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Improved performance of Elecsys CSF Abeta measurement achieved using the simple, unified Routine-Use protocol for CSF collection

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Introduction

- Cerebrospinal fluid (CSF) biomarkers amyloid-β1–42 (Abeta42), amyloid-β1–40 (Abeta40), phospho-tau (pTau) and total tau (tTau) can support diagnosis of Alzheimer's disease (AD).[1–6]
- Pre-analytical handling differences are a known source of variability in measured levels of these biomarkers.[7–10]
- An industry consortium chaired by the Alzheimer's Disease Association developed a simplified Routine-Use CSF sample handling protocol intended for universal adoption in clinical practice.[11]

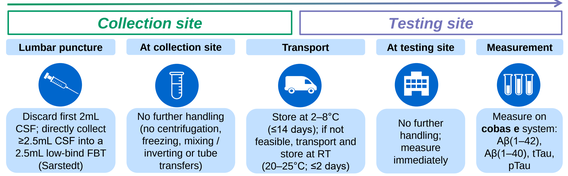
Objective

- **We conducted an analysis to compare biomarker levels and aliquot-to-aliquot variability in samples using a newly developed Routine-Use protocol (Figure 1) versus two clinical trial protocols (BioFINDER and Roche Trial) for measurement of CSF biomarkers to support diagnosis of AD.**

Methods

- CSF samples were prospectively collected from a consecutive series of patients aged >18 years old undergoing routine diagnostic procedure at a Memory Clinic for diagnosis of suspected normal pressure hydrocephalus from June 2019 to February 2020 per the Routine-Use protocol for fresh CSF (n=3 aliquots per patient measured) and two clinical trial protocols (BioFINDER and Roche Trial) for frozen CSF (n=4 aliquots per patient measured).
- Abeta42, pTau and tTau concentrations were quantified by commercially available Elecsys® (Gen1) immunoassays (Roche Diagnostics, Rotkreuz, Switzerland). Abeta40 was measured using a research-use only prototype immunoassay. All biomarkers were measured on the cobas e system. Individual patient results are presented as means of 3(4) aliquots. Incomplete (missing/invalid) results were excluded.
- Routine-Use samples were refrigerated (2–8°C) and measured fresh for each biomarker within 48 hours of collection. BioFINDER and Roche Trial samples were frozen and stored (<–60°C; ≥1 week) before biomarker measurement.
- Freshly measured Routine-Use results were evaluated versus comparator frozen protocol results by calculating mean percentage difference and aliquot-to-aliquot variability for all biomarkers.
- Similar analyses comparing Routine-Use results remeasured following one freeze-thaw cycle (frozen at –20°C; thawed ≥1 week later while being roller mixed) were performed using an updated Elecsys Abeta42 CSF II (Gen2) assay currently under development by Roche Diagnostics (technical performance presented separately).
- Mean and coefficient of variation (CV) were presented for each protocol/assay combination for each patient. Mean percentage bias between methods was calculated for each patient, based on complete cases. Weighted Deming regression and Passing-Bablok regression were used for method comparison and comparison of fresh with frozen samples (Routine-Use protocol), respectively.

Figure 1. Pre-analytical protocol for AD biomarkers in fresh CSF samples[11]



AD, Alzheimer's disease; CSF, cerebrospinal fluid; FBT, false bottom tube; RT, room temperature.

Results

Analysis population

- Of the 26 patients included in this interim analysis, mean (SD) age was 76.6 (6.0) years; n (%) male was 15 (58%); n (%) apolipoprotein E4 (*APOE4)* genotype was 1 (4%) type 23, 2 (8%) type 24, 18 (69%) type 33, 4 (15%) type 34, and 1 (4%) unknown; and mean (SD) mini mental state examination score was 25.9 (3.0).

