or high Aβ42 pools) were commutable for all immunoassay combinations and for the immunoassay and SRM method combinations as their values were within the 95% PI (Figure 1). The low Aβ CSF pool with spiked Aβ42 was commutable within the clinical individual sample range for most comparisons. The formats that contained Tween were the least commutable. The aCSF spiked with Aβ42 was only commutable for a few methods (mainly WAKO and AlzBio3) and the measured concentration of Aβ42 spiked PBS was close to the lower limit of detection for most methods. However, the opposite was found in PBS and aCSF when detergent was added, rendering the Aβ42 concentration close to the upper limit of detection in some of the methods.

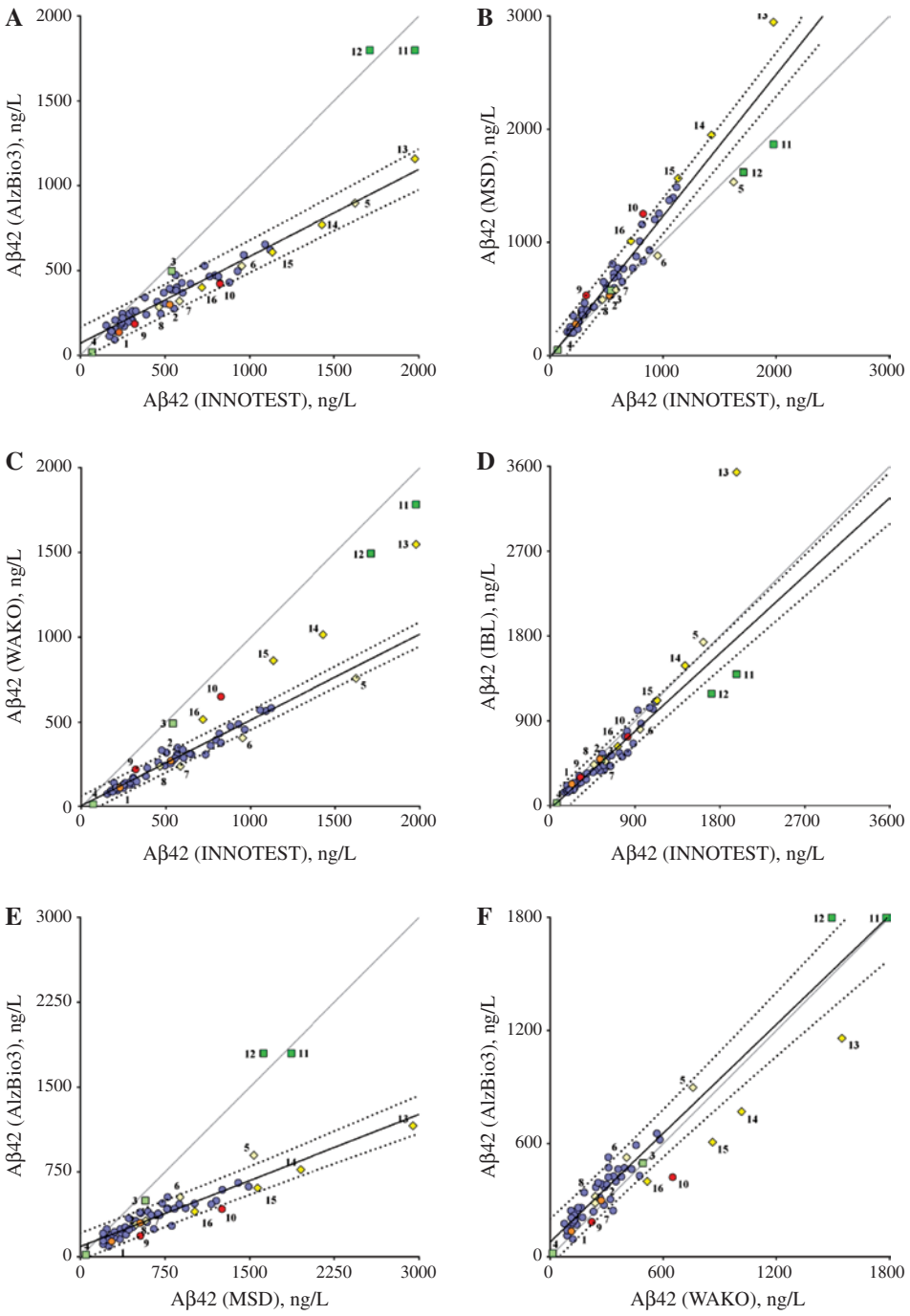

Figure 1: Linear regression analysis – commutability I.

