On-Well array immunoassay: an alternative to 96-well ELISA for biomarker quantification in biofluids

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Introduction

It is estimated that worldwide, more than 50 million people are currently living with Alzheimer's Disease (AD) or related disorders, and there is foreseen a doubling of this number every 20 years as the population ages. Early diagnosis is critical for the detection of pre-symptomatic stages of the disease. It enhances its accurate monitoring and prediction, paving the road for developing new therapeutic and preventive approaches. Molecular diagnosis using blood biomarkers is less invasive than traditional imaging methods or cerebrospinal fluid tests. It also provides an opportunity for routine and widespread screening at a lower price than current methodologies.

This research aims **to develop an enhanced format for monitoring biomarkers from noninvasive samples, such as blood plasma, based on 96-well plate On-Well microarrays. Thus, we aim to reduce sample volumes, reagent consumption, and assay times while enhancing scalability.**

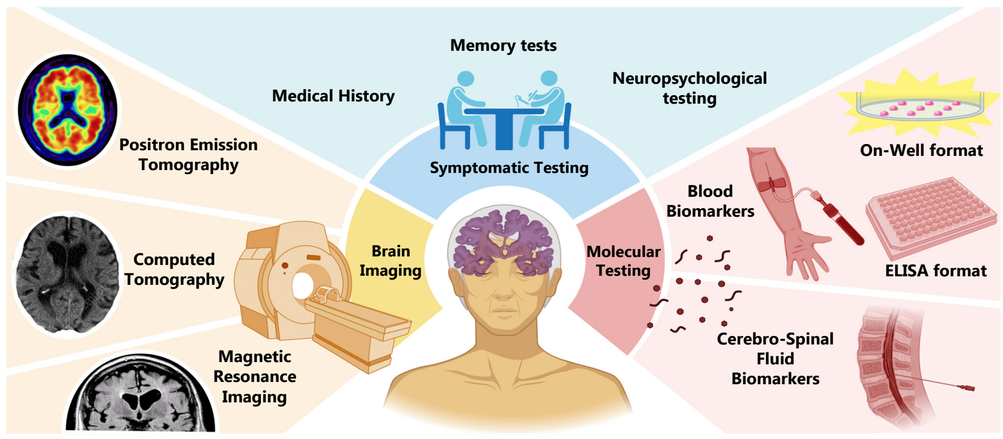


Figure 1. Diagram of Alzheimer's Disease testing techniques. Current detection methods involving cerebrospinal fluid biomarkers or neuro-imaging techniques are costly and not easily accessible for widespread monitoring due to their invasive nature. Presently, molecular diagnoses are conducted using ELISA protocols, where samples are recognized and quantified in separate wells of a 96-well microtiter plate coated with capture antibodies. On-Well arrays provide a new, less invasive blood-based testing option.

