OP-21: Optimization of initial nanobiotechnology procedures to design a gold nanoparticle-based nucleic acid lateral flow assay to detect BRAF V600E mutation in papillary thyroid carcinoma

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Introduction: Lateral flow assays (LFAs) have significant applications in point-of-care detection of cancer biomarkers. Proper validation of each step in their development process is crucial in achieving the final outcome. This study aimed to develop optimised protocols to design a gold nanoparticle (AuNP)-based nucleic acid lateral flow assay (NALFA) to detect the BRAF V600E mutation for prognostication of papillary thyroid carcinoma (PTC).

Methods: Design of primers for the multiplex polymerase chain reaction (PCR) and oligonucleotide probes for LFA and optimization of multiplex PCR to amplify fragments of *BRAF* and *GAPDH* were carried out. Characterization of AuNPs, evaluation of their stability overtime and assessment of their conjugation to detection probes (DPs) were performed.

Results: PCR conditions were optimised to amplify a 255 bp fragment of *BRAF*, flanking the V600E mutation site and a 208 bp fragment of *GAPDH* flanking exon 2. A λmax of 525 nm was obtained for AuNPs indicating an average diameter of approximately 20 nm. The sharp peak and absence of secondary spectral features in the localised surface plasmon resonance (LSPR) spectrum revealed a narrow particle size distribution and good shape homogeneity. These properties were retained until two weeks post synthesis assuring stable AuNPs. Four weeks later, λmax ranged from 528-530 nm and the peak of LSPR spectrum broadened towards longer wavelengths, indicating AuNP agglomeration. The expected shift in λmax averaged 3nm, was observed for the DPs specific for *BRAF* and *GAPDH* PCR fragments when conjugated with AuNPs. This indicated successful conjugation of DPs to AuNPs.

Conclusions: Since expected results were obtained for the initial steps carried out, it is possible to proceed with determination of the hybridization conditions and subsequent analyses on sensitivity and specificity of the LFA. These findings will contribute to the development of a LFA to detect BRAF V600E mutation status of patients having PTC for efficient prognostication.

Keywords: BRAF V600E, gold nanoparticle-based nucleic acid lateral flow assay, multiplex polymerase chain reaction, papillary thyroid carcinoma