Commutability study II

Method comparisons

For the immunoassays, the mean CVs for the CSF individual samples varied between 1.4% and 8.0%, while the mean CVs for the pooled CSF samples were between 2.8% and 7.6%. For the SRM method, the mean CV for the individual CSF and the pooled CSF samples was 11.4% and 13.8%, respectively. There were no significant differences between the intra-assay CVs of the individual and non-individual samples for the above mentioned methods, with the exceptions of EUROIMMUN (p=0.01) and IBL (p=0.04) for which the median CVs of non-individual

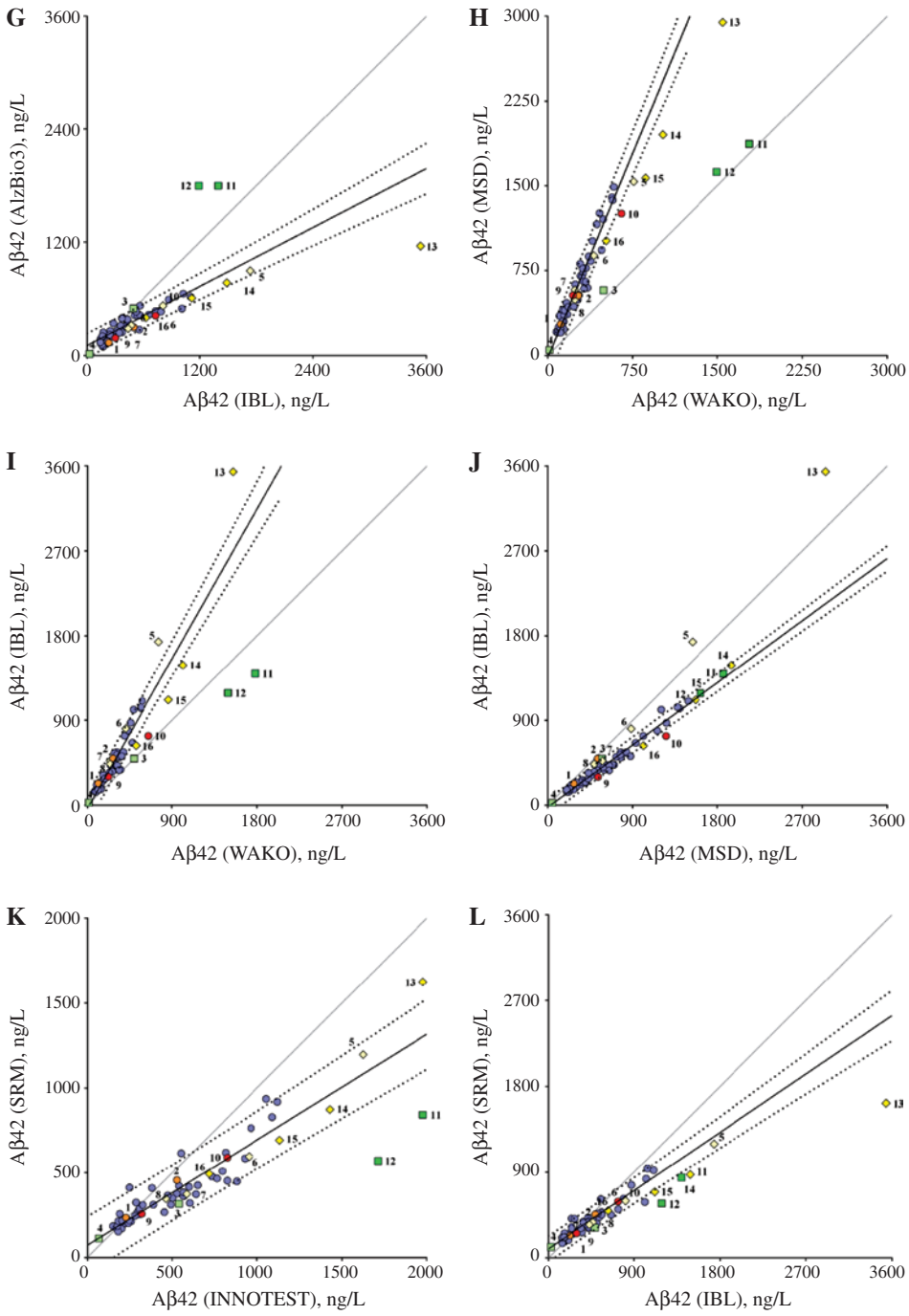

Figure 1 (continued)

samples were significantly higher (median CV 5.6% and 2.7%, respectively) though under 10%.

