**Supplementary Material**

**Supplementary Table 1.** Validation parameters of the calibration method.

|  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- |
|  | *ABtest40* | | | | | | | | | | | | | | | | | | | | | | | | | | | |
|  | **Run I** | | |  | | **Run II** | | |  | | **Run III** | | |  | | **Run IV** | | |  | | **Run V** | | |  | | **Run VI** | | |
| pg/ml | RE (%) | CV (%) |  | | RE (%) | | CV (%) |  | | RE (%) | | CV (%) |  | | RE (%) | | CV (%) |  | | RE (%) | | CV (%) |  | | RE (%) | | CV (%) |
| 200 | -0.1 | 0.5 |  | | 0.8 | | 2.4 |  | | 1.5 | | 1.4 |  | | 0.9 | | 3.2 |  | | 0.1 | | 2.9 |  | | 0.5 | | 1.8 |
| 100 | 0.6 | 6.5 |  | | -3.4 | | 3.6 |  | | -3.2 | | 4.0 |  | | -3.6 | | 4.8 |  | | -0.1 | | 5.7 |  | | -3.4 | | 5.4 |
| 50 | -1.8 | 7.7 |  | | 2.1 | | 4.7 |  | | 2.0 | | 3.2 |  | | 1.8 | | 5.5 |  | | -1.1 | | 5.2 |  | | -0.4 | | 4.9 |
| 25 | 1.0 | 5.5 |  | | 5.1 | | 4.7 |  | | 6.5 | | 8.5 |  | | 4.7 | | 6.7 |  | | 2.0 | | 5.3 |  | | 0.6 | | 0.6 |
| 12.5 | -0.7 | 7.1 |  | | 9.5 | | 5.5 |  | | 12.4 | | 5.7 |  | | 7.6 | | 2.9 |  | | 3.8 | | 2.5 |  | | 5.2 | | 3.6 |
| 6.25 | 9.9 | 11.7 |  | | -16.6 | | 5.7 |  | | 4.3 | | 7.8 |  | | 10.0 | | 4.7 |  | | -8.6 | | 4.2 |  | | -9.9 | | 10.2 |
| 3.125 | -17.2 | 6.7 |  | | -1.7 | | 2.1 |  | | -3.8 | | 9.9 |  | | 3.6 | | 8.6 |  | | -6.4 | | 11.5 |  | | -9.6 | | 2.3 |
|  |  |  |  | |  | |  |  | |  | |  |  | |  | |  |  | |  | |  |  | |  | |  |
|  | *ABtest42* | | | | | | | | | | | | | | | | | | | | | | | | | | | |
|  | **Run I** | | |  | | **Run II** | | |  | | **Run III** | | |  | | **Run IV** | | |  | | **Run V** | | |  | | **Run VI** | | |
| pg/ml | RE (%) | CV (%) |  | | RE (%) | | CV (%) |  | | RE (%) | | CV (%) |  | | RE (%) | | CV (%) |  | | RE (%) | | CV (%) |  | | RE (%) | | CV (%) |
| 200 | 0.4 | 4.7 |  | | 0.0 | | 4.6 |  | | 0.2 | | 0.2 |  | | 1.4 | | 3.8 |  | | -2.4 | | 8.4 |  | | 0.0 | | 6.4 |
| 100 | -3.4 | 1.5 |  | | 0.4 | | 5.7 |  | | -0.4 | | 3.1 |  | | -3.2 | | 4.9 |  | | -2.4 | | 2.5 |  | | 0.0 | | 0.4 |
| 50 | 1.1 | 3.3 |  | | -1.5 | | 5.4 |  | | 0.3 | | 1.3 |  | | 1.1 | | 4.1 |  | | 1.4 | | 2.6 |  | | -0.3 | | 2.4 |
| 25 | 2.0 | 3.3 |  | | 1.4 | | 4.0 |  | | 3.0 | | 5.2 |  | | 4.0 | | 4.6 |  | | 5.6 | | 2.3 |  | | 0.9 | | 4.5 |
| 12.5 | 7.8 | 1.1 |  | | 3.6 | | 2.0 |  | | -2.8 | | 1.0 |  | | 6.0 | | 1.9 |  | | 8.4 | | 3.8 |  | | -0.6 | | 0.6 |
| 6.25 | -2.9 | 8.5 |  | | -7.9 | | 1.8 |  | | -0.9 | | 3.3 |  | | -5.0 | | 8.0 |  | | -12.4 | | 5.3 |  | | -1.8 | | 2.3 |
| 3.125 | -12.1 | 19.7 |  | | 4.6 | | 16.2 |  | | -1.9 | | 1.1 |  | | -5.0 | | 2.0 |  | | -2.0 | | 3.6 |  | | 0.4 | | 6.7 |

RE, relative error of the back-calculated concentration regarding the theoretical concentration of the calibration; CV, coefficient of variation of the duplicates at each concentration level of the quantification range (from 3.125 to 200 pg/ml of Aβ40 or Aβ42 standards).

