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Design of Experiment for Optimizing Immunoassay Microarray Printing

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Abstract : Immunoassays have been utilized for several applications, including the detection of pathogens. Our laboratory is in the development of a tier 1 biothreat panel utilizing Vertical Flow Assay (VFA) technology for simultaneous detection of pathogens and toxins. One method of manufacturing VFA membranes is with non-contact piezoelectric dispensing, which provides advantages, such as low-volume and rapid dispensing without compromising the structural integrity of antibody or substrate. Challenges of this processinclude premature discontinuation of dispensing and misaligned spotting. Preliminary data revealed the Yp 11C7 mAb (11C7)reagent to exhibit a large angle of failure during printing which may have contributed to variable printing outputs. A Design of Experiment (DOE) was executed using this reagent to investigate the effects of hydrostatic pressure and reagent concentration on microarray printing outputs. A Nano-plotter 2.1 (GeSIM, Germany) was used for printing antibody reagents ontonitrocellulose membrane sheets in a clean room environment. A spotting plan was executed using Spot-Front-End software to dispense volumes of 11C7 reagent (20-50 droplets; 1.5-5 mg/mL) in a 6-test spot array at 50 target membrane locations. Hydrostatic pressure was controlled by raising the Pressure Compensation Vessel (PCV) above or lowering it below our current working level. It was hypothesized that raising or lowering the PCV 6 inches would be sufficient to cause either liquid accumulation at the tip or discontinue droplet formation. After aspirating 11C7 reagent, we tested this hypothesis under stroboscope.75% of the effective raised PCV height and of our hypothesized lowered PCV height were used. Humidity (55%) was maintained using an Airwin BO-CT1 humidifier. The number and quality of membranes was assessed after staining printed membranes with dye. The droplet angle of failure was recorded before and after printing to determine a “stroboscope score” for each run. The DOE set was analyzed using JMP software. Hydrostatic pressure and reagent concentration had a significant effect on the number of membranes output. As hydrostatic pressure was increased by raising the PCV 3.75 inches or decreased by lowering the PCV -4.5 inches, membrane output decreased. However, with the hydrostatic pressure closest to equilibrium, our current working level, membrane output, reached the 50-membrane target. As the reagent concentration increased from 1.5 to 5 mg/mL, the membrane output also increased. Reagent concentration likely effected the number of membrane output due to the associated dispensing volume needed to saturate the membranes. However, only hydrostatic pressure had a significant effect on stroboscope score, which could be due to discontinuation of dispensing, and thus the stroboscope check could not find a droplet to record. Our JMP predictive model had a high degree of agreement with our observed results. The JMP model predicted that dispensing the highest concentration of 11C7 at our current PCV working level would yield the highest number of quality membranes, which correlated with our results. Acknowledgements: This work was supported by the Chemical Biological Technologies Directorate (Contract # HDTRA1-16-C-0026) and the Advanced Technology International (Contract # MCDC-18-04