All methods correlated significantly ($p<0.0001$), with R^2 values between 0.67 and 0.98 for the immunoassay comparisons of the individual CSF samples when assessed by Passing-Bablok. The R^2 values for the comparisons between the SRM and immunoassays were found to be in the range of 0.71–0.97; for exact values see the lower left corner of Table 3. The coefficients of determination were ≥0.93 for all comparisons except comparisons including AlzBio3 and INNOTEST for which the coefficients of determination were 0.91. These results indicate that the newly developed methods (MSD, Saladax, EUROIMMUN, SRM, Simoa, Elecsys and IBL) correlate well with each other as do the more established methods (INNOTEST and AlzBio3). The correlation between the

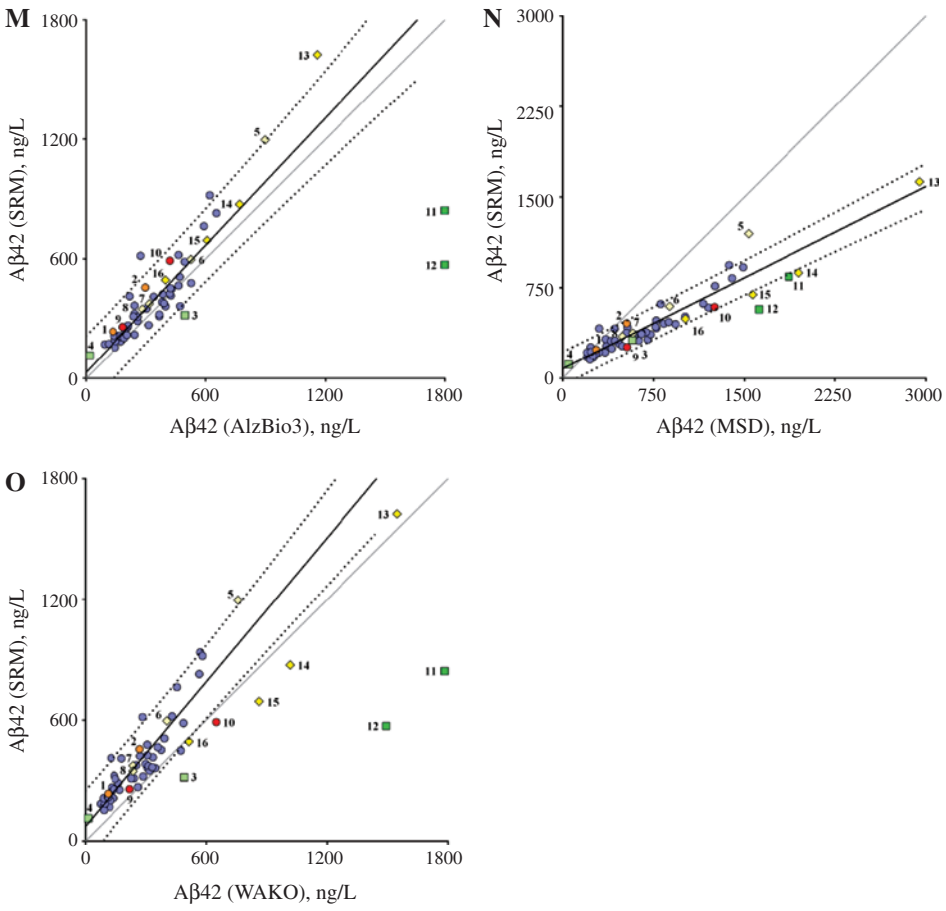

Figure 1 (continued)

more established and newly developed methods was somewhat more moderate with the coefficients of determination ranging between 0.81 and 0.92 for INNOTEST and between 0.67 and 0.91 for AlzBio3. The slopes for the different method comparisons varied substantially from 0.15 to 6.6 (Table 4).

Commutability of candidate CRM formats

The commutability of the candidate CRM formats was evaluated using different statistical approaches. The results obtained using Passing-Bablok regression and comparisons of the means of the results for the formats with the PI are shown in Figure 2. In general, the non-spiked neat CSF pool was highly commutable and the most promising candidate CRM. It was found to behave comparable to the patient CSF samples (i.e. falling within the 95% PI) for almost all method comparisons, except for the comparison between Simoa/MSD, Simoa/SRM and SRM/IBL. For these method combinations the neat CSF pool was found to lack commutability based on its position outside the 95% PI (Figure 2E, AA and AD, respectively, and Table 5). Furthermore, the neat CSF pool was also assessed with respect to its relative distance from the Passing-Bablok regression line. In more than half of the comparisons it was found to be within 5% relative distance to the regression line, while for the rest of the comparisons the neat CSF pool was between 5% and 10% away from the regression line. The exceptions were the method comparisons between Simoa/MSD and MSD/IBL where the neat CSF pool was found at a relative distance of 10%–15% (Table 6A). The CSF pool with the lowest spiked concentration of Aβ42 (spiked level 1) was also highly commutable with respect to falling within the 95% PI for most of the method comparisons, except for Elecsys/EUROIMMUN, Elecsys/AlzBio3, Elecsys/SRM, SRM/Simoa, Simoa/AlzBio3, and AlzBio3/Saladax (Figure 2 and Table 5). This material was also highly commutable with respect to the relative distance from the regression line in half of the method comparisons that ended up within 5% of the relative distance from the line. For approximately 30% of method comparisons the material had a relative distance of 5%–10%, while for the remaining comparisons the material was 10%–15% away

Table 3: Linear regression of the mean values for the commutability II individual samples.

