A NEW COST-EFFECTIVE METHOD FOR QUANTIFICATION OF TOTAL ApoE IN HUMAN PLASMA SAMPLES

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BACKGROUND

Apolipoprotein E (ApoE) is a 34 kDa glycoprotein involved in lipid metabolism. Three common codominant alleles (ε2, ε3 and ε4) encode three ApoE protein isoforms (E2, E3 and E4). The presence of one or two ε4 alleles of the *APOE* gene is accepted as a reliable biomarker and risk factor for developing late onset Alzheimer´s Disease (AD). Recently, we have developed a non-genetic, cost-effective and highly reliable method to detect the presence of ApoE4 in human plasma using high-throughput clinical chemistry analysers. This non-quantitative method is based on the use of a ***proprietary coating agent (BX agent)***, which allows the capture of ApoE4 from plasma samples without using capture antibodies.

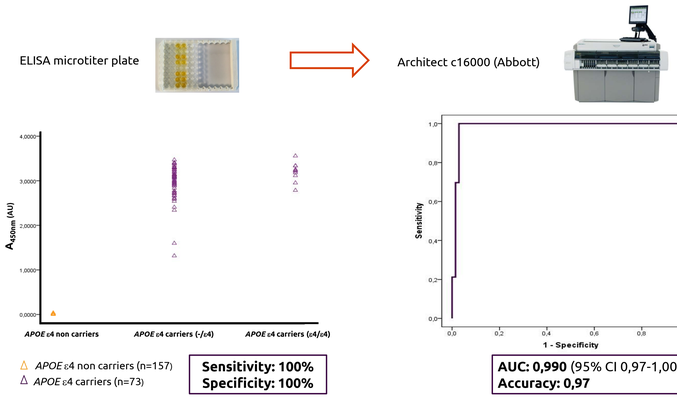

Figure 1. ApoE4 isoform can be detected with 100% accuracy in ELISA plates coated with BX agent. The presence/absence of ApoE4 was detected in 230 plasma samples from individuals previously genotyped by Real-Time PCR. A total of 157 *APOE* ε4 non carriers (ε2/ε3, n=16 and ε3/ε3, n=141) and 73 *APOE* ε4 carriers (ε2/ε4, n=4, ε3/ε4, n=59 and ε4/ε4, n=10) were analyzed. The method developed by Biocross revealed 100% concordance with *APOE* genotyping by Real-Time PCR [1].

Figure 2. The methodology was successfully adapted to an immunoturbidimetry assay for its use in clinical chemistry platforms. Receiver Operator characteristic (ROC) curve for the *APOE* ε4 carriership analysis of 102 plasma samples (70 *APOE* ε4 non carriers and 32 *APOE* ε4 carriers), AUC (Area under the curve, with 95% confidence intervals) and accuracy (measured as 1-missclasification rate) values are provided. Accuracy was calculated assuming a 100% sensitivity and 96% specificity.

OBJECTIVE

Recent evidence shows that ApoE plasma concentration is associated with increased risk of AD and dementia, independently of *APOE* genotype[2,3]; suggesting that plasma ApoE is an easily accessible pre-clinical biomarker of AD. In this work, we explored if our methodology (capturing ApoE from plasma with BX agent) can be used for the quantification of human plasma ApoE.

RESULTS

1. ApoE BINDING STABILITY

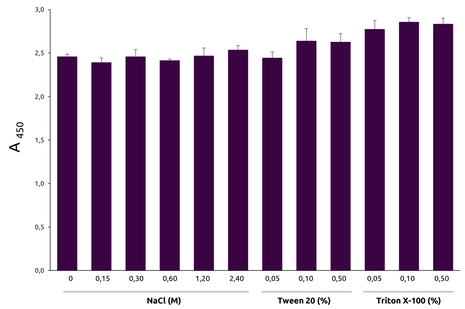

Figure 3. Stable binding of ApoE to BX agent-coated ELISA plates after treatment with different concentrations of salt or detergents. The ability of different buffers containing salt or detergent (polysorbate 20 (tween 20) or Triton X-100) to remove the apoE bound to BX agent-coated ELISA plates was analyzed. For this purpose, ELISA plates were coated overnight with BX agent. Then, plates were washed and allow to bind the apoE present in plasma (1:200 dilution) for 1 hour. After washing, the wells were incubated for 1 hour at RT with the different buffers, washed and the remaining ApoE was revealed by the combination of a polyclonal anti-apoE antibody (Santa Cruz, H-223) and an anti-rabbit IgG-peroxidase.
The presence of increasing concentration of either NaCl or detergents did not affect the detection of ApoE compared to the control without salt or detergent.

