Araclon BioTech

Mission is to research and develop immunotherapies and diagnostic methods used in the treatment of degenerative diseases.

• Araclon has been an affiliate of Grifols Group since March 2012
• Araclon is based in Spain and in the US.
• A company dedicated to the research and development of therapies and diagnostic methods to be applied to degenerative diseases, currently focusing on Alzheimer’s disease
• Current lines of investigation are:
  1. Early diagnosis of Alzheimer’s disease
  2. Therapy for Alzheimer’s disease (preventive immunotherapy).
ABtest Service: For the quantification of Aβ40 and Aβ42 for both free and total fractions in plasma.

- An ELISA colorimetric assay, coupled with an automated testing system
- All components developed in-house
- As a Service we can control the conditions of each and every test.
ABtest40 and ABtest42. Validation parameters

Accuracy

Precision was assessed with the same five samples repeated in the same and additional runs with 3 different ABtest batches. The coefficient of variation was always below 10%, for all intra-assay, inter-assay and inter-batch, in the entire range of concentration levels of the assay.

Abtest kits are specific for the target Aβ40 or Aβ42, with no cross-reactivity with similar molecules. Other Aβ peptides do not produce a signal including Aβ40 in ABtest42 and vice versa. LLOQ for ABtest40 is 7.6pg/ml and 3.6pg/ml for ABtest42. This has been empirically validated following Clinical and Laboratory Institute guidelines.

Precision

Specificity

Accuracy was evaluated with five samples covering the entire quantification range of the assay. The relative error was within the acceptance criteria, with average values always below 15% independent of the concentration level measured.

Sensitivity

<table>
<thead>
<tr>
<th>LLOQ</th>
<th>Precision (CV)</th>
<th>Accuracy (RE)</th>
</tr>
</thead>
<tbody>
<tr>
<td>ABtest40</td>
<td>7.60pg/ml</td>
<td>21.6%</td>
</tr>
<tr>
<td>ABtest42</td>
<td>3.60pg/ml</td>
<td>18.1%</td>
</tr>
</tbody>
</table>
We analyzed plasma samples from 104 healthy control individuals at the 18, 36 and 54 month time points (55 with 3 a-PET scans, 13 with 2 a-PET scans and 36 with 1 a-PET; from visit 2 to visit 4).

The plasma assays were performed blinded to the characteristics of the individuals.
ROC comparison: AIBL

Model: Age + APOE4

Model: log(TP42/40) + Age + APOE4
Optimization of PPV: AIBL

**ABtest PPV model:**
Optimized ABtest PPV with relation to prevalence and a model using APOE4 status and age.
**AB255 – A multi center ABtest trial**

<table>
<thead>
<tr>
<th></th>
<th>N</th>
<th>Baseline</th>
<th>12 months</th>
<th>24 months</th>
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<tbody>
<tr>
<td>HC</td>
<td>83</td>
<td>80</td>
<td>76</td>
<td></td>
</tr>
<tr>
<td>MCI-FDGneg</td>
<td>104</td>
<td>96</td>
<td>91</td>
<td></td>
</tr>
<tr>
<td>MCI-FDGpos</td>
<td>39</td>
<td>31</td>
<td>30</td>
<td></td>
</tr>
</tbody>
</table>

**Correlation FDG / ABtest:** An inverse relationship between TP42/40 and risk of AD as measured by FDG PET, supporting amyloid PET findings.

**Progressors vs Stable:** Baseline levels of TP42/40 are predictive of MCI who later progress to AD in the 24 months follow up, progressors have significantly lower levels of TP42/40 at baseline.

* = p<0.05; ** = p<0.01; *** = p<0.001 In U Mann-Whitney test
**Plasma biomarkers in middle-aged individuals at risk for Alzheimer Disease (ACS-Araclon study).**

P value for the interaction term between the plasma variable and the central biomarker (slope or intercept).

<table>
<thead>
<tr>
<th></th>
<th>ACS TP42 / TP40</th>
<th>ACS FP42 / TP42</th>
</tr>
</thead>
<tbody>
<tr>
<td>MCSUVR intercept</td>
<td>0.022</td>
<td>nsa</td>
</tr>
<tr>
<td>MCSUVR slope</td>
<td>nsa</td>
<td>0.00081</td>
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<tr>
<td>CSF Aβ42 intercept</td>
<td>0.0251</td>
<td>0.0238</td>
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<tr>
<td>CSF Aβ42 slope</td>
<td>nsa</td>
<td>nsa</td>
</tr>
<tr>
<td>Tau intercept</td>
<td>0.0292</td>
<td>nsa</td>
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<tr>
<td>Tau slope</td>
<td>0.00307</td>
<td>0.000876</td>
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<tr>
<td>pTau intercept</td>
<td>0.0324</td>
<td>nsa</td>
</tr>
<tr>
<td>pTau slope</td>
<td>0.000572</td>
<td>0.0066</td>
</tr>
</tbody>
</table>

**MCSUVR:** mean cortical standardized uptake value ratio. (positivity defined as > 1.42). **nsa:** no significant association.

ACS linear mixed-effects models adjusted for: time, ApoE genotype, age, gender and family history (+/-). AIBL linear mixed-effects models adjusted for: time, ApoE genotype. ACS age ± SD at base line: 62 ± 8; AIBL age ± SD at base line: 72 ± 7.
Correlations between plasma and brain beta-amyloid levels in individuals with subjective cognitive decline: the Fundació ACE Healthy Brain Initiative (FACEHBI).


Fundació ACE, Barcelona Alzheimer Treatment, Servei de Medicina Nuclear, Hospital Clinic i Provincial, Barcelona, Spain.
Regulatory Pathway:

Europe: CE Marked IVD for the quantification of beta amyloid proteins in blood.

USA: 1. CLIA Lab (San Marcos, Texas)
      2. LDT
      3. IUO
      4. Mid to long term goal is to have ABtest positioned as an IVD as an effective intervention becomes available.

Supporting Material: AIBL, ACS, ADNI, A4, LEARN, DPUK, EPAD, FACEHBI, AB255 and others.
CONCLUSIONS

✓ The TP42/40 ratio shows promise as a useful first-step screening tool for cohort enrichment in clinical trials to recruit individuals at increased risk of developing Alzheimer’s disease.

✓ The TP42/40 shows promise as a tool to enrich cohorts for amyloid PET positivity

✓ Evaluation of plasma FP42/TP42 may be informative as a first-step screening tool to identify preclinical AD in cognitively normal middle-aged individuals.
Availability:

We do not sell kits, we provide a service.

The ABtest Service is commercially accessible from anywhere in the world. We have two approved laboratories, Zaragoza, Spain and San Marcos, Texas.

Samples can be shipped in from anywhere in the world at -80°C