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Second-generation Elecsys cerebrospinal fluid immunoassays aid diagnosis of early Alzheimer's disease

https://doi.org/10.1515/cclm-2022-0516
Received May 27, 2022; accepted October 10, 2022;
published online October 24, 2022

Abstract

Objectives: Timely diagnosis of Alzheimer's disease (AD) is critical for appropriate treatment/patient management. Cerebrospinal fluid (CSF) biomarker analysis is often used to aid diagnosis. We assessed analytical performance of second-generation (Gen II) Elecsys® CSF immunoassays (Roche Diagnostics International Ltd), and adjusted existing cut-offs, to evaluate their potential utility in clinical routine.
Methods: Analytical performance was assessed using CSF samples measured with Elecsys CSF Gen II immunoassays on cobas e analyzers. Aβ42 Gen I/Gen II immunoassay method comparisons were performed (Passing-Bablok regression). Cut-off values were adjusted using estimated bias in biomarker levels between BioFINDER protocol aliquots/Gen I immunoassays and Gen II protocol aliquots/immunoassays. Distribution of Gen II immunoassay values was evaluated in AD, mild cognitive impairment (MCI), and cognitively normal cohorts; percentage observations outside the measuring range were derived.
Results: The Gen II immunoassays demonstrated good analytical performance, including repeatability, intermediate precision, lot-to-lot agreement (Pearson's r: ≥0.999), and platform agreement (Pearson's r: ≥0.995). Aβ42 Gen I/Gen II immunoassay measurements were strongly correlated (Pearson's r: 0.985–0.999). Aβ42 Gen II immunoassay cut-offs were adjusted to 1,030 and 800 ng/L, and pTau181/Aβ42 ratio cut-offs to 0.023 and 0.029, for Gen II and I protocols, respectively. No observations were below the lower limit of the measuring range; above the upper limit, there were none from the AD cohort, and 2.6 and 6.8% from the MCI and cognitively normal cohorts, respectively.
Conclusions: Our findings suggest that the Gen II immunoassays have potential utility in clinical routine to aid diagnosis of AD.

Keywords: Alzheimer's disease; beta-amyloid (1–42); biomarkers; cerebrospinal fluid; phosphorylated tau 181P; total tau.

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Introduction

The pathological processes underlying Alzheimer's disease (AD) begin many years before clinical presentation [1]. Ongoing drug discovery efforts in AD focus on developing disease-modifying treatments aiming to delay onset or progression of dementia and must be initiated early in the disease process [2]. Aducanumab was recently approved by the US Food and Drug Administration for treatment of AD in patients with mild cognitive impairment or early-stage dementia [3]. Early diagnosis of AD, possibly before symptom onset, is important for timely and effective intervention [4].

Cerebrospinal fluid (CSF) biomarkers beta-amyloid (1–42) (Aβ42), phosphorylated tau 181P (pTau181), and total tau (tTau) accurately identify early AD by detecting pathophysiological changes underlying the disease [1, 4, 5]. Benefits of CSF analysis for diagnosis of AD include lower running costs compared with amyloid positron emission tomography (PET), greater accessibility for patients, and a standardized and simple procedure for sample handling and shipment [6]. Increasing evidence suggests that CSF Aβ42/beta-amyloid (1–40), pTau181/Aβ42, and tTau/Aβ42 ratios perform better than CSF Aβ42 alone in diagnosing AD [7–9]. These ratios demonstrate high concordance with amyloid PET and are predictive of future clinical progression to AD [7–11].

CSF biomarkers should be measured robustly to aid diagnosis of AD; however, there are difficulties with CSF measurement, including pre-analytical and analytical factors, and patient-related and confounding factors to consider [12]. Such factors could affect CSF measurement and influence diagnostic accuracy. Pre-analytical factors that can affect CSF measurement include sample collection, handling, storage, tube type, collected volume, and transportation [12]. CSF Aβ42 measurement is particularly susceptible to pre-analytical variations as Aβ42 is prone to adhesion to surfaces [13–15]. Introduction of a global standard pre-analytical protocol would reduce pre-analytical variations and is critical to establishing universal cut-off values for measurement of CSF biomarker levels, particularly Aβ42 [14], which may support the adoption of CSF biomarkers in clinical routine and ease results interpretation.

Recently, unified pre-analytical protocols were developed and reported for handling and measurement of fresh CSF samples for use in routine clinical diagnosis and to maximize recovery of Aβ42 [13, 14, 16]. Existing CSF immunoassays for AD diagnosis are often limited by analytical factors such as lot-to-lot and inter-laboratory variations, which have hindered widespread introduction of CSF biomarkers into clinical practice [17]. The introduction of automated measurement of CSF biomarkers has resulted in higher precision, better lot-to-lot consistency, and lower inter-laboratory variations [13]. There are benefits of a unified pre-analytical protocol and reduced analytical variability, including high diagnostic performance, minimized patient misclassification rates, and interstudy comparisons, and thus would support CSF biomarker use in routine clinical practice [14]. Additionally, to reduce differences in results between vendors, the International Federation of Clinical Chemistry and Laboratory Medicine Working Group for CSF proteins developed certified reference materials (CRMs), intended for calibration of diagnostic assays for Aβ42 [14, 15].