Linear regression	R^2 and number of individual samples							
	Saladax	EUROIMMUN	INNOTEST	SRM	Simoa	Elecsys	AlzBio3	IBL
MSD	0.93	0.95	0.81	0.95	0.95	0.98	0.67	0.96
	32	32	32	32	32	32	32	32
Saladax	x	0.98	0.92	0.96	0.97	0.96	0.81	0.98
		32	32	32	32	32	32	32
EUROIMMUN		x	0.89	0.95	0.96	0.98	0.77	0.97
			32	32	32	32	32	32
INNOTEST			x	0.87	0.87	0.84	0.91	0.87
				32	32	32	32	32
SRM				x	0.96	0.95	0.71	0.97
					32	32	32	32
Simoa					x	0.97	0.71	0.98
						32	32	32
Elecsys						x	0.71	0.98
							32	32
AlzBio3							x	0.73
								32

Values represent correlation coefficient. R^2 and number of individual samples included in analyses. All correlations were significant ($p<0.0001$).

Table 4: Slopes of Passing-Bablok regression analyses of pair-wise comparisons between methods.

Passing-Bablok regression	Dependent value (x-axis)								
	IBL	Saladax	EUROIMMUN	INNOTEST	SRM	Simoa	Elecsys	MSD	AlzBio3
Independent value (y-axis)									
IBL	x	1.28	1.04	1.20	0.67	0.82	0.62	1.77	3.75
Saladax	0.78	x	0.82	0.96	0.51	0.62	0.49	1.37	2.96
EUROIMMUN	0.96	1.21	x	1.14	0.64	0.77	0.60	1.68	3.63
INNOTEST	0.83	1.04	0.87	x	0.51	0.64	0.52	1.49	3.21
SRM	1.49	1.94	1.56	0.67	x	1.18	0.90	2.70	2.26
Simoa	1.22	1.62	1.30	1.96	0.85	x	0.77	2.28	6.55
Elecsys	1.61	2.06	1.66	1.55	1.11	1.30	x	2.73	5.15
MSD	0.56	0.73	0.60	0.67	0.37	0.44	0.37	x	6.15
AlzBio3	0.27	0.34	0.28	0.31	0.15	0.19	0.16	0.44	x

Green: 0.50–1.50, yellow: 0.00–0.49 and 1.51–2.00, orange: 2.01–2.50, red: >2.51.

from the regression line (Table 6B). The other two materials were spiked with concentrations of Aβ42 (spiked level 2 and 3) that put them in the higher end of the clinical sample interval and were found to lack commutability for most method comparisons with regard to the 95% PI as well as the distance to the Passing-Bablok regression line (Tables 5 and 6C,D and Figure 2).

Discussion

In the present study it was shown that pooled neat CSF is commutable, and has good potential as a CRM format for the calibration of methods used for Aβ42 quantification in CSF. It was also shown that the results of methods for Aβ42 are highly correlated, which is a prerequisite for being able to achieve comparability of results obtained with different methods.

Standardization efforts are ongoing in the International Federation of Clinical Chemistry and Laboratory Medicine Working Group for CSF proteins (IFCC WG-CSF), and the Alzheimer's Association Global Biomarker Standardization Consortium (GBSC). The aim of the IFCC WG-CSF is to develop reference measurement procedures (RMPs) and CRMs for the AD CSF biomarkers [30, 31]. One important step in this standardization