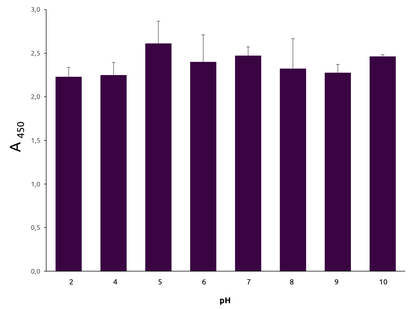

Figure 4. Stable binding of ApoE to BX agent-coated ELISA plates after treatment with different pH.
The stability of the binding of ApoE to BX agent-coated ELISA plates was also analyzed as a function of the pH in the range from pH 2 to 10. Wells were blocked with BX agent overnight and washed. Then, BX agent-coated plates were incubated for 1h with human plasma to allow binding of ApoE. Wells were then washed and incubated with different pH solutions and the assay was continued as described before.
The binding of apoE to the BX agent-coated wells was fairly unaffected by the pH in the range studied.

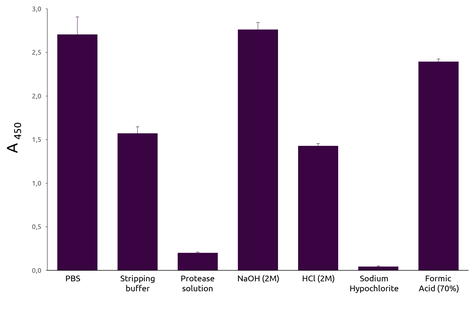

Figure 5. Resilience of ApoE binding to BX agent-coated ELISA plates.
Resilience of ApoE binding to more stringent conditions were also assayed by treatment at 56 ºC for 1 hour either with PBS, Stripping buffer (a combination of anionic detergent and a reducing agent (2% SDS and 0.7% 2-mercaptoethanol)), enzymatic detergent solution (Coulter Clenz® cleaning agent, Beckman Coulter)), strong acids (2 M HCl or 70% formic acid), strong bases (2 M NaOH) or sodium hypochlorite (20,000 ppm).
The only reagents that were able to remove completely the apoE bound to the plate coated with BX agent were those that destroy the protein either by digestion (enzymatic detergent) or by oxidation (sodium hypochlorite).

2. ApoE DYNAMIC RANGE

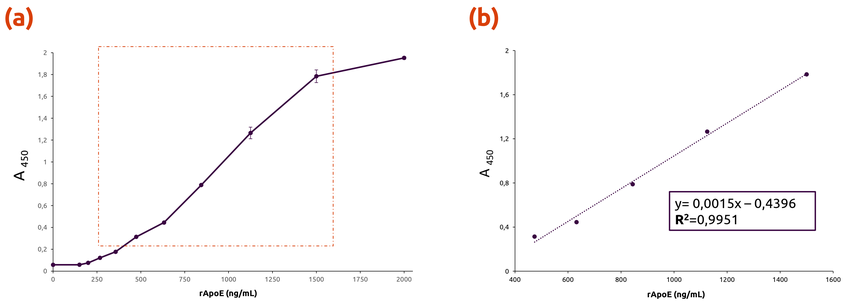

Figure 6. Standard curve constructed with 11 different concentrations of recombinant ApoE (rApoE).
BX agent-coated ELISA plates were incubated for 1 hour with 11 different concentrations of rApoE (from 0 to 2000 ng/mL) and then the assay was continued as previously described to measure the absorbance of each concentration. A representative standard curve is shown in (a).
The curve shows an intermediate portion (dashed rectangle in (a), corresponding to 474 to 1500 ng/mL) where a lineal relationship between absorbance and rApoE concentration can be observed (R^2= 0,9951). This intermediate portion is plotted separately in (b). This dynamic range was confirmed in two additional independent experiments (data not shown).

CONCLUSION

Our preliminary results suggest that human plasma ApoE can be stably bound to surfaces coated with BX agent. Furthermore, this binding has an acceptable dynamic range, which indicates that our methodology can be used for ApoE quantification. The adaptation of the method to immunoturbidimetry will allow the development of a fast and cost-effective method for quantitative measurement of ApoE in human plasma samples.

REFERENCES

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