The fully automated Elecsys® β-Amyloid(1–42) CSF, Phospho-Tau (181P) CSF, and Total-Tau CSF immunoassays (Roche Diagnostics International Ltd, Rotkreuz, Switzerland) were developed to robustly measure CSF biomarkers, considering the above mentioned pre-analytical and analytical factors. As electrochemiluminescence immunoassays, they utilize monoclonal antibodies via a sandwich test principle. First-generation (Gen I) Elecsys CSF immunoassays are Conformité Européen-approved *in vitro* diagnostic devices. The Elecsys β-Amyloid(1–42) CSF immunoassay has superior inter-laboratory variation (coefficient of variation [CV]: ~4%) compared with existing manual CSF immunoassays (CVs: >15%) [7]. Second-generation (Gen II) Elecsys CSF immunoassays were developed to improve analytical performance. The Elecsys β-Amyloid(1–42) CSF II immunoassay has an extended measuring range and is re-standardized according to CRM ERM-DA480/-481/-482 [15, 18]. All three Elecsys CSF Gen II immunoassays (the Elecsys β-Amyloid(1–42) CSF II immunoassay and updated Elecsys Phospho-Tau (181P) CSF and Total-Tau CSF immunoassays; hereafter referred to as the Elecsys CSF Aβ42, pTau181, and tTau Gen II immunoassays) have higher thresholds for biotin interference and are designed to run on a broader range of analyzers compared with their corresponding Gen I versions. A new simplified pre-analytical handling procedure was developed for use with the Elecsys CSF Aβ42 Gen II immunoassay in clinical routine to reduce pre-analytical variability [13]. With improved technical performance and reduced variability, these immunoassays could provide a solution towards unified biomarker testing and timely diagnosis for patients with early AD.

Therefore, we aimed to assess analytical performance of the Elecsys CSF Gen II immunoassays, including comparisons with the corresponding Gen I immunoassays, and adjust the existing assay cut-off values for use with the simplified pre-analytical protocol [13].

Materials and methods

This study complied with all relevant national regulations and institutional policies, and was performed in accordance with the principles of the Declaration of Helsinki. Detailed methods are provided in Supplementary Table S1.

Analytical performance

Analytical performance was evaluated at the Roche Penzberg site. Samples were generated from uncharacterized CSF, purchased from third-party vendors, or remnant anonymized samples from previous

Table 1: Summary of the different pre-analytical protocols used in the study.

	Pre-analytical protocol		
	BioFINDER protocol	**Gen I protocol**	**Gen II protocol**
Sample type	Frozen	Frozen	Fresh
Total CSF volume, mL	20	12	≥2.5
Number of aliquots	18	20	≥1
Aliquot volume, mL	1.0	0.5	2.5
Handling	Two primary collection tubes, centrifugation (10 min/2,000 g/4 °C), transfer into one tube, mix by rotating 3–4 times, aliquoting in storage tubes, freeze	One primary collection tube, centrifugation (10 min/2,000 g/4 °C), aliquoting in storage tubes, freeze	No centrifugation, freezing, mixing/inverting, or tube transfers
Storage	<–60 °C	–80 °C	2–8 °C (up to 15 days)

CSF, cerebrospinal fluid; Gen I, first-generation; Gen II, second-generation.

Roche-sponsored clinical studies. Where applicable, samples were processed (sample pooling, spiking, and dilution) and frozen/stored at –80 °C prior to measurement. Samples used for the analytical performance experiments were not collected using the new 'Gen II' pre-analytical protocol and partially contrived for generation of sample sets with appropriate volume/concentration for performance evaluation. The different pre-analytical protocols used in this study are summarized in Table 1.

Samples were analyzed using Elecsys CSF Aβ42, pTau181, and tTau Gen II immunoassays on the cobas e 801 analyzer (some experiments were performed on other cobas e analyzers).

Repeatability and intermediate precision: Repeatability and intermediate precision were evaluated per Clinical and Laboratory Standards Institute (CLSI) EP05-A3 guidelines using two assay-specific PreciControl samples and a panel of native and spiked CSF samples (n≥5). One run, with two parts, was performed per day with two aliquots per part over 21 days (n=84). Repeatability and intermediate precision were calculated using variance component analysis.

Method comparisons: Method comparisons between lots and platforms were conducted per CLSI-EP09, using Passing-Bablok regression and Pearson's correlation. Based on regression fit, percentage bias was estimated at a concentration of 1,030 ng/L.

For lot-to-lot comparisons, native and spiked (<10% of all samples) CSF samples were analyzed using two reagent lots (A and B) with the three Elecsys CSF Gen II immunoassays on the cobas e 801 analyzer. For between-platform comparisons, CSF samples (n>100) were analyzed using a single reagent lot with the three immunoassays on cobas e 801, e 402, e 601, and e 411 analyzers. Sample aliquots were measured on the different platforms over several days.

Elecsys CSF Gen II immunoassay linearity: Linearity of the Elecsys CSF Aβ42 Gen II immunoassay was determined using native and spiked (to cover the higher end of the measuring range) CSF samples (n=9) on the cobas e 801 analyzer. Linearity of the Elecsys CSF pTau181 and tTau Gen II immunoassays was determined using native CSF samples (n=3) on cobas e 601 and e 801 analyzers. All samples were measured in triplicate. Theoretical and observed concentrations were compared using ordinary least squares linear regression and Pearson's correlation was calculated.

Pre-analytical bridging study

Samples for the pre-analytical bridging study were prospectively collected at the Clinical Memory Research Unit, Lund University (Malmö, Sweden) and measured at the University of Gothenburg laboratory (Mölndal, Sweden) to evaluate systematic differences between biomarker levels measured in samples prepared according to different pre-analytical protocols and using different immunoassay versions. The study was designed to adjust Elecsys CSF Gen II immunoassay cut-off values if necessary and results were also used to compare aliquot-aliquot variability between the pre-analytical protocols and perform method comparison of Elecsys CSF Gen I vs. Gen II immunoassay versions.