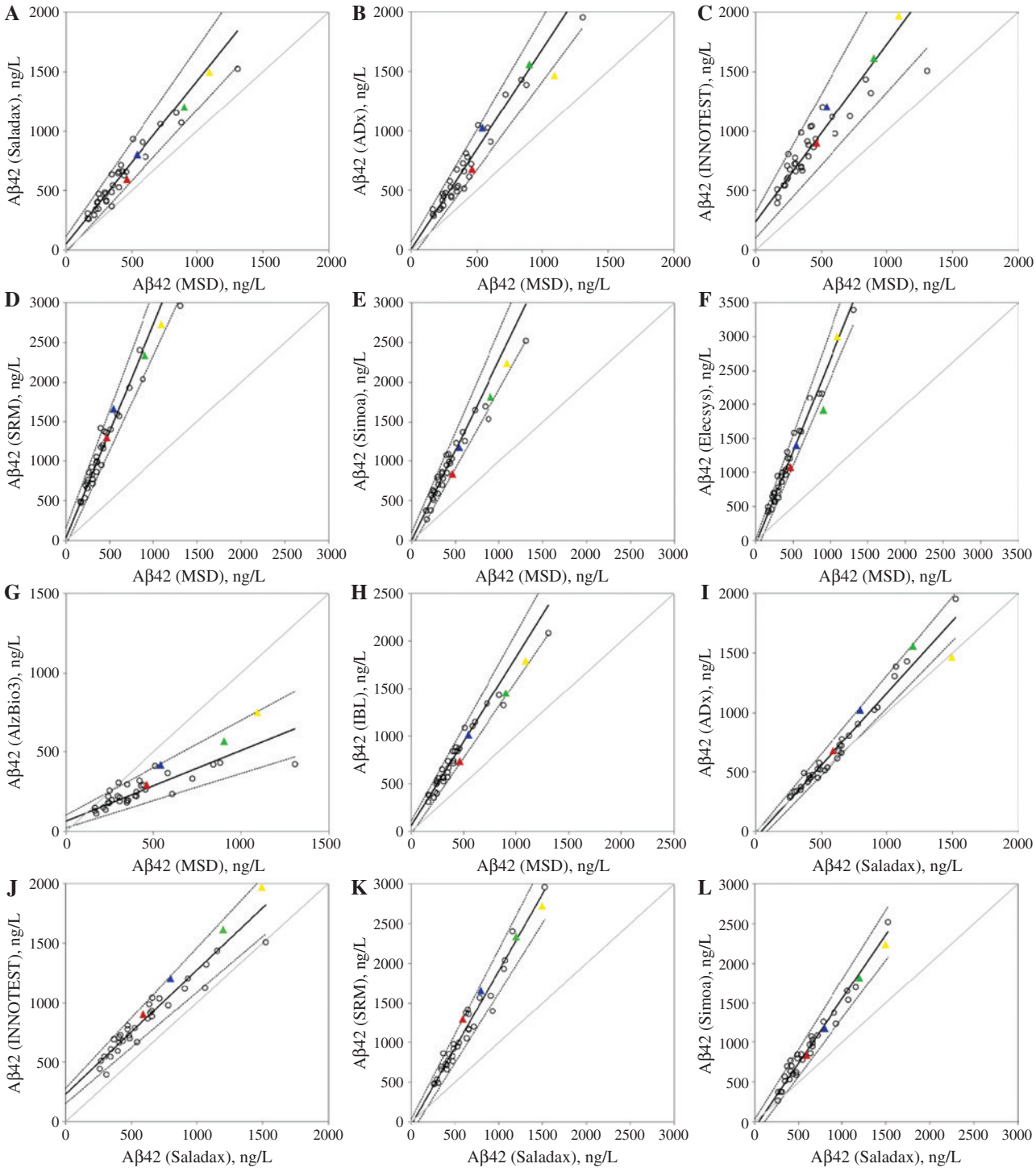

Figure 2: Passing-Bablok regression analysis – commutability II.

process is to evaluate the commutability of candidate CRMs for different analytical methods that are used within the field. If the methods give correlating results, the use of a commutable CRM for calibration should make it possible to produce values that are comparable irrespective of analytical method, time or place of measurement [32]. In the present study, it was shown that the majority of the results of the different immunoassays correlate well; and in addition, they also correlate with results from the SRM-based method, which has now been published as candidate SRM-based RMP [27, 33].