Assay Formats

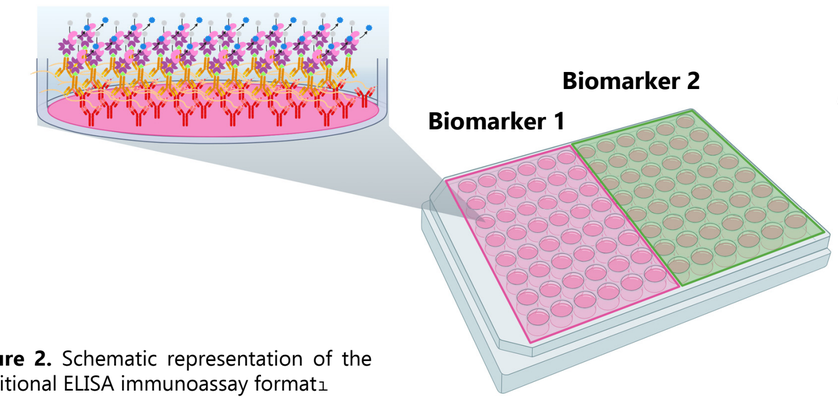
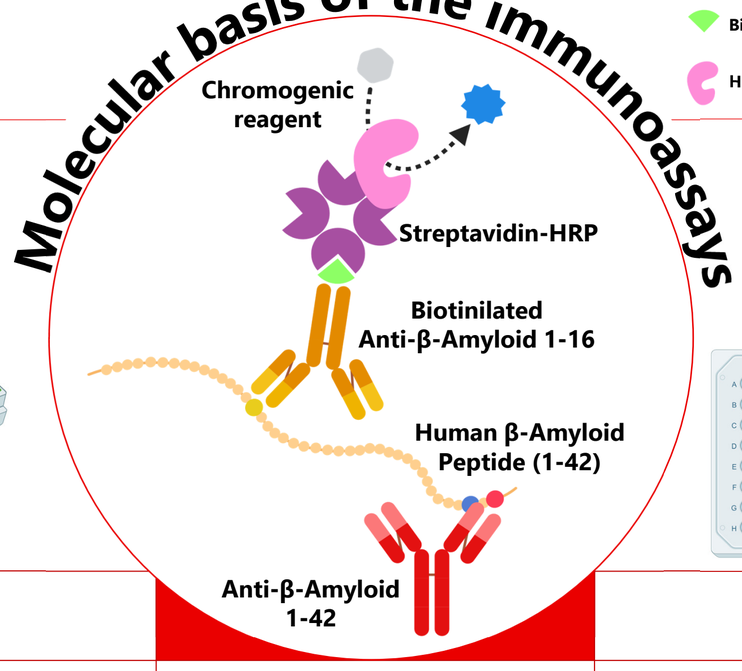


Figure 2. Schematic representation of the traditional ELISA immunoassay format[1]



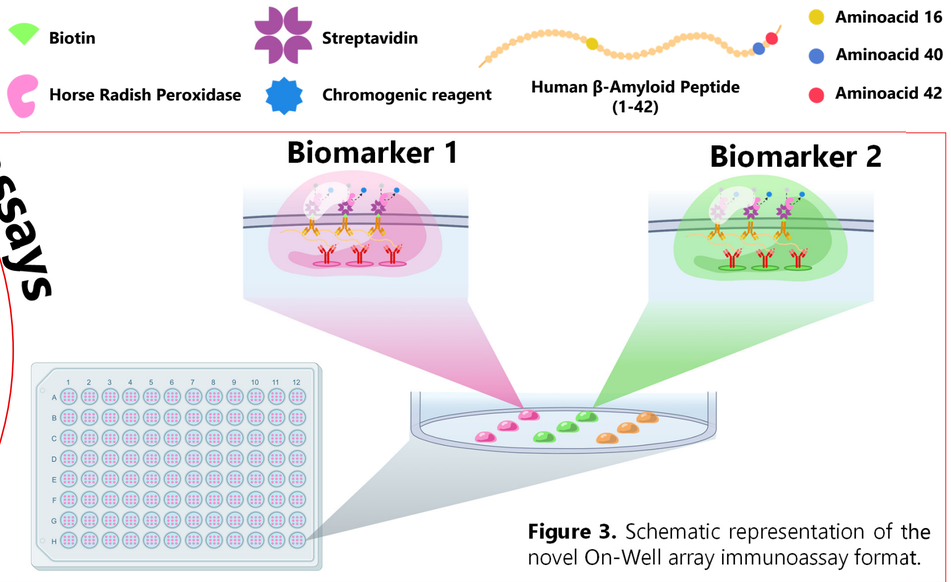


Figure 3. Schematic representation of the novel On-Well array immunoassay format.

