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

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Introduction
Alzheimer’s disease (AD), the most prevalent cause of adult onset dementia, is notoriously difficult to diagnose. Research has suggested that monitoring levels of amyloid beta (Aβ), total-tau (T-tau), and phospho-tau (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 ANDIQA. 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 is a fluorimetric assay for the determination of Aβ42, T-tau, and P-tau in human CSF. Aβ42 is captured selectively by monoclonal antibody 4G8, T-tau by monoclonal antibody AT100, 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 phycoerythrin-labeled streptavidin conjugate. Leaks excite fluorophores embedded within the microsphere and biological reactants bound to the microsphere surface. The range of quantitation is 20 to 1407 pg/mL (Aβ42), 12 to 1535 pg/mL (T-tau), 10 to 1407 pg/mL (P-tau) for 50 beads, 10 to 1407 pg/mL for 25 beads, and 10 to 1407 pg/mL for 10 beads. The antigen-antibody complex is then detected by a phycoerythrin-labeled streptavidin conjugate. Leaks excite fluorophores embedded within the microsphere and biological reactants bound to the microsphere surface. The range of quantitation is 20 to 1407 pg/mL (Aβ42), 12 to 1535 pg/mL (T-tau), 10 to 1407 pg/mL (P-tau)

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**Results**

**VALIDATION SUMMARY, 100 BC REFERENCE**

<table>
<thead>
<tr>
<th>Analyte</th>
<th>Analyte Characteristic</th>
<th>A priori Acceptance Criteria</th>
<th>Method Summary</th>
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<tbody>
<tr>
<td>Aβ42</td>
<td>Mean of Differences (D)</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>±10% Bias (≤ 20% at the ULOQ)</td>
<td>±20% CV</td>
<td>% Bias: -2.40 to 13.04</td>
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<tr>
<td>T-tau</td>
<td>Mean of Differences (D)</td>
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<td></td>
</tr>
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<tr>
<td>P-tau</td>
<td>Mean of Differences (D)</td>
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<td></td>
</tr>
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**BLAND-ALTMAN ANALYSIS**

**ANDIQA RESULTS**

**ANALYSIS**

**Comparison**

<table>
<thead>
<tr>
<th>Analyte</th>
<th>Calibration Model</th>
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<tr>
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</tr>
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</tbody>
</table>

**Statistical Analyses**

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

**FREQUENCY DISTRIBUTION OF CV RANGES**

Frequency of CV values greater than 30% 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, decreasing concordance coefficient values and greater standard deviation suggest 25 BC or less should not be used in analysis.

**Conclusions**

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.

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