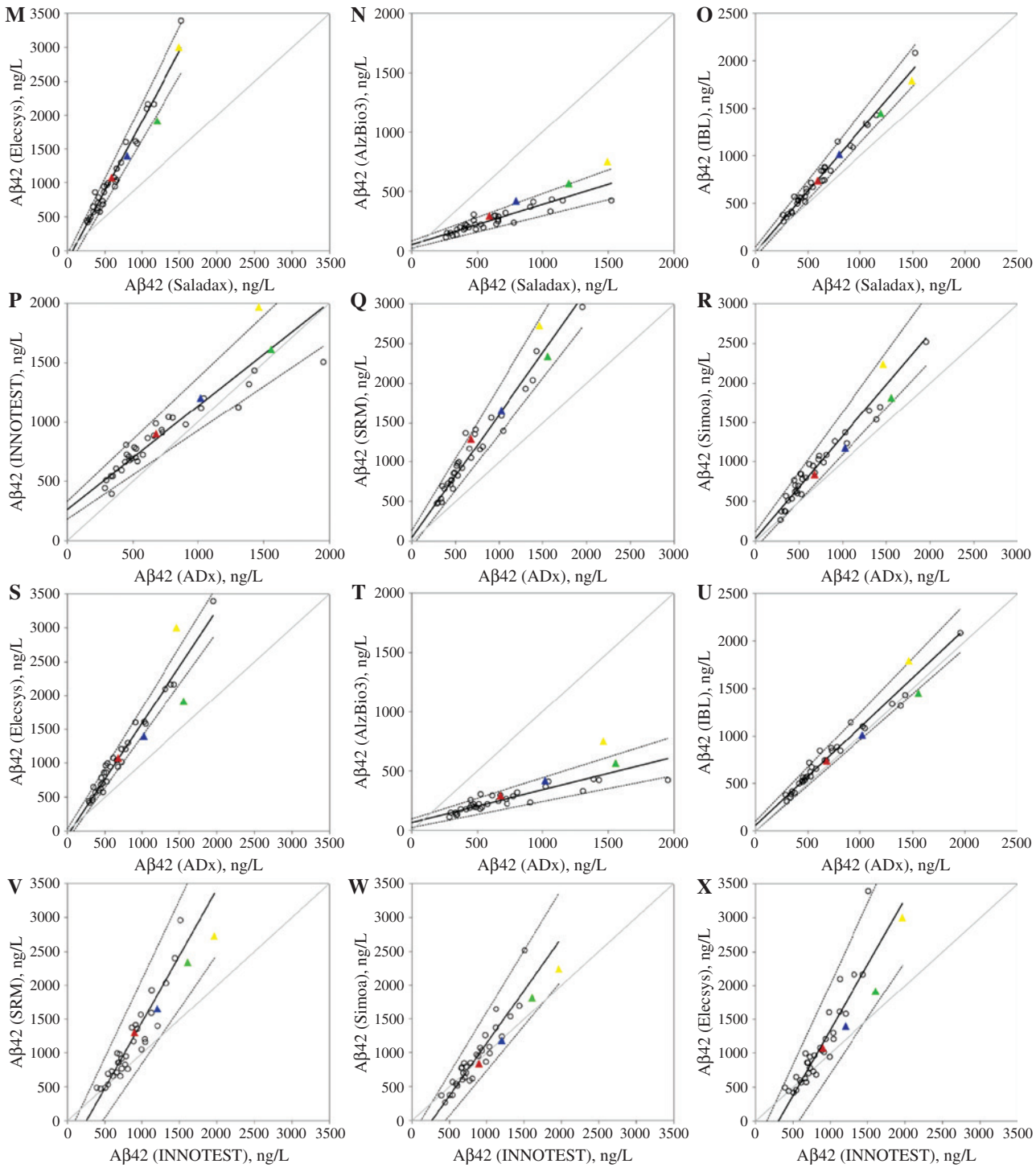

Figure 2 (continued)

The first substudy confirmed that the majority of methods tested were highly correlated (R^2>0.9) as shown previously [19]. It is well recognized in the research community that the various available methods for Aβ42 give highly variable concentrations. However, as long as the methods are highly correlated this problem can be solved by the introduction of a commutable CRM that can be used to calibrate the methods. For most of the comparisons the neat CSF pool behaves as the clinical samples and can be found within the PI and in close proximity to

