Fluorescent-based Lateral Flow Point of Care Detection of Cervical Cancer Biomarkers in Serum

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***Abstract*— Point-of-care (POC) diagnostics tools provide low-cost methods of disease detection in low resource settings. Our current platform detects antibodies in serum to recombinant protein biomarkers on glass substrate. Most commercial POC tests utilize nitrocellulose lateral flow format with colorimetric detection. Nitrocellulose enables fluid handling, serum filtration, better protein immobilization, rapid detection time, and affordability. Colorimetry limits sensitivity and provides only qualitative results. We aim to use fluorescent-based detection on a nitrocellulose lateral flow format to achieve higher sensitivity and semi-quantitative results to detect cervical cancer biomarkers. As a model, an EBNA-1 assay is used to evaluate nitrocellulose as a substrate for multi-step fluorescent-based detection of antibodies in serum.**

I. Introduction

Point-of-care (POC) diagnostics tools are either hand-held or benchtop devices that meet the ASSURED criteria (Affordable, Sensitive, Specific, User-friendly, Robust/Rapid, Equipment-free, Deliverable) [6]. They serve as an effective solution for providing low-cost, sensitive diagnostic tests to low resource settings. The global market for POC diagnostics was predicted to reach $27.5 billion by 2018 [4]. Thus, the objective is to develop a compatible lateral flow assay and device to read clinically significant results for detecting cervical cancer biomarkers from serum.

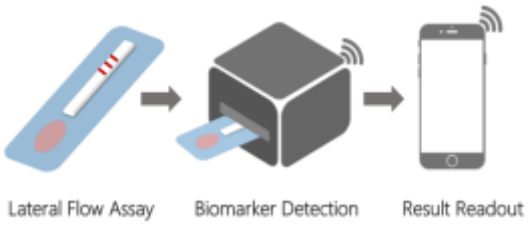

Figure 1. Proposed Diagnostic Test Model

Our POC device, shown in Figure 2, was developed to detect antibodies in serum to HPV16 biomarkers (E2, E6, E7) for cervical and head and neck cancers. The current POC model has been optimized for detecting antibodies in serum to recombinant biomarkers immobilized on a glass substrate. The POC device uses cheap green LEDs and optical filters to semi-quantitatively measure the fluorescence of captured and labeled antibodies. Captured antibodies are tagged with a fluorophore-conjugated secondary antibody, Dylight 549 conjugated goat anti-human IgG [3]. Although a glass substrate provides low or negligible auto-fluorescence, it requires blood samples to be pre-processed and provides no microfluidic advantages. Furthermore, glass requires additional disposal methods with safety hazards. Therefore, we aim to investigate nitrocellulose as an optimal substrate for enabling fluid handling and serum filtration while keeping the same or similar sensitivity for our POC test using fluorescence [1,5].

Figure 2. Current Point of Care Device

Thus, Hi Flow Plus 75 from Millipore was identified as an ideal nitrocellulose substrate for its high flow rate and transparent backing. Generally, high flow rate is associated with lower sensitivity and larger pore size in nitrocellulose membranes. Hi Flow Plus 75 has a capillary flow rate of 75 seconds to travel 4 cm. Its pore size is estimated to be 15-20 μm in diameter. Because the nitrocellulose membrane is not as optically transparent as glass, fluorophore dyes are not suitable for fluorescent detection [2]. Instead, latex microspheres filled with fluorophore dyes are used as the secondary label. 0.5 μm latex microsphere beads with excitation/emission spectrum of 555/570 nm, similar to Dylight 549, were selected as a suitable bead size to match the Hi Flow Plus 75 membrane. The latex microspheres were conjugated to goat anti-human IgG antibodies.

To initially characterize nitrocellulose as an optimal substrate for fluorescent signal detection of antibodies in serum, EBNA-1 assay experiments were conducted to determine the optimal concentration of microspheres, serum dilution, blocking conditions, and immobilized EBNA-1 antigen. Initial results present dilution curves for the serum and secondary label and indicate challenges in effectively blocking the nitrocellulose membrane to reduce nonspecific binding.

References

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