The impact of decreased bead count to determine MFI values for total-tau, amyloid beta$_{(1-42)}$, and phospho-tau$_{181}$ in human cerebrospinal fluid by flow based fluorometric immunoassay

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Introduction

Alzheimer's disease (AD), the most prevalent cause of adult onset dimensia, is notoriously difficult to diagnose. Research has suggested that monitoring levels of amyloid beta$_{(1-42)}$(Aβ42), total-tau (T-tau), and phospho-tau$_{181}$ (P-tau) may be useful in identifying AD. The present study describes the original validation data under the manufacturer's specifications and compares it to data produced under decreased bead count (BC) parameters using ANOVA, Bland-Altman analysis, and frequency of distribution of CV ranges of duplicate wells.

Equivalence was demonstrated between 50 and 100 BC, however data indicate that BC of 25 or less do not produce reliable results in determination of concentrations of Aβ42, T-tau, and P-tau in human cerebrospinal fluid (CSF)

Objectives

***Summarize accuracy and precision data from an original GLP validation at 100 BC parameter (reference)**

***Re-analyze original raw data from 100 BC validation under decreased BC parameters**

***Perform statistical analyses to compare each new set of data to 100 BC reference**

***Determine degree of equivalency at each BC parameter, and which BCs are optimal for purposes of analysis**

Materials and Methods

THE INNO-BIA® AlzBio3 ASSAY

The INNO-BIA® AlzBio3 test is a fluorimetric assay for the determination of Aβ42, T-tau, and P-tau in human CSF. Aβ42 is captured selectively by monoclonal antibody 4D7A3, T-tau by monoclonal antibody AT120, and P-tau by monoclonal antibody AT270. The sample is further incubated with the biotinylated detector antibody 3D6 for Aβ42 and HT7 for T-tau and P-tau. The antigen-antibody complex is then detected by a phycoerthrin-labeled streptavidin conjugate. Lasers excite fluorochromes embedded within the microsphere and biological reactants bound at the microsphere surface. The range of quantitation is 20 to 1407 pg/mL (Aβ42), 12 to 1535 pg/mL (T-tau), and 6 to 205 pg/mL (P-tau).

VALIDATION OF THE AlzBio3 ASSAY

A fit-for-purpose approach for biomarker method validation was performed based on 100 BC.

STATISTICAL ANALYSES: a full re-analysis of the original raw data was performed using 3, 10, 25, and 50 BC; data were compared using the following statistical analyses

ANOVA: Differences between least squares means (LSM) expressed as differences or percent ratios were calculated and tested. P-values and 99% confidence intervals (CI) were also reported.

BLAND-ALTMAN ANALYSIS: Differences between the paired concentration values were used to calculate the mean differences (D) and the standard deviation of the differences (S). The limits (D±2S) were calculated and reported.

FREQUENCY OF %CV: The correlation coefficient (CV) for each set of sample replicates was categorized into four groups, 0-5% , 6-10%, 11-20%, and > 20%.

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Results

VALIDATION SUMMARY, 100 BC REFERENCE

Analyte	Assay Characteristic	A priori Acceptance Criteria	Method Summary
Aβ42, T-tau, and P-tau	Calibration Model:	± 15% Bias (≤ 20% at the LLOQ and ULOQ) ≤ 20% CV	% Bias: -2.43 to 13.04 % CV: 0.00 to 4.17
	Accuracy (Inter-run) and Relative Accuracy:	± 30% Bias ± 35% Bias for the LLOQ and ULOQ Validation samples	% Bias: -9.52 to 5.33 % Bias (RA): -16.57 to 4.48
	Precision: Intrarun (repeatability)	≤ 20% CV for Pooled QCs	% CV: 0.00 to 14.43
	Precision: Inter-run (intermediate precision)	≤ 25% CV for all Validation Samples	% CV: 2.97 to 22.95
	Total Error (Inter-run):	[|% Bias| + % CV] ≤ 40% for all Validation Samples	% Total Error: 3.76 to 31.52