**Supplementary Table 2.** Specificity of the antibodies involved in ABtest40 and ABtest42.

|  |  |  |
| --- | --- | --- |
|  | | **Cross-reactivity (%)** |
| **Anti N-terminal** | |  |
| Aβ2-40 | | 97.0% |
| Aβ3-40 | | 149.6% |
| [Pyr3] Aβ3-40 | | 129.5% |
| Aβ4-40 | | 3.8% |
| Aβ11-40 | 0.5% | |
| Murine Aβ1-40 | | 1.2% |
| **Anti Aβ40** | |  |
| Aβ1-37 | | 0.6% |
| Aβ1-38 | | 1.4% |
| Aβ1-42 | | 0.0% |
| Aβ1-43 | | 0.0% |
| **Anti Aβ42** | |  |
| Aβ1-37 | | 4.1% |
| Aβ1-38 | | 1.8% |
| Aβ1-40 | | 0.9% |
| Aβ1-43 | | 0.8% |

Anti N-terminal: monoclonal 1F3 antibody. Anti Aβ40: pAB002. Anti Aβ42: pAB031. Percentage of cross reactivity, regarding the target Aβ species signal (100%) of 200 pg/ml of Aβ peptides serially truncated in N- and C-terminal.

**Supplementary Table 3.** Limit of blank (LoB) and limit of detection (LoD) estimated for ABtest40 and ABtest42.

|  |  |  |  |  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- | --- | --- | --- | --- |
|  | **Limit of blank** | | | |  | **Limit of detection** | | | |
|  | ABtest40 | | ABtest42 | |  | ABtest40 | | ABtest42 | |
| Batch | LoB (pg/ml) | SDL | LoB (pg/ml) | SDL |  | LoD (pg/ml) | SDL | LoD (pg/ml) | SDL |
| #1 | 1.94 | 2.72 | 2,74 | 1.62 |  | 7.56 | 1.51 | 3.51 | 0.93 |
| #2 | 7.44 | 2.17 | 1,26 | 1.65 |  | 7.93 | 1.74 | 3.88 | 1.15 |
| #3 | 5.83 | 2.64 | 1.93 | 1.14 |  | 7.01 | 1.17 | 3.32 | 0.81 |
| **Mean** | **5.07** |  | **1.98** |  |  | **7.50** |  | **3.57** |  |

LoB was estimated with 18 replicates of four samples without Aβ with three different batches of ABtest40 and ABtest42 (batch#1, batch#2, and batch#3). Variability of the determinations was represented as the global standard deviation, . LoD was established from the previously determined LoB after repetition (18 replicates) of the quantification of four samples with Aβ40 or Aβ42 levels close to LoB using three different ABtest batches.

**Supplementary Table 4.** Limit of quantification of ABtest40 and ABtest42.

|  |  |  |  |  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- | --- | --- | --- | --- |
|  | **ABtest40** | | | |  | **ABtest42** | | | |
|  | Concentration tested | | CV (%) | RE (%) |  | Concentration tested | | CV (%) | RE (%) |
| #1 | 7.60 pg/ml | | 22.1 | -2.4 |  | 3.60 pg/ml | | 14.4 | 14.5 |
| #2 | 7.60 pg/ml | | 21.2 | 4.4 |  | 3.60 pg/ml | | 21.8 | 19.1 |
| Mean |  |  | 21.6 | 3.4 |  |  |  | 18.1 | 16.8 |

LoQ was determined as the lowest concentration of Aβ40 and Aβ42 fulfilling the accuracy (RE ≤25%) and precision (CV ≤25%) criteria. The quantification of 7.60 pg/ml in ABtest40 and 3.60 pg/ml in ABtest42 met the acceptance criteria assaying 44 replicates in a single run with two different ABtest40 and ABtest42 batches.

**Supplementary Table 5.** Accuracy validation results for each concentration level.

|  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- |
|  | *Mean RE %* | | | | |
|  | **LLOQ** | **Low** | **Mid** | **High** | **ULOQ** |
| **ABtest40** | 12.2 | 6.5 | 8.8 | 4.8 | 3.7 |
| **ABtest42** | 10.4 | 7.5 | 4.5 | 8.0 | 5.3 |

RE, relative error as the mean of the difference between each of the 36 determinations carried out at each concentration level regarding the theoretical concentration. LLOQ, concentration close to the lower limit of quantification of the dynamic range of the assay. MID, middle concentration. ULOQ, concentration close to the upper limit of quantification of the dynamic range.