Traditional ELISA		On-Well Immunoarray
96 per plate	**Number of assays**	864 (96 x 9) per plate
1 (x 1 replicate)	**Reagents per well**	3 (x3 replicates)
50 µL antibody / 50 µL sample	**Volume per well**	0.04 µL antibody / 25 µL sample

Results

Single-biomarker approach

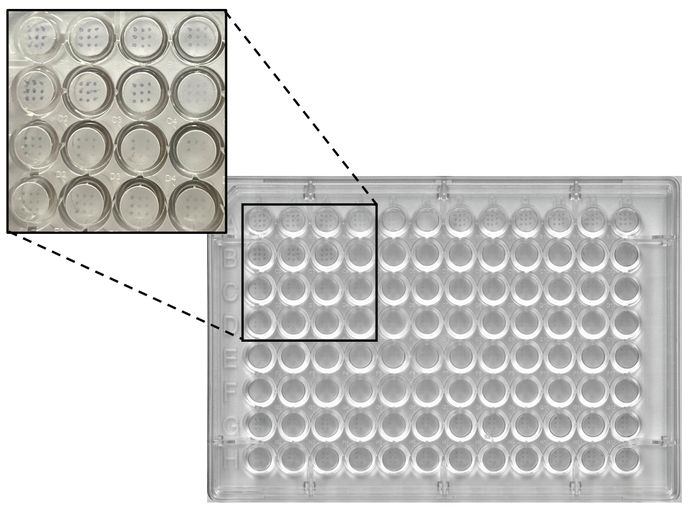


Figure 4. Naked-eye detection and zoom into the On-Well array format (nine replicates) for immunosensing of Amyloid-β peptide (1-42) using a chromogenic reagent.

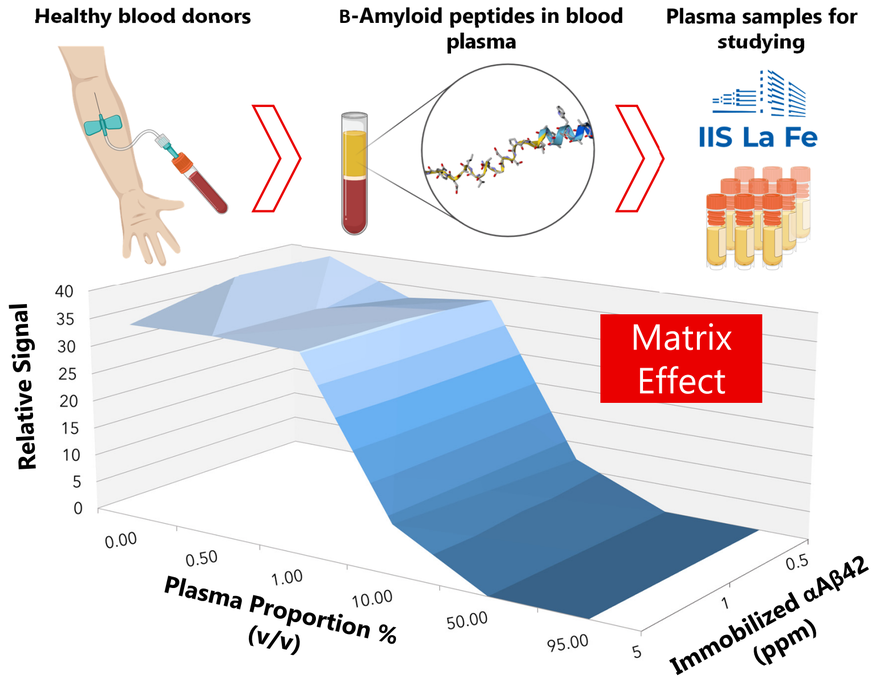


Figure 6. (Top) Schematic representation of serum samples drawn from healthy donors at IIS-La FE. **(Bottom)** Matrix effect study of blood plasma for the detection of Amyloid-β peptide (1–42) using the On-Well format at three different concentrations for the immobilized antibody(three replicates). A serum concentration of 1% (v/v) shows similar detection efficiency as a plasma-free sample. The optimal serum proportion for the On-Well array might be between 1 and 10% (v/v) of plasma.