BLAND-ALTMAN ANALYSIS

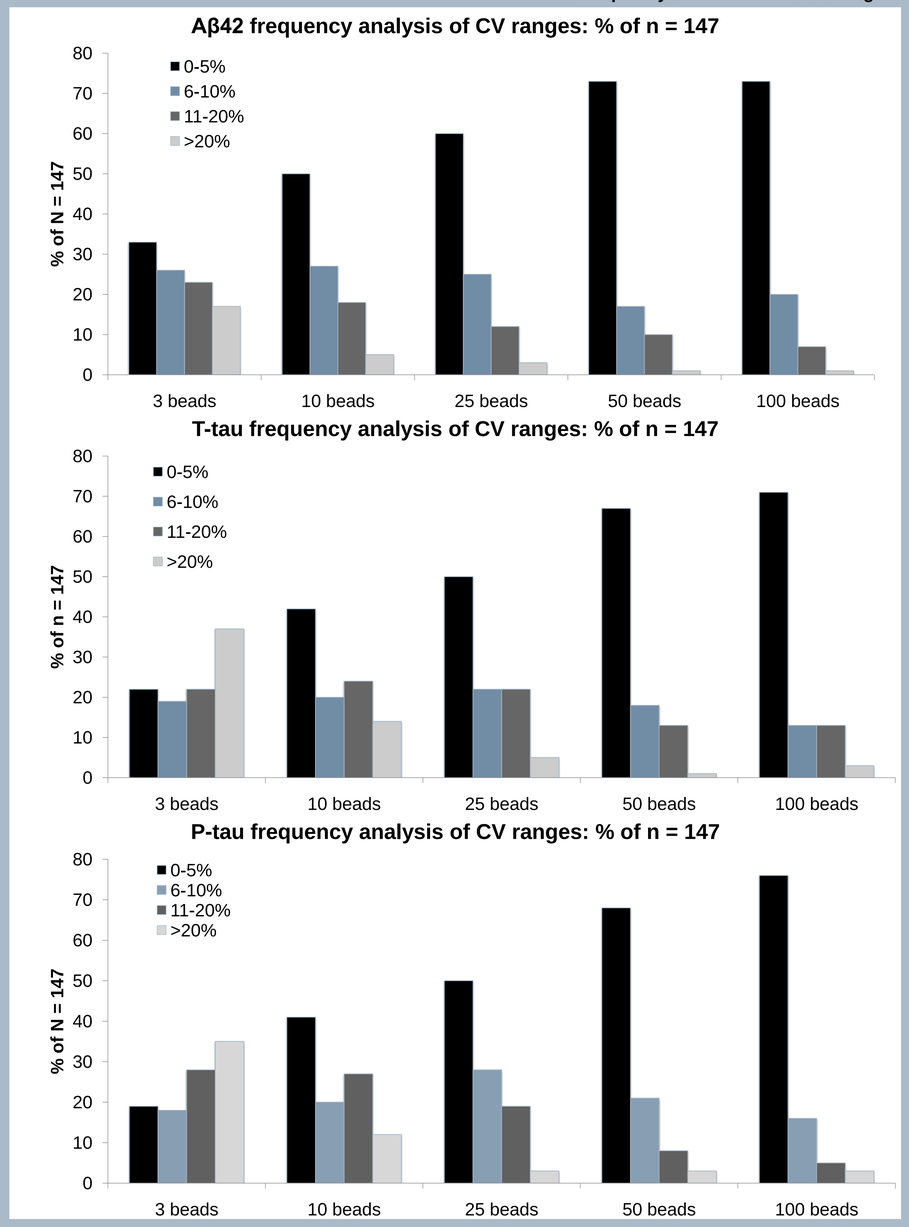
Analyte	Statistical Parameter	50 vs 100 BC	25 vs 100 BC	10 vs 100 BC	3 vs 100 BC
Aβ42	N	139	139	139	139
	Mean of Differences (D)	-1.6	0.3	-9.3	-7.4
	Standard Deviation of Differences	11.0	35.6	38.0	57.0
	D-2S	-23.5	-71.0	-85.4	-121.4
	D+2S	20.3	71.5	66.7	106.6
T-tau	N	137	137	137	137
	Mean of Differences (D)	0.4	-0.2	-14.7	-13.0
	Standard Deviation of Differences	6.1	21.5	91.1	94.7
	D-2S	-11.9	-43.2	-197.0	-202.5
	D+2S	12.7	42.9	167.5	176.4
P-Tau	N	138	138	138	138
	Mean of Differences (D)	-0.2	-0.3	-0.4	0.8
	Standard Deviation of Differences	1.5	2.6	4.7	7.3
	D-2S	-3.2	-5.6	-9.7	-13.9
	D+2S	2.8	5.0	9.0	15.4

ANOVA RESULTS

Analyte	Comparisons	LSMean [a] Test	LSMean [a] Ref	Diff [b]	Ratio (%)[c] (Test/Ref)	99% CI [d] Lower	99% CI [d] Upper	p-value[e]
Aβ42	3 vs. 100 BC	346.2	353.6	-7.4	97.9	93.1	102.7	0.3126
	10 vs. 100 BC	344.3	353.6	-9.3	97.4	92.5	102.2	0.2027
	25 vs. 100 BC	353.9	353.6	0.3	100.1	95.3	104.9	0.9686
	50 vs. 100 BC	352.0	353.6	-1.6	99.6	94.7	104.4	0.8261
T-Tau	3 vs. 100 BC	348.7	361.8	-13.1	96.4	92.1	100.7	0.0501
	10 vs. 100 BC	347.0	361.8	-14.8	95.9	91.6	100.2	0.0267
	25 vs. 100 BC	361.6	361.8	-0.2	100.0	95.7	104.2	0.9781
	50 vs. 100 BC	362.2	361.8	0.4	100.1	95.8	104.4	0.9527
P-Tau	3 vs. 100 BC	62.5	61.6	0.9	101.4	98.1	104.6	0.3249
	10 vs. 100 BC	61.2	61.6	-0.4	99.3	96.0	102.5	0.5936
	25 vs. 100 BC	61.4	61.6	-0.2	99.7	96.4	102.9	0.8095
	50 vs. 100 BC	61.4	61.6	-0.2	99.7	96.4	103.0	0.8361

a = Least Squares Mean for the Test (3, 10, 25, or 50 BC) and Ref (100 BC)
b = Difference = LS Mean (Test) - LS Mean (Ref)
c = Ratio(%) = LS Mean (Test)/LS Mean (Ref)
d = 99% Confidence Interval
e = p-value for the difference; Significant difference defined *a priori* as $p < 0.05$

Frequency Distribution of CV Ranges



Conclusions

***FULL GLP VALIDATION: Accuracy and precision data were acceptable for 100 BC reference.**

***FREQUENCY DISTRIBUTION OF CV RANGES**

Frequency of CV values greater than 20% increased with decreasing BC; conversely, CV values of 5%or less decreased with decreasing BC.

***BLAND-ALTMAN ANALYSIS**

Equivalence between 25, 50, and 100 BC was demonstrated, however decreaing concordance coefficient values and greater standard deviation suggest 25 BC or less should not be used in analysis.

***ANOVA**

A general trend of decreasing p-values in comparison of decreasing BC compared to reference reflects a decrease in agreement between decreasing BC parameters as compared to reference.

****Statistical analysis indicate BCs of less than 25 should not be used as a basis for determination**