Method comparison

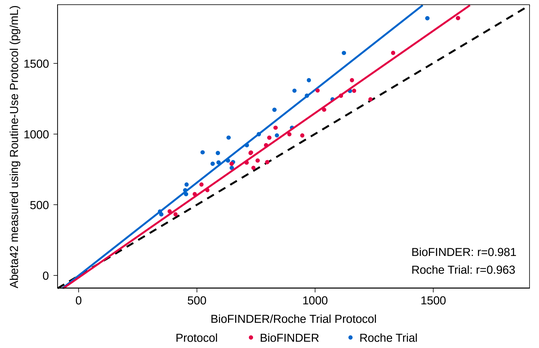
- Abeta40, pTau and tTau concentrations were similar across sample handling protocols, while Abeta42 concentrations differed across sample handling protocols (**Table 1**).
- Abeta42 concentrations in Routine-Use results were on average 14.2% (95% CI 11.0%, 17.4%; p<0.0001) and 32.4% (95% CI 27.1%, 37.6%; p<0.0001) higher than BioFINDER and Roche Trial results, respectively (**Figure 2**).

Table 1. Comparison of AD CSF biomarker concentration results in samples prepared according to BioFINDER, Roche Trial, and Routine-Use protocols

CSF biomarker	BioFINDER n [mean (SD)]	Roche Trial n [mean (SD)]	Routine-Use n [mean (SD)]
Abeta42 (pg/mL)	26 [844.8 (298.2)]	26 [736.5 (276.4)]	25 [976.1 (343.3)]
pTau (pg/mL)	23 [12.28 (2.64)]	23 [12.31 (2.63)]	22 [12.33 (2.63)]
tTau (pg/mL)	25 [144.6 (32.25)]	25 [145.5 (32.61)]	24 [145.0 (32.40)]
Abeta40 (ng/mL)	26 [10.61 (2.68)]	26 [9.88 (2.60)]	25 [11.12 (3.04)]

All biomarkers were measured using Elecsys Gen1 immunoassays on the cobas e system.
AD, Alzheimer's disease; CSF, cerebrospinal fluid; SD, standard deviation.

Figure 2. Regression analysis for method comparison of the Routine-Use vs. BioFINDER and Roche Trial protocols for measurement of Abeta42

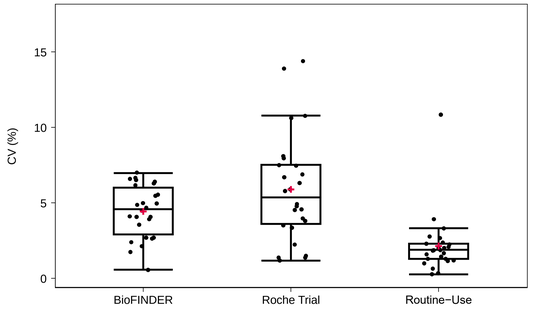


Dashed line represents the identity line. Colored solid lines represent the weighted Deming regression lines for comparison to the respective protocol. (Slopes = 1.16 and 1.31 for BioFINDER [red] and Roche Trial [blue], respectively. The intercept estimates were not significantly different from 0.)

Aliquot-to-aliquot variability

- Lower aliquot-to-aliquot variability (%CV) was observed for Abeta42 (**Figure 3**), Abeta40, Abeta42/40 ratio and pTau/Abeta42 ratio results with the Routine-Use protocol versus results for the comparator protocols (**Table 2**). Aliquot-to-aliquot variability for pTau and tTau was similar across protocols with mean CV below 1.5% for all protocols.

Figure 3. Aliquot-aliquot variability for Abeta42 measured after sample preparation according to Routine-Use vs. BioFINDER and Roche Trial protocols



Abeta42 was measured using Elecsys Gen1 immunoassay on the cobas e system. Red cross symbol represents mean CV.
CV, coefficient of variation.

