Name(s)
Tarun S. Chiruvolu

Project Number
S1304

Project Title
Point-of-Care Detection of Mutations: A Lateral Flow Assay for Detecting NSCLC in Humans

Abstract

Objectives/Goals
Lung cancer (NSCLC) causes >200,000 deaths in US annually. Mutation detection costs 1000s of dollars and takes days. Rapid, low-cost point-of-care (POC) devices for cancer mutation can aid treatments, and with new therapies reduce mortalities. Lateral flow assays (LFA) commercialized for pregnancy/glucose tests offer a low-cost option for mutation detection. Combining microfluidic flow, and precision of nucleic acid hybridization, this project aims to show T790M mutation detection in NSCLC using model oligonucleotides (ON). The goal is to develop a simple, easy to use, and repeatable LFA by validating basic streptavidin-biotin binding assay for test strip (TS) design and use it to show ON hybridization assay for point mutation detection.

Methods/Materials
Various TS designs, and >20 tests were used to optimize a 4mmx40mm strip and assay conditions on Whatman (1CHR chromatography paper). 40nm Au-nanoparticle-streptavidin (SG) reporter, biotin-bovine serum albumin (B-BSA) capture molecule, and 1xPBS wash buffer were used to show site-specific binding from 5ul of SG to B-BSA. This was applied to small oligos-wild type(Control-CO,1mM), T790M with a point mutation (Test-TO,1mM), and biotin-ON probe (PO, 1mM) complimentary to TO and CO in 1xPBS. PO was incubated with SG as reporter in the assay to bind to test(TL) and control lines(CL). Different concentrations of PO and TO were tested to identify conditions for reporter binding to TL and CL. Negative control with SG only (no PO) was used to show binding was specific. Tests were done to get repeatable results. Actual tests needed <1hr (<1min to blot PO-SG reporter, <1hr for wicking, signal).

Results
SG-B-BSA LFA successfully showed repeated high-level binding. From over 100 ON LFA tests, several showed binding to both TL and CL, but further research can fully validate the assay at lower detection limits.

Conclusions/Discussion
This project expands utility of LFA from clinic to the field by successfully mimicking a lab test and allowing mutation detection at ~0.5mM. Further development of a complimentary probe for cell's genome would enhance this method's success and utility.

Summary Statement
By detecting point mutations, this project lays foundation for advancing POC for lung cancer diagnosis and allows cross-application to other epithelial cancers or hereditary diseases, streamlining treatment methods.

Help Received
Dr. Debjani Roy for guidance, support, and parents for help with supplies, printing, board.