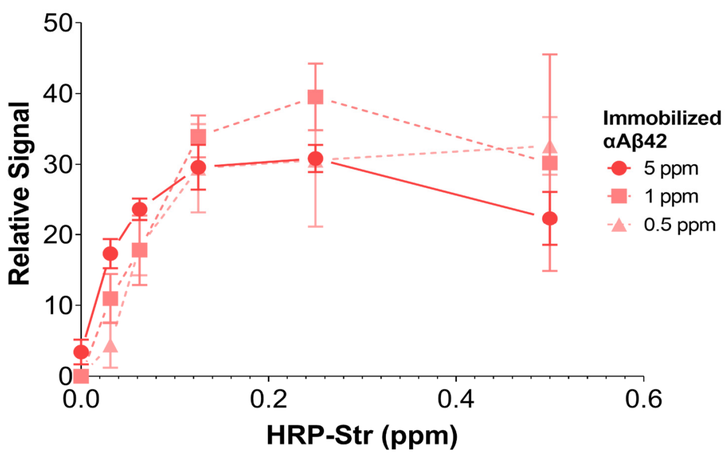


Figure 5. Optimization study for the Horse Radish Peroxidase-Streptavidin conjugate (HRP-Str) at three different concentrations of immobilized antibody for the On-Well format.

Multiplexed approach

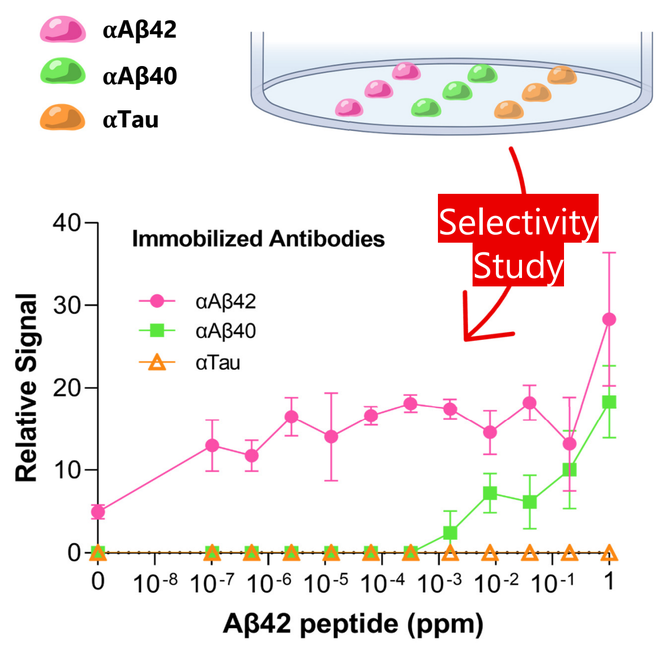


Figure 7. (Top) Schematic of the On-Well assay format for parallel testing of three different analytes using anti-Amyloid-β peptides 1-40 and 1-42 antibodies (αAβ40 and αAB42, respectively) and an anti-Tau protein antibody(αTau). **(Bottom)** Selectivity study of the multiplex assay format against Amyloid-β peptide (1-42) for the chosen antibodies, each at a concentration of 5 ppm. The On-Well multiplexed format showed good selectivity, specifically at low peptide concentrations.

Conclusions

- The preliminary results indicated a high antibody immobilization density, adequate sensitivity, and detection ability, which allows the use of minute-volume samples.
- The On-Well microarray biosensing format can simultaneously detect up to three biomarkers in a single essay, showing good selectivity at low analyte concentrations. Thus, this format offers a convenient approach to analyzing AD biomarker profiles.
- This approach further allows testing minimal-invasivity samples, such as blood plasma, reducing costs and patient discomfort associated with traditional testing methodologies.
- The On-Well format allows for cost-effective scalability, promoting its translation into the healthcare system and aiding the development of new preventive and therapeutic approaches.

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References

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