**Supplementary Table 6.** Recovery percentages of Aβ added in plasma.

|  |  |  |  |  |  |  |  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- |
|  |  | **ABtest40** | | | | |  | **ABtest42** | | | | |
|  |  | **Undiluted plasma** | |  | **Diluted plasma** | |  | **Undiluted plasma** | |  | **Diluted plasma** | |
| **Aβ spiked concentration** | | Recovery (%) | SD |  | Recovery (%) | SD |  | Recovery (%) | SD |  | Recovery (%) | SD |
| 150 pg/ml | | 59.6 | 2.0 |  | 87.1 | 4.7 |  | 64.9 | 4.9 |  | 93.6 | 10.6 |
| 75p g/ml | | 63.5 | 4.1 |  | 93.3 | 6.3 |  | 66.9 | 5.9 |  | 97.4 | 7.3 |
| 20 pg/ml | | 58.2 | 5.6 |  | 101.7 | 8.5 |  | 59.0 | 4.9 |  | 93.6 | 7.7 |
| **Mean** |  | **60.4** | **3.9** |  | **94.0** | **6.5** |  | **63.6** | **5.3** |  | **94.9** | **8.6** |

Recovery, average percentage of recovery of known concentration of Aβ added to three different undiluted and three-fold diluted plasma samples in Sample/Standard Diluent. SD, standard deviation of % recovery in the different samples. Three different concentrations levels were added to each sample in order to guarantee recovery in the whole range of the assay.

**Supplementary Table 7.** Further dilution linearity study.

|  |  |  |  |  |  |  |  |  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- |
|  | **ABtest40** | | | | | | | | | | | | |
|  | **Sample 1** | | | | **Sample 2** | | | | **Sample 3** | | | | **Mean recovery (%)** |
| Dilution | Obs. Conc | CV | Theor. Conc | % recovery | Obs. Conc | CV | Theor. Conc | % recovery | Obs. Conc | CV | Theor. Conc | % recovery |
| 1/3 | 181.14 | 4.3 | 181.14 | 100,0 | 164.4 | 4.9 | 164.4 | 100,0 | 154.69 | 1.6 | 154.69 | 100,0 |  |
| 1/6 | 94.61 | 3.2 | 90.57 | 104.5 | 83.35 | 4.7 | 82.2 | 101.4 | 79.74 | 1.6 | 77.35 | 103.1 | **103.0** |
| 1/12 | 44.93 | 5.2 | 45.28 | 99.2 | 39.85 | 6.1 | 41.1 | 97,0 | 38.31 | 2,0 | 38.67 | 99.1 | **98.4** |
| 1/24 | 22,00 | 3.9 | 22.64 | 97.2 | 17.98 | 5.7 | 20.55 | 87.5 | 17.52 | 5.2 | 19.34 | 90.6 | **91.8** |
| 1/48 | 9.55 | 13.9 | 11.32 | 84.4 | 8.06 | 18.6 | 10.27 | 78.4 | 6.6 | 19.4 | 9.67 | 68.2 | **77.0** |
|  |  |  |  |  |  |  |  |  |  |  |  |  |  |
|  | **ABtest42** | | | | | | | | | | | | |
|  | **Sample 1** | | | | **Sample 2** | | | | **Sample 3** | | | | **Mean recovery (%)** |
| Dilution | Obs. Conc | CV | Theor. Conc | % recovery | Obs. Conc | CV | Theor. Conc | % recovery | Obs. Conc | CV | Theor. Conc | % recovery |
| 1/3 | 188.91 | 4.3 | 188.91 | 100,0 | 179.4 | 5.3 | 179.4 | 100,0 | 178.98 | 3.4 | 178.98 | 100,0 |  |
| 1/6 | 100.66 | 1.8 | 94.45 | 106.6 | 95.45 | 6.3 | 89.7 | 106.4 | 95.63 | 1.6 | 89.49 | 106.9 | **106.6** |
| 1/12 | 48.41 | 2.1 | 47.23 | 102.5 | 45.45 | 6.1 | 44.85 | 101.3 | 46.84 | 2.1 | 44.74 | 104.7 | **102.8** |
| 1/24 | 24.22 | 2.9 | 23.61 | 102.6 | 23.32 | 7.3 | 22.42 | 104,0 | 23.64 | 4.2 | 22.37 | 105.7 | **104.1** |
| 1/48 | 11.74 | 1.1 | 11.81 | 99.4 | 11.21 | 14.1 | 11.21 | 100,0 | 11.68 | 7.6 | 11.19 | 104.4 | **101.3** |