CSF samples from 26 patients with suspected normal pressure hydrocephalus were collected per the Gen II protocol for fresh CSF (n=3 aliquots/patient) [14], and two clinical trial protocols (BioFINDER and Gen I) for frozen CSF (<–60 °C; n=4 aliquots/patient) [7]. Aliquots from one patient prepared according to the Gen II protocol were hemolyzed and excluded from the analysis. The Gen II protocol is intended for measurement of fresh samples and has a larger volume per aliquot (2.5 mL) than the Gen I protocol (0.5 mL). CSF biomarker concentrations were measured using Elecsys CSF Gen I and Gen II immunoassays on cobas e 601; measurements were taken over several months.

Aliquot-aliquot variability: The variability of analyte concentrations measured in aliquots collected from one subject, termed 'aliquot-aliquot variability' (CV [%]), was calculated for each pre-analytical protocol and immunoassay version on the cobas e 601 analyzer. Aliquot-aliquot variability includes within-run error and the variability of analyte concentration in aliquots due to pre-analytical handling.

Adjustment of cut-off values for Elecsys CSF Gen II immunoassays: Cut-off values for Elecsys CSF Gen II immunoassays for use with the Gen II protocol were adjusted using the estimated bias between biomarker concentrations measured in BioFINDER aliquots using

Elecsys CSF Gen I immunoassays vs. concentrations measured in Gen II protocol aliquots using Elecsys CSF Gen II immunoassays on the cobas e 601 analyzer. Concentration bias between the Gen II and Gen I protocols was used to adjust cut-off values for Elecsys CSF Gen II immunoassays with the Gen I protocol.

Comparison of Elecsys CSF Aβ42 Gen I vs. Gen II: Sample concentrations measured using Elecsys CSF Aβ42 Gen I and Gen II immunoassays and averaged per patient and pre-analytical protocol were analyzed using Passing-Bablok regression and Pearson's correlation.

Additional method comparison studies for the Elecsys CSF Aβ42 Gen I vs. Gen II immunoassay

In-house method comparisons: At the Roche Penzberg site, Elecsys CSF Aβ42 Gen I and Gen II method comparisons were performed per CLSI-EP09 on the cobas e 601 analyzer using a single reagent lot; 103 frozen native CSF samples measured in one run. Data were analyzed using Passing-Bablok regression and Pearson's correlation.

Method comparisons using BioFINDER2 samples: We used Aβ42 values generated using Elecsys CSF Aβ42 Gen I and Gen II immunoassays for CSF samples (n=546) measured during the BioFINDER2 study [19]. Measurements were performed over several months using ≥2 lots for Gen I and Gen II immunoassays. Data were analyzed using Passing-Bablok regression and Pearson's correlation.

Coverage of the extended measuring range of the Elecsys CSF Aβ42 Gen II immunoassay

Simulation of expanded patient population for the Elecsys CSF Aβ42 Gen II immunoassay: A simulation was conducted to investigate coverage of the Elecsys CSF Aβ42 Gen II immunoassay extended measuring range (150–2,500 ng/L) in samples prepared using the Gen II protocol, as the measurement range of the Elecsys CSF Aβ42 Gen I immunoassay is narrow. The simulation was based on available Elecsys CSF Aβ42 Gen I immunoassay measurements in the BioFINDER1 cohort, specifically in a subset of CSF samples collected in Sarstedt tubes [7]. Concentrations above the upper limit were calculated using signals and the extrapolated calibration curve. This subset comprised samples from 237 cognitively normal patients (control), 431 patients with mild cognitive impairment (MCI), and 60 patients with AD.

Bias estimate between BioFINDER and Gen II protocols was used to transform original Aβ42 measurements from the BioFINDER1 cohort. Percentage of observations outside the measuring range of the Gen II immunoassay was derived.

CSF biomarker distribution in BioFINDER2 samples: Distribution of Elecsys CSF Gen II immunoassay measurements was evaluated from 706 CSF BioFINDER2 samples (prepared using the Gen II protocol). The BioFINDER2 population comprised cognitively normal individuals and patients with subjective cognitive decline, MCI, AD, and other dementia types. Percentages of observations outside the measuring range of the Gen II immunoassays were derived.

Table 2: Method comparisons of the Elecsys CSF Gen II immunoassays.

Elecsys CSF immunoassay	Lot-to-lot comparison (cobas e 801)			Platform comparison									
				cobas e 801 vs. cobas e 402			cobas e 601 vs. cobas e 801			cobas e 601 vs. cobas e 411			
	n	Passing-Bablok fit	Pearson's r	n	Passing-Bablok fit	Pearson's r	n	Passing-Bablok fit	Pearson's r	n	Passing-Bablok fit	Pearson's r
Aβ42	131	−4.90 + 1.026*x	1.000	133	−6.70 + 1.036*x	0.999	131	27.6 + 0.949*x	0.997	130	−11.6 + 1.003*x	0.995
pTau181	130	1.05 + 0.905*x	0.999	127	−0.119 + 1.038*x	1.000	130	−0.046 + 0.991*x	1.000	128	−0.591 + 0.984*x	0.999
tTau	114	2.48 + 1.019*x	1.000	109	−2.58 + 1.055*x	1.000	108	−1.36 + 0.935*x	0.999	108	3.91 + 0.951*x	0.999

Aβ42, beta-amyloid (1–42); CSF, cerebrospinal fluid; Gen II, second-generation; n, number; pTau181, phospho-tau 181; tTau, total tau.

Results

Analytical performance

Repeatability and intermediate precision estimates (CVs and standard deviations [SDs]) for each Elecsys CSF Gen II immunoassay on cobas e 801 and e 601 met pre-defined acceptance criteria (Supplementary Tables S2 and S3).

