To date only the presence of one or two alleles c4 of the APOE gene is accepted as a reliable biomarker and risk factor of developing late onset Alzheimer’s Disease (AD) (3). The presence of one allele c4 of the APOE gene increases the risk of suffering AD by 3-5 fold, while the presence in homozygosis increases the risk by 15-20 fold (3). Furthermore, APOE c4 carriers progress faster from the precocious stage of AD to the AD c4 non-carriers (1). Therefore, APOE c4 carriers clearly satisfy a target population where research targets and prevention strategies should be focused. Despite its clear clinical utility, apolipoprotein E4 analysis is not required by neurologists in a routine basis mainly for logistic and bureaucratic reasons: It involves DNA analysis, which in Europe, requires an informed consent, and the standard analysis technique is PCR which requires additional processing of the sample to extract the DNA. Furthermore, PCR is a method of analysis more complex and expensive than the techniques normally used in a clinical laboratory setting.

### Background

Biocross developed a test where the ApoE4 present in plasma is specifically captured by the ELISA microplate with the aid of a special coating agent developed by Biocross. Then, the use of a highly specific antibody for ApoE4 that does not recognize the rest of apolipoprotein E isoforms (E2 and E3) - allows the discrimination of APOE c4 carriers (patients that carry at least one allele c4 of the APOE gene) from APOE c4 non-carriers with 100% sensitivity and 100% specificity.

### Adaptation to Turbidimetry & Technical Verification

Biocross adapter the method to a turbidimetric-based kit. The kit consists of two main reagents: R1 (which contains a highly specific anti-apoE4 antibody) and R2 (a solution containing latex (polystyrene beads)). During the assay, the anti-apoE4 antibody induces the agglutination of latex beads in the presence of apoE4 in the sample. This agglutination is proportional to the concentration of the apoE4 and can be measured spectrophotometrically. The main studies carried out to verify the test parameters are summarized below. These studies were performed using the platform Selectra Vital Junior.

#### 1. Interference

One plasma sample from one APOE c4 carrier (e4/e4), pooled and one plasma sample from one APOE c4 non-carrier (c4/c2), were tested in duplicate either in the absence (dotted line) or in the presence (solid line) of one or several concentrations of classic interference factors (haemoglobin, bilirubin, triglycerides or rheumatoid factor). Values are mean ± SD of duplicates. Red line represents positivity cut-off for the test (121 WAOA).

#### 2. Performance

ApoE4 ISOFROM CAN BE DETECTED WITH 100% SENSITIVITY AND 100% SPECIFICITY IN ELISA

#### 3. Stability

Figure 8. RT and R2 were stored at 2-8°C and one sample (one apoE4 c4 carrier and one apoE4 c4 non-carrier) were analyzed 159 days. The results showed 100% concordance (a solution containing 400 mg/dL of haemoglobin, 100 mg/dL of bilirubin, 200 mg/dL of triglycerides or 100 mg/dL of rheumatoid factor). Values are mean ± SD of duplicates. Red line represents positivity cut-off for the test (121 WAOA).

#### 4. Linearity

Figure 7. Receiver Operating Characteristic (ROC) curve for the apoE4 carrier analysis of 114 plasma samples (55 apoE4 c4 carriers and 59 apoE4 c4 non-carriers). The optimal cut-off and sensitivity were calculated according to a receiver-operating characteristic (ROC) curve for each concentration, determining specificity (true negative rate) and sensitivity (true positive rate). Values are mean ± SD of duplicates. Red line represents positivity cut-off for the test (121 WAOA).

### Clinical Utility

The ApoE4 blood marker assay could be easily incorporated into routine dementia test profiles, allowing a fast identification of APOE c4 carriers that can be integrated into the clinical routine setting of hospitals.

### Conclusion

Our results show that ApoE4 blood marker assay is a cost effective and highly reliable method to identify APOE c4 carriers that can be integrated into the clinical routine setting of hospitals.

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**REFERENCES**