Assessment of dilution linearity starting from 1/3 dilution, which is considered as the reference concentration (maximum dilution effect, total Aβ quantifiable in ABtest). Three high Aβ concentration samples were serially diluted in triplicates using Sample/Standard Diluent. The theoretical concentration corresponds to the reference concentration of the 1/3 sample serially divided by the dilution factor. These results showed that linearity is maintained beyond the 1/3 dilution of the plasma in Sample/Standard Diluent, further supporting that maximal Aβ concentration in plasma is measured at the 1/3 dilution. Obs. Conc, observed concentration (pg/ml); CV, coefficient of variation of replicates of the same sample (%); Theor. Conc, theoretical concentration (pg/ml). % recovery=100x(Obs. Conc/Theor. Conc).

**Supplementary Table 8.** Validation parameters of different plasma Aβ quantification assays.

|  |  |  |  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- | --- | --- | --- |
|  | **Intra-assay CV** | | **Inter-assay CV** | | **LLOQ (pg/ml)** | | **Quantification range (pg/ml)** | |
| **Study** | Aβ40 | Aβ42 | Aβ40 | Aβ42 | Aβ40 | Aβ42 | Aβ40 | Aβ42 |
| ABtest validation | 3.9-12.0% | 3.8-7.9% | 3.6-15.0% | 4.6-11.0% | 7.6 | 3.6 | 3.125-200 | 3.125-200 |
| Vanderstichele et al., 2000† [1] | |  |  | 7.1% |  | 5 |  |  |
| Mehta et al., 2000† [2] | 8-16% | | 10-18% | | 20 | 40 |  |  |
| Mayeux et al., 2003† [3] |  |  |  |  | 5 | 10 |  |  |
| van Oijen et al., 2006‡ [4] | 4.4% | 4.9% | 10.1% | 14.8% |  |  | 10-1000 | 5-100 |
| Schupf et al., 2008† [5] |  |  | *<10%* | | 9 | 10 |  |  |
| Blasko et al., 2008† [6] |  | 8.1% |  | 17.3% |  |  |  | 5-1000 |
| Blennow et al., 2009\* [7] |  |  | *3-7.3%* | *1.9-7.2%* |  |  | 30-3000 | 15-600 |
| Hansson et al., 2010\* [8] |  |  | *6.4%* | *2.7%* |  |  |  |  |
| Lewzuck et al., 2010\* [9] | 1.8-4.1% | 1.3-3.8% | 2.2-10.4% | 2.3-11.5% |  |  |  |  |
| Yaffe et al., 2011\* [10] | 3.5% | 2.5% | 9.9% | 9.3% |  |  |  |  |
| Figurski et al., 2012\* [11] | 7.2% | 4.5% | 6.4% | 4.8% |  |  | 3.75-1256 | 1.25-530 |
| Shah et al., 2012† [12] |  |  | 3.1-7.9% | 12-20% |  |  | 3.9-250 | 0.49-125 |
| Lachno et al., 2012\* [13] | 2-9% | 1-7% | 7-10% | 5-17% | 8.0 | 6.3 | 8.4-1545 | 7.6-1485 |
| Lachno et al., 2013\* [14] | 2-8% | 2-7% | 8-9% | 7-11% |  |  | 15.6-750 | 43.9-750 |
| Kaffashian et al., 2015\* [15] |  |  | *1.8%* | *11.2%* |  |  |  |  |
| Chouraki et al., 2015\* [16] | 3.2% | 2.6% | 10.5% | 7.6% |  |  |  |  |

CV, coefficient of variation; LLOQ, lower limit of quantification. Data in italics refer to global variability of the assay. This review of validation parameters of different assays was based on PubMed search of the key words “validation OR performance AND amyloid AND plasma AND immunoassay” and a result of data extraction from plasma Aβ quantification studies that included this information about the test. The assay methodology used in every study is presented as follows: †double-antibody colorimetric sandwich ELISA; ‡DELFIA time-resolved fluorescence (TRF) immunodetection in ELISA; \*Luminex xMAP technology using INNO-BIA plasma Aβ forms assays.

**Supplementary Figure 1**. Average global precision of ABtest40 and ABtest42.



Average intra-assay, inter-assay, and inter-batch precision. CV, coefficient of variation.

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