Data obtained for lot-to-lot comparisons on cobas e 801 were strongly correlated (Pearson's r: 0.999–1.000), as were between-platform comparisons for cobas e 801 vs. e 402 (Pearson's r: 0.999–1.000), cobas e 601 vs. e 801 (Pearson's r: 0.997–1.000), and cobas e 601 vs. e 411 (Pearson's r: 0.995–0.999). For all lot-to-lot and between-platform comparisons, slope estimates were within 1.00 ± 0.10 (Table 2).

High linearity beyond the measuring range was shown between theoretical expected concentration and measured concentration for the Elecsys CSF Aβ42 Gen II immunoassay on cobas e 801 (Pearson's r: 0.999; Figure 1). High linearity beyond the measuring range was observed for Elecsys CSF pTau181 (cobas e 601 and 801, Pearson's r: 0.999) and tTau (cobas e 601, Pearson's r: 0.999; cobas e 801, Pearson's r: 0.998) Gen II immunoassays.

Pre-analytical bridging study

Aliquot-aliquot variability was similar for Elecsys CSF Aβ42 Gen I and Gen II immunoassays within each protocol, but lower for the Gen II vs. Gen I protocol. Mean aliquot-aliquot variability with the Gen II protocol was 2.15 and 2.05% for Elecsys CSF Aβ42 Gen I and Gen II immunoassays, respectively. Mean aliquot-aliquot variability with the Gen I protocol was 5.88 and 6.63% for Elecsys CSF Aβ42 Gen I and Gen II immunoassays, respectively (Supplementary Figure S1).

No clinically meaningful differences between pre-analytical protocols and immunoassay generations were obtained for pTau181 or tTau. Overall mean percentage difference between concentrations of CSF Aβ42 measured using the BioFINDER protocol/Gen I immunoassay vs. the Gen II protocol/Gen II immunoassay was −6.32% (95% confidence interval [CI]: −8.73 to −3.90%). Consequently, an adjustment factor of 0.937 was applied to the original BioFINDER1 cut-off for Aβ42 for the Gen II protocol/Gen II immunoassays, and an inverted adjustment factor of 0.937^{-1} was used for adjustment of cut-off values for the pTau181/Aβ42 and tTau/Aβ42 ratios. Overall mean percentage difference between Gen II vs. Gen I protocols for the Elecsys CSF Aβ42

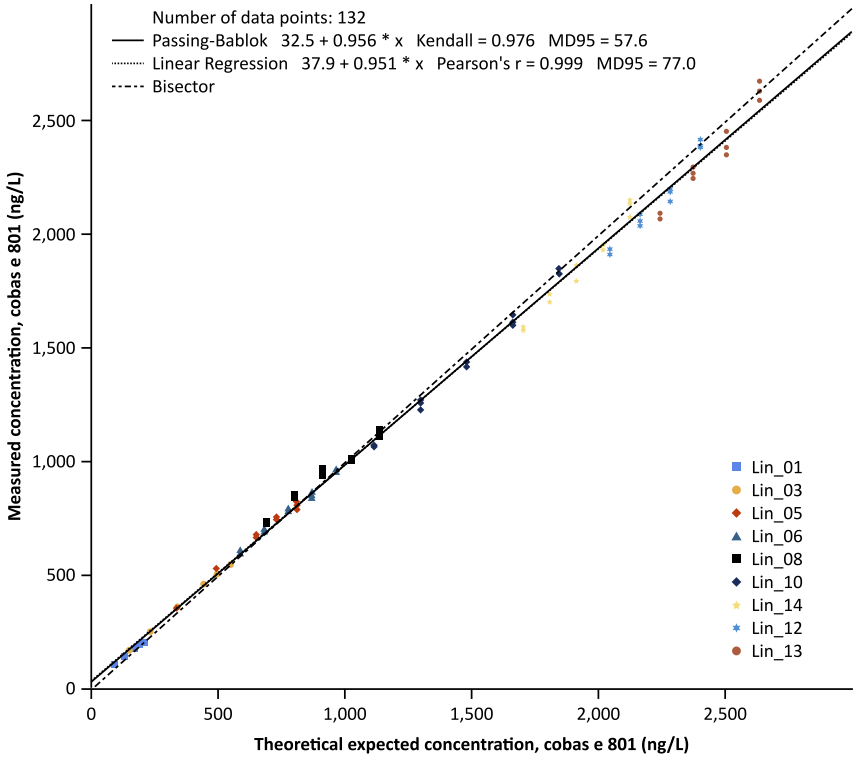

Figure 1: Regression analysis of the Elecsys CSF Aβ42 Gen II immunoassay linearity experiment on the cobas e 801 analyzer. Aβ42, beta-amyloid (1–42); CSF, cerebrospinal fluid; Gen II, second-generation; MD, median distance.

Table 3: Original cut-off values and adjusted cut-off values determined for the Elecsys CSF Gen II immunoassays and different pre-analytical protocols.

CSF biomarker	Pre-analytical protocol and generation of immunoassay		
	BioFINDER protocol/Gen I immunoassays (original cut-off values)	Gen II protocol/Gen II immunoassays (adjusted cut-off values)	Gen I protocol/Gen II immunoassays (adjusted cut-off values)
Aβ42	1,100 ng/L	1,030 ng/L	800 ng/L
pTau181/Aβ42 ratio	0.022	0.023	0.029
tTau/Aβ42 ratio	0.26	0.28	0.36

Aβ42, beta-amyloid (1–42); CSF, cerebrospinal fluid; Gen I, first-generation; Gen II, second-generation; pTau181, phospho-tau 181; tTau, total-tau.