Table 2. Aliquot-aliquot variability (%CV) of AD CSF biomarker results in samples prepared according to BioFINDER, Roche Trial, and Routine-Use protocols

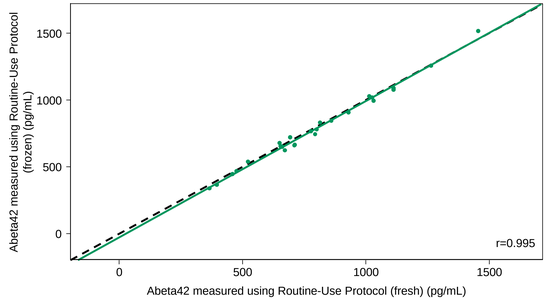
CSF biomarker	BioFINDER	Roche Trial	Routine-Use
Abeta42			
n	26	26	25
Mean	4.42	5.88	2.15
Median (p25, p75)	4.57 (2.89, 6.00)	5.35 (3.56, 7.47)	1.87 (1.28, 2.26)
Range, min–max	0.546–6.98	1.16–14.39	0.260–10.83
Abeta40			
n	26	26	25
Mean	3.05	3.28	1.45
Median (p25, p75)	3.19 (2.16, 4.04)	2.59 (2.06, 4.55)	1.03 (0.840, 1.58)
Range, min–max	0.368–5.11	0.840–7.05	0.314–6.96
pTau/Abeta42			
n	23	23	22
Mean	5.04	5.85	2.07
Median (p25, p75)	5.30 (3.88; 6.31)	5.39 (3.39; 7.69)	1.87 (0.952; 2.75)
Range, min–max	1.62–8.20	0.664–15.01	0.00–7.92
Abeta42/40			
n	26	26	25
Mean	5.15	7.63	2.06
Median (p25, p75)	5.12 (3.70; 6.65)	6.98 (5.10; 9.95)	1.53 (1.24; 2.41)
Range, min, max	0.893–9.14	2.04–16.36	0.564–6.38

All biomarkers were measured using Elecsys Gen1 immunoassays on the cobas e system.
AD, Alzheimer's disease; CSF, cerebrospinal fluid; CV, coefficient of variation.

Fresh-frozen effect

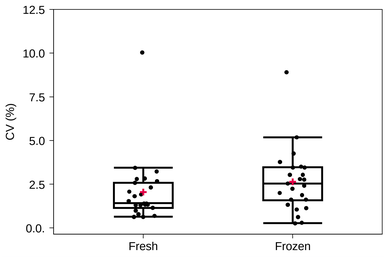
- Freeze-thawing of Routine-Use samples using the Gen2 assay resulted in a –1.95% (95% CI –3.53%, –0.382%; p=0.017; r=0.995) mean change in Abeta42 concentrations (**Figure 4**).
- A slightly elevated aliquot-to-aliquot variability was observed with the frozen versus fresh samples (mean CV 2.64% versus 2.05%, respectively) (**Figure 5**).
- pTau and tTau levels and the aliquot-to-aliquot variability were not significantly affected by freezing (data not shown).

Figure 4. Comparison of Abeta42 results from fresh versus frozen CSF samples measured after sample preparation according to Routine-Use protocol



Dashed line represents the identity line. Green line is the Passing-Bablok regression line for the comparison of fresh to frozen concentrations (the estimated bias in terms of slope and intercept was not significant). Data from 25 patients. CSF, cerebrospinal fluid.

Figure 5. Aliquot-aliquot variability of Abeta42 from fresh versus frozen CSF samples measured after sample preparation according to Routine-Use protocol



	Fresh	Frozen
n	25	25
Mean (SD)	2.05 (1.87)	2.64 (1.79)
Median (p25, p75)	1.42 (1.14, 2.58)	2.54 (1.61, 3.46)
Range, min, max	0.639, 10.05	0.279, 8.91

Abeta42 was measured using Elecsys Gen2 immunoassay on the cobas e system. Data from 25 patients.
CV, coefficient of variation; CSF, cerebrospinal fluid; SD, standard deviation.

Conclusions

- **The Routine-Use protocol is reliable and robust (low aliquot-to-aliquot variability fresh-frozen effect), and has higher analyte recovery for Abeta42 and less aliquot-to-aliquot variability versus comparator protocols.**
- **These findings support the use of the Routine-Use protocol for measurement of CSF biomarkers to support diagnosis of AD.**

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