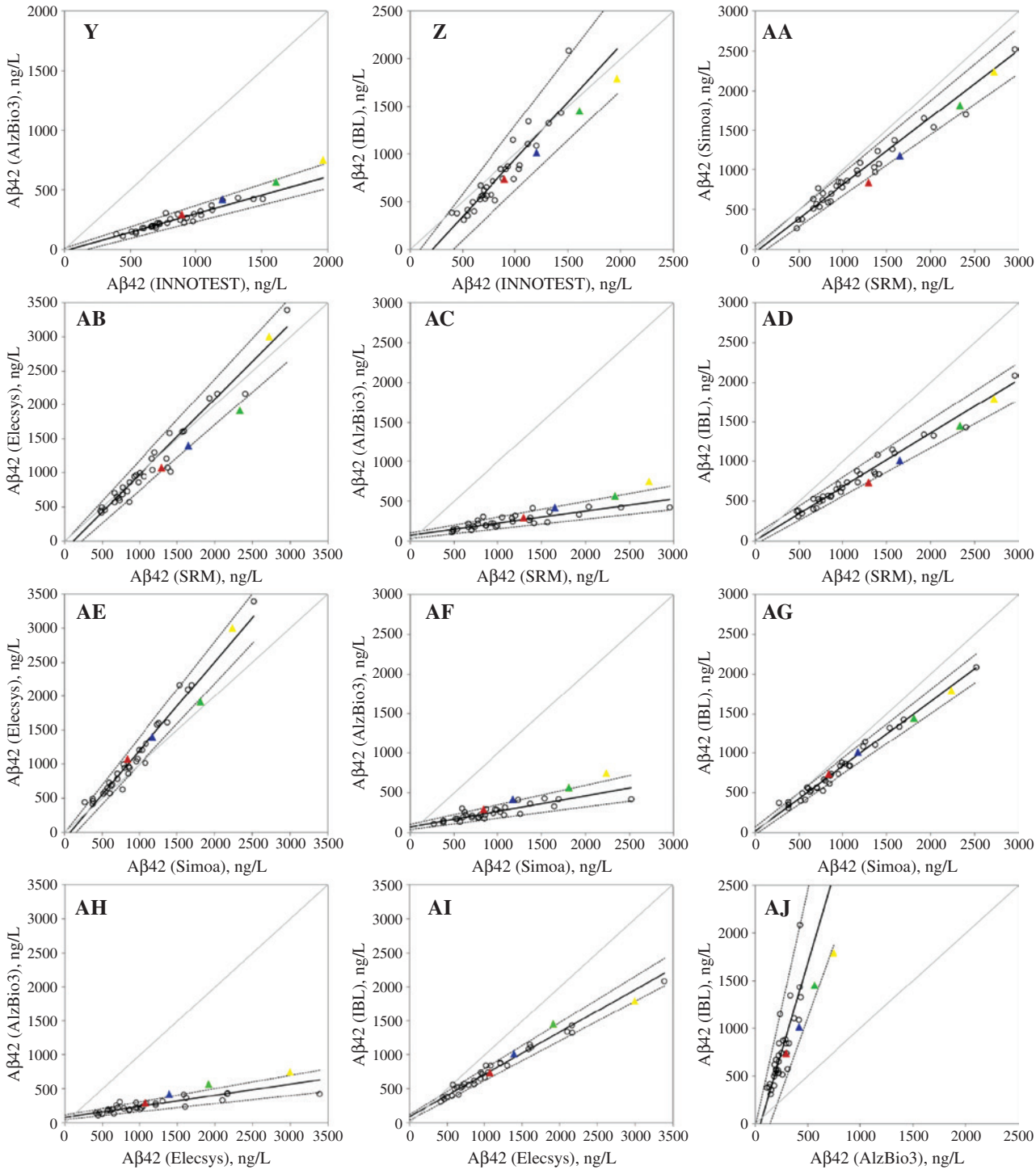

Figure 2 (continued)

the regression line. There were only three comparisons, out of 36, for which the neat CSF pool did not pass the specified criteria for commutability. However, the 95% PI imply that two out of 36 comparisons should fail to be within the acceptance range. In addition, the neat CSF pool candidate CRM was within a relative distance of <15% from the regression line for the three comparisons that did not pass.

The neat CSF and neat CSF spiked with Aβ42 were the candidates that showed the most promise from the first

Table 5: Summary of results for Passing-Bablok regression of mean values; assessment of neat CRM commutability (upper right) and number of candidate CRMs within 95% PI (lower left).

	Dependent values (x-axis)								
	MSD	**Saladax**	**EUROIMMUN**	**INNOTEST**	**SRM**	**Simoa**	**Elecsys**	**AlzBio3**	**IBL**
Independent values (y-axis)									
MSD	x	1	1	1	1	0	1	1	1
Saladax	4	x	1	1	1	1	1	1	1
EUROIMMUN	3	3	x	1	1	1	1	1	1
INNOTEST	4	4	3	x	1	1	1	1	1
SRM	4	4	4	4	x	0	1	1	0
Simoa	3	4	4	4	2	x	1	1	1
Elecsys	3	3	1	4	2	3	x	1	1
AlzBio3	4	1	3	3	2	1	1	x	1
IBL	4	4	2	4	3	4	2	3	x

Upper right corner: 1 indicates commutability, 0 indicates lack of commutability.

Table 6: Relative distance from regression line.

(A) Neat CSF.

Passing-Bablok regression	**Dependent value (x-axis)**								
	Elecsys	**IBL**	**EUROIMMUN**	**INNOTEST**	**SRM**	**Saladax**	**AlzBio3**	**MSD**	**Simoa**
Independent value (y-axis)									
Elecsys	x	2.5	3.1	1.1	3.7	1.3	4.2	4.7	6.8
IBL	2.4	x	0.7	3.1	3.7	3.1	7.5	10.7	3.5
EUROIMMUN	2.8	0.9	x	1.4	4.0	2.3	7.5	10.0	5.5
INNOTEST	1.3	3.3	2.0	x	1.4	0.6	5.0	5.1	6.0
SRM	3.7	3.9	4.2	1.4	x	0.4	5.2	5.0	8.6
Saladax	0.7	3.2	2.9	0.6	0.3	x	5.9	7.2	6.3
AlzBio3	4.6	7.1	6.9	5.3	5.7	5.6	x	4.7	9.3
MSD	4.2	11.2	9.5	5.3	5.0	7.2	4.4	x	13.1
Simoa	6.9	3.5	5.6	5.5	8.8	6.5	8.6	13.3	x

(B) Spiked level 1.

Passing-Bablok regression	**Dependent value (x-axis)**								
	MSD	**Saladax**	**EUROIMMUN**	**IBL**	**SRM**	**Simoa**	**Elecsys**	**AlzBio3**	**INNOTEST**
Independent value (y-axis)									
MSD	x	0.1	0.6	3.5	3.1	5.0	3.0	14.4	7.6
Saladax	0.1	x	0.04	2.5	4.3	4.5	5.9	9.5	7.8
EUROIMMUN	0.1	0.6	x	2.7	3.1	5.7	5.9	8.9	7.3
IBL	3.0	2.4	2.6	x	1.2	1.7	4.2	10.0	10.4
SRM	3.1	3.7	3.0	1.1	x	2.0	5.2	10.1	13.3
Simoa	5.2	4.8	5.7	1.7	2.2	x	1.9	10.9	12.5
Elecsys	3.5	6.5	5.7	4.1	5.2	1.9	x	9.4	12.1
AlzBio3	14.7	9.2	8.3	9.5	10.5	11.6	9.9	x	3.6
INNOTEST	7.7	7.9	7.8	10.6	13.3	13.0	11.9	3.4	x

(C) Spiked level 2.