Gen II immunoassay was -21.5% (95% CI: -23.9 to -19/1%), necessitating an adjustment factor of 0.780 to adjust the Gen II cut-off to the Gen I protocol and an inverted adjustment factor of 0.780^{-1} for adjustment of cut-off values for the pTau181/Aβ42 and tTau/Aβ42 ratios. The resulting cut-off values for Gen II protocol/Gen II immunoassays and Gen I protocol/Gen II immunoassays are shown in Table 3.

Measurements of Elecsys CSF Aβ42 Gen I and Gen II immunoassays were strongly correlated (Pearson's r: 0.986). Regression analysis yielded a slope of 0.791 (95% CI: 0.763–0.826); intercept was 29.7 (95% CI: 7.69–54.4). Bias at an Aβ42 concentration of 1,030 ng/L was –18.0% (95% CI: –18.8 to –16.3). However, bias estimates may be slightly influenced by potentially confounded lot/run effects.

Additional method comparison studies for the Elecsys Aβ42 Gen I vs. Gen II immunoassay

In total, 103 samples were included in in-house method comparisons of Elecsys CSF Aβ42 Gen I vs. Gen II immunoassays. Measurements taken with the two assay generations were strongly correlated (Pearson's r: 0.999) (Figure 2). Regression analysis yielded a slope of 0.747 (95% CI: 0.740–0.751); intercept was 45.3 (95% CI: 38.5–52.9). Bias at an Aβ42 concentration of 1,030 ng/L was –20.7% (95% CI: –21.0 to –20.5).

In total, 546 samples from the BioFINDER2 study had available Gen I and Gen II Aβ42 measurements. One sample

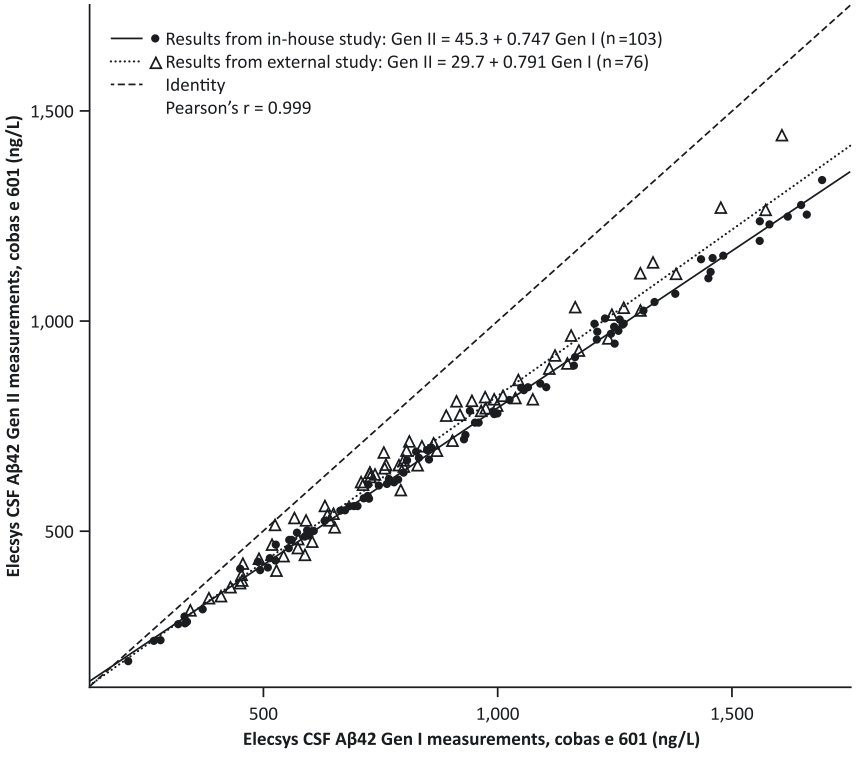

Figure 2: Passing-Bablok regression analysis of the method comparison studies for the Elecsys CSF Aβ42 Gen I and Gen II immunoassays on the cobas e 601 analyzer. Aβ42, beta-amyloid (1–42); CSF, cerebrospinal fluid; Gen I, first-generation; Gen II, second-generation.

was below the measuring ranges of the Gen I and Gen II immunoassays. Of 546 samples, 160 (29%) had Aβ42 values above the upper limit of the Gen I immunoassay, and 14 (2.5%) were above the upper limit of the Gen II immunoassay. The remaining 372 samples had values within the measuring ranges of the Gen I and Gen II immunoassays. The Elecsys CSF Aβ42 Gen I and Gen II immunoassays were strongly correlated (Pearson's r: 0.985) (Supplementary Figure S2).

Coverage of the extended measuring range of the Elecsys CSF Aβ42 Gen II immunoassay

Figure 3 shows simulated distributions for the expanded patient population of the Elecsys CSF Aβ42 Gen II immunoassay, split by clinical cohort. No simulated observations were below the lower limit of the measuring range. Regarding simulated observations above the upper limit, there were none from the AD cohort, 2.6% from the MCI cohort, and 6.8% from the cognitively normal cohort, suggesting the updated measuring range is broad enough to cover the intended-use population (AD and MCI). In the cognitively normal cohort, only a few observations were outside of the new measuring range.

The distribution of Elecsys CSF Aβ42 Gen II immunoassay measurements (n=706) in BioFINDER2 samples (Figure 4) showed that the extended measuring range was sufficient, not only for the intended-use population (MCI and AD), but also for the cognitively normal population: no samples fell below the lower limit of the measuring range and 2.2% were above the upper limit.