Passing-Bablok regression	Dependent value (x-axis)								
	Simoa	Saladax	IBL	INNOTEST	MSD	SRM	EUROIMMUN	AlzBio3	Elecsys
Independent value (y-axis)									
Simoa	x	2.6	2.3	10.7	9.0	1.4	8.7	11.0	10.1
Saladax	2.4	x	3.7	8.2	6.3	5.4	5.9	10.8	10.5
IBL	2.3	3.5	x	11.3	10.0	1.1	9.6	11.7	8.2
INNOTEST	11.2	8.3	11.6	x	1.6	14.9	2.3	4.8	16.8
MSD	8.8	6.3	10.6	1.4	x	9.3	0.1	10.3	12.4
SRM	1.6	4.8	1.0	14.8	9.3	x	9.8	12.0	9.6
EUROIMMUN	8.6	6.5	9.7	1.7	0.6	9.9	x	6.2	16.9
AlzBio3	11.7	10.5	11.2	5.0	10.6	12.4	5.5	x	13.4
Elecsys	10.2	11.1	8.1	16.9	12.9	9.6	16.6	13.0	x

(D) Spiked level 3.

Passing-Bablok regression	Dependent value (x-axis)								
	Elecsys	MSD	Saladax	EUROIMMUN	Simoa	IBL	INNOTEST	SRM	AlzBio3
Independent value (y-axis)									
Elecsys	x	1.6	2.2	1.6	4.6	6.7	5.7	8.4	8.2
MSD	2.1	x	2.8	2.8	5.6	7.8	6.0	8.8	18.0
Saladax	2.7	2.8	x	5.6	2.2	4.3	10.3	7.8	15.0
EUROIMMUN	1.8	2.3	6.1	x	7.7	9.8	3.9	12.2	10.0
Simoa	4.7	5.8	2.4	7.8	x	3.3	12.5	4.3	14.2
IBL	6.5	7.3	4.2	9.7	3.3	x	13.8	3.0	15.7
INNOTEST	5.5	6.1	10.4	4.5	13.0	14.0	x	19.3	7.2
SRM	8.4	8.7	7.2	12.1	4.5	2.9	19.3	x	16.4
AlzBio3	8.6	18.3	14.7	9.4	14.9	15.2	7.5	16.8	x

Green: 0.0%–5.0%, yellow: 5.1%–10.0%, orange: 10.1%–15.0%, red: 15.1%–20.0%. Comparison of relative distance of the neat CSF pool and the three spike levels (candidate CRM formats) to the Passing-Bablok regression lines. The line equations were calculated with Analyze-it® for Microsoft Excel (version 2.30). The fields of the tables below were colour coded to group assay correlations according to the distance.

round of the commutability assessments and therefore they were further evaluated in the second commutability study. None of the artificial CRM formats tested was found to be commutable. Neither the aCSF nor the PBS spiked with Aβ42 showed any promise as CRMs. The PBS spiked with 1000 ng/L of recombinant Aβ42 ended up below the clinical sample range when assessed by various methods and the spiked aCSF was often outside the PI. The low commutability for the CSF pools and the artificial systems (spiked aCSF and PBS) that contained detergent might be explained by the fact that the clinical samples did not contain any extra additives, except for what is present in the buffers provided with the various immunoassays. If the CRM format should be commutable for more method comparisons by adding detergents to the neat CSF, the clinical procedure of the CSF sampling would have to be changed and any influence on the candidate RMPs would need to be investigated. This would have a major impact on already ongoing studies and would increase the burden of sample storage. If no other option could have been found this route would have to be further investigated. However, since the neat CSF pool seems to behave well for almost all method comparisons this path was not pursued.

Conclusions

Multiple candidate CRM formats (neat and spiked CSF) were evaluated for commutability of Aβ42 measurements across eight immunoassays and SRM. The commutability across the immunoassays and SRM is a prerequisite for

harmonization of Aβ42 cut-off values for different measurement methods. With regard to the candidate CRM formats that were evaluated, the non-spiked candidate CRM was found to be commutable for all comparisons with the exception of the comparisons between IBL/SRM, Simoa/SRM and Simoa/MSD. However, the relative distance from the regression line for these comparisons was less than 15%. The neat CSF can therefore be regarded as the most commutable candidate CRM format for the methods evaluated herein. Since the candidate RMP is based on LC-MS SRM, it can be used to set the value of the neat CSF candidate CRM format, which can be used for value assignment of the kit calibrators. Spiking of neat CSF with recombinant Aβ42 reduced the commutability and is therefore not considered as a candidate CRM format.

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