Distributions of Elecsys CSF pTau181 and tTau Gen II immunoassay measurements in BioFINDER2 samples are shown in Supplementary Figure S3.

Discussion

The Elecsys CSF Gen II immunoassays demonstrated good analytical performance, supporting their use in clinical routine, and provide additional benefits over the respective Gen I immunoassays without compromising technical performance. Method comparison experiments showed strong agreement between lots and platforms. The Elecsys CSF Aβ42 Gen II immunoassay compared well with the Gen I immunoassay in terms of precision and demonstrated linearity across the measuring range.

Modifications implemented into the Elecsys CSF Gen II immunoassays, together with the new pre-analytical protocol, confer improved robustness in measurement of CSF biomarkers, supporting use of these immunoassays to aid diagnosis of early AD. In the pre-analytical bridging study, clinically meaningful differences between immunoassay generations and pre-analytical protocols were found for

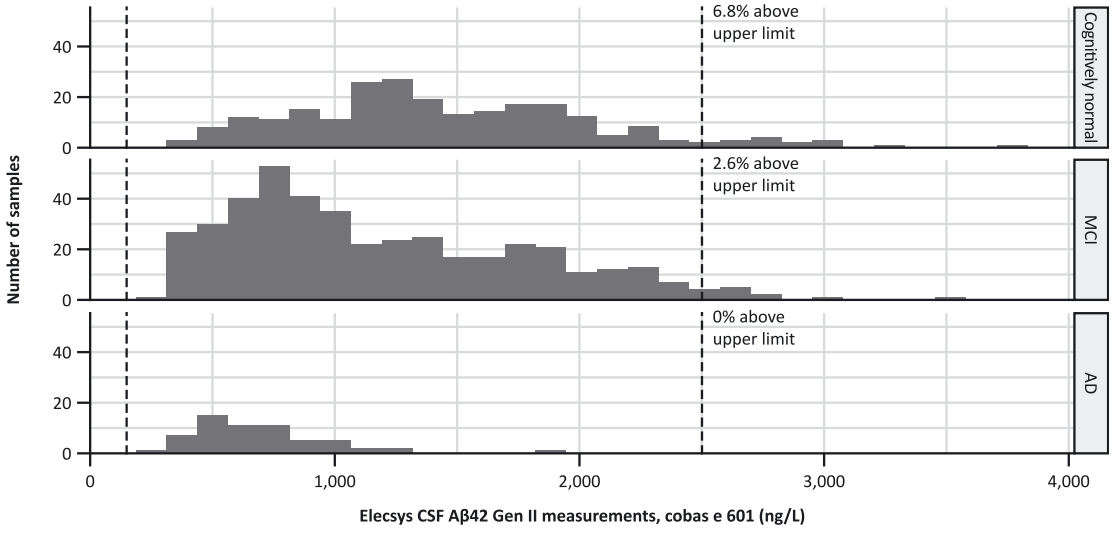

Figure 3: Simulated distribution for the expanded patient population of the Elecsys CSF Aβ42 Gen II immunoassay split by clinical cohort[a]. Note: The vertical dashed lines represent the lower (150 ng/L) and upper (2,500 ng/L) limits of the Elecsys CSF Aβ42 Gen II immunoassay measuring range. [a]The CSF Aβ42 concentrations measured in BioFINDER1 samples using the Elecsys CSF Aβ42 Gen I immunoassay were transformed using the results of the pre-analytical bridging study. Therefore, simulated Elecsys CSF Aβ42 Gen II immunoassay measurements in Gen II protocol samples=0.937* Elecsys CSF Aβ42 Gen I immunoassay measurements in BioFINDER1 samples. Please see the section 'Simulation of expanded patient population for the Elecsys CSF Aβ42 Gen II immunoassay' in Materials and methods. Aβ42, beta-amyloid (1–42); AD, Alzheimer's disease; CSF, cerebrospinal fluid; Gen I, first-generation; Gen II, second-generation; MCI, mild cognitive impairment.

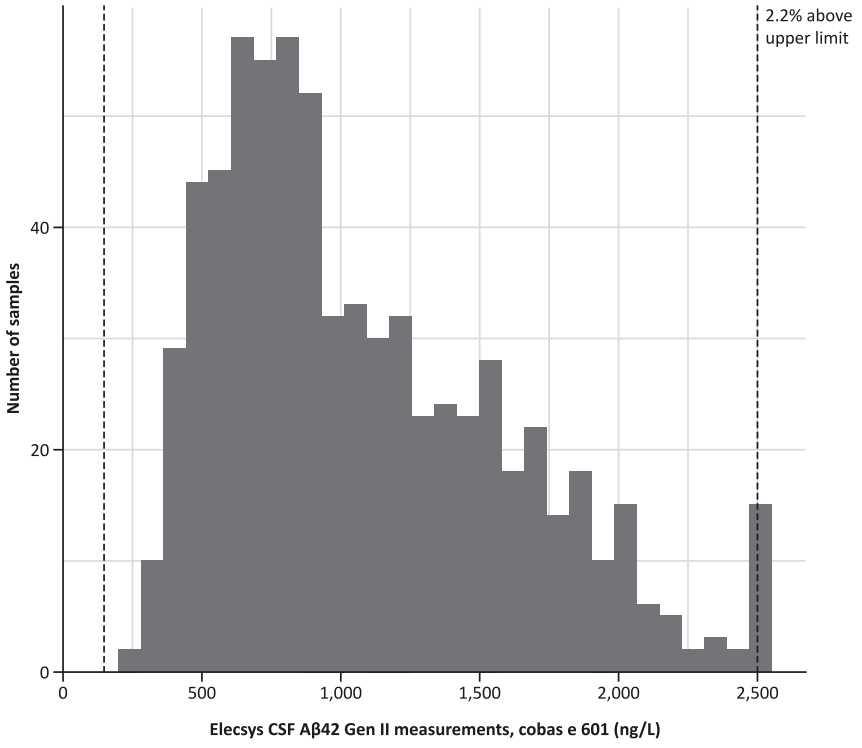

Figure 4: Aβ42 measurement distribution in BioFINDER2 samples using the Gen II protocol and Elecsys CSF Aβ42 Gen II immunoassay (n=706). Note: The vertical dashed lines represent the lower (150 ng/L) and upper (2,500 ng/L) limits of the Elecsys CSF Aβ42 Gen II immunoassay measuring range. Aβ42, beta-amyloid (1–42); CSF, cerebrospinal fluid; Gen II, second-generation.

measuring Aβ42, but not pTau181 or tTau. Adjusted cut-off values were derived from overall mean percentage differences between CSF Aβ42 concentrations measured using the BioFINDER protocol and Gen I immunoassay vs. the Gen II protocol and Gen II immunoassay. Using these adjusted cut-off values and the Gen II/Gen I protocols, clinical performance of Elecsys CSF Gen II immunoassays (concordance with amyloid PET) is expected to be the same as for the respective Gen I immunoassays. The extended measuring range for the Elecsys CSF Aβ42 Gen II immunoassay (150–2,500 ng/L) could permit expansion of use to earlier disease stages, compared with the Gen I immunoassay (measuring range 200–1,700 ng/L), as knowledge about exact biomarker concentration is more beneficial than values simply being above the cut-off.

Aliquot-aliquot variability for Elecsys CSF Aβ42 Gen I and II immunoassays on samples prepared using the Gen I protocol was mitigated by the Gen II protocol [14]. Further studies are warranted to confirm how reductions in aliquot-aliquot variability may improve clinical performance. The Gen II protocol is recommended for use with the Elecsys CSF Aβ42 Gen II immunoassay as less Aβ42 is lost during handling steps [14]; thus, higher Aβ42 concentrations are measured. Conversely, tau results are unaffected by pre-analytical protocol [13]. Similar results were obtained in in-house and two external (pre-analytical bridging and BioFINDER2) method comparison experiments for the Elecsys CSF Aβ42 Gen I vs. Gen II immunoassays.

Our findings have several implications for clinical practice. The data indicate improved robustness of CSF biomarker measurements using the Gen II protocol, particularly regarding lower aliquot-aliquot variability. Immunoassay improvements, combined with the standardized pre-analytical protocol, are expected to reduce patient misclassification and give clinicians greater confidence when using measured levels of CSF biomarkers in AD diagnosis [14]. Furthermore, the standardized pre-analytical protocol, if adopted broadly, could enable establishment of global cut-off values, facilitating amyloid positivity testing in routine clinical settings. The new pre-analytical protocol developed for collection of samples for the Gen II protocol may not be suitable for research and clinical trials. The Gen I protocol may be more appropriate due to smaller aliquot size, as less storage space is required at −80 °C. This is important, as for research and clinical trials many backup aliquots are desired. Additionally, larger aliquots are not optimal for long-term freezing.

Elecsys CSF Gen II immunoassays demonstrate improved on-board stability of reagents for low-throughput scenarios on cobas e 801 (≥16 weeks) compared with cobas e 601 (≥28 days) and e 411 (≥28 days), and are available on a wider variety of platforms for a broader range of users. The Gen II immunoassays have practical advantages, such as ease-of-use, universal cut-offs, fast turnaround time, and fewer processing steps vs. the Gen I immunoassays. When Elecsys CSF Gen II immunoassays are used with the new pre-analytical

protocol, lower volumes of CSF (≥2.5 mL) are drawn from patients in lumbar puncture for results vs. the Gen I protocol (10 mL).

One strength of our study is that the Elecsys CSF Gen I and Gen II immunoassay comparisons and distribution analyses were performed in a large, broad heterogeneous cohort. Another strength is the prospectively collected, fresh CSF samples from patients with suspected normal pressure hydrocephalus (not the intended-use population) used for the pre-analytical bridging study. Furthermore, standardization of the Elecsys CSF Aβ42 Gen II immunoassay to CRMs resulted in a systematic difference in CSF Aβ42 levels measured by the Gen I and Gen II immunoassays. Future studies should be conducted in a broader population to demonstrate PET concordance and verify the adjusted cut-offs.

Conclusions

The Elecsys CSF Gen II immunoassays demonstrated good analytical performance on extended cobas e platforms, including precision, lot-to-lot comparisons, and platform agreement, supporting their use in clinical routine to aid diagnosis of early AD. Elecsys CSF Gen II immunoassays provide additional benefits over the Gen I immunoassays, without compromising technical performance. Good technical performance of the immunoassays, plus reduced preanalytical variability (a result of the new protocol), may provide a solution towards unified biomarker testing and timely diagnosis for patients with early AD.

Acknowledgments: The authors would like to thank Martina Wittig, Gudrun Geiselmann, Franziska Mayr, and Sevinj Aliyeva for conducting the internal experiments. The authors would also like to thank Femke Bouwman and Charlotte Teunissen for their input to the critical review of the manuscript. Third-party medical writing assistance, under the direction of the authors, was provided by Sophie Lavelle, MSc of Ashfield MedComms (Macclesfield, UK), an Inizio company, and was funded by Roche Diagnostics International Ltd (Rotkreuz, Switzerland). COBAS, COBAS E, and ELECSYS are trademarks of Roche. All other product names and trademarks are the property of their respective owners. The Elecsys β-Amyloid(1–42) CSF II, Phospho-Tau (181P) CSF, and Total-Tau CSF immunoassays are approved for clinical use in CE-mark accepting countries.

Research funding: This study was supported by Roche Diagnostics International Ltd (Rotkreuz, Switzerland). The BioFINDER study was supported by the Knut and Alice Wallenberg foundation (2017-0383), the Marianne and Marcus Wallenberg foundation (2015.0125), the Parkinson foundation of Sweden (1280/20), Regionalt Forskningsstöd (2020-0314), the Skåne University Hospital Foundation (2020-O000028), the Strategic Research Area MultiPark (Multidisciplinary Research in Parkinson's disease) at Lund University, the Swedish Alzheimer Foundation (AF-939932), the Swedish Brain Foundation (FO2021-0293), the Swedish federal government under the ALF agreement (2018-Projekt0279), and the Swedish Research Council (2016-00906).

Author contributions: Kaj Blennow, Niels Borlinghaus, Veronika Corradini, Sandra Rutz, and Oskar Hansson contributed to study conception/design and data acquisition. Erik Stomrud and Henrik Zetterberg contributed to data acquisition and data analysis/interpretation. Laura Müller-Hübner contributed to data acquisition. Ekaterina Manuilova and Frances-Catherine Quevenco contributed to data analysis/interpretation. All authors contributed to manuscript writing and/or critical review and approved the final version for submission.

Competing interests: Kaj Blennow served as a consultant at advisory boards and data monitoring committees for Abcam, Axon, Biogen, JOMDD/Shimadzu, Julius Clinical, Lilly, MagQu, Novartis, Prothena, Roche Diagnostics, and Siemens Healthineers, and is also the co-founder of Brain Biomarker Solutions in Gothenburg AB (BBS), which is a part of the GU Ventures Incubator Program. Dr Blennow is supported by the ALF-agreement (#ALFGBG-715986), the Alzheimer's Association 2021 Zenith Award (ZEN-21-848495), the Alzheimer Drug Discovery Foundation (ADDF; USA; #RDAPB-201809-2016615), the European Union Joint Program for Neurodegenerative Disorders (JPND2019-466-236), Hjärnfonden (Sweden; #FO2017-0243), the National Institute of Health (NIH; USA; grant #1R01AG068398-01), the Swedish Alzheimer Foundation (#AF-742881), the Swedish Research Council (#2017-00915), and the Swedish state under the agreement between the Swedish government and the county councils. Erik Stomrud reports no conflicts of interest. Henrik Zetterberg is a Wallenberg Scholar supported by grants from the Swedish Research Council (#2018-02532), the European Research Council (#681712 and #101053962), Swedish State Support for Clinical Research (#ALFGBG-71320), the Alzheimer Drug Discovery Foundation (ADDF), USA (#201809-2016862), the AD Strategic Fund and the Alzheimer's Association (#ADSF-21-831376-C, #ADSF-21-831381-C and #ADSF-21-831377-C), the Olav Thon Foundation, the Erling-Persson Family Foundation, Stiftelsen för Gamla Tjänarinnor, Hjärnfonden, Sweden (#FO2019-0228), the European Union's Horizon 2020 Research and Innovation Programme under the Marie Skłodowska-Curie grant agreement No. 860197 (MIRIADE),

the European Union Joint Programme – Neurodegenerative Disease Research (JPND2021-00694), and the UK Dementia Research Institute at UCL (UKDRI-1003). Prof. Zetterberg also served at scientific advisory boards and/or as a consultant for Abbvie, Alector, Annexon, Apellis, Artery Therapeutics, AZTherapies, CogRx, Denali, Eisai, Nervgen, Novo Nordisk, Pinteon Therapeutics, Red Abbey Labs, Passage Bio, Roche, Samumed, Siemens Healthineers, Triplet Therapeutics, and Wave, has given lectures in symposia sponsored by Cellectricon, Fujirebio, Alzecure, Biogen, and Roche, and is a co-founder of Brain Biomarker Solutions in Gothenburg AB (BBS), which is a part of the GU Ventures Incubator Program. Niels Borlinghaus, Ekaterina Manuilova, Laura Müller-Hübner, and Sandra Rutz are employees of Roche Diagnostics GmbH (Penzberg, Germany) and hold shares in F. Hoffmann-La Roche. Veronika Corradini and Frances-Catherine Quevenco are employees of Roche Diagnostics International Ltd (Rotkreuz, Switzerland); Veronika also holds shares in F. Hoffmann-La Roche. Oskar Hansson has acquired research support (for his institution) from AVID Radiopharmaceuticals, Biogen, Eli Lilly, Eisai, GE Healthcare, Pfizer, and Roche. In the past two years, Prof. Hansson has received consultancy/speaker fees from Alzpath, Biogen, Cerveau, Genentech, Roche, and Siemens.

Informed consent: Not applicable.

Ethical approval: This study complied with all relevant national regulations and institutional policies, and was performed in accordance with the principles of the Declaration of Helsinki. For the pre-analytical bridging study, ethical approval was received from the regional Ethical Review Board of Lund prior to study initiation and written informed consent in accordance with the Code of Federal Regulations Title 21 Part 50 was obtained from each eligible patient prior to study enrollment. No ethical consent was required for the experiments performed at the Roche Penzberg site, which used fully anonymized samples purchased from commercial vendors.

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Supplementary Material: The online version of this article offers supplementary material (https://doi.org/10.1515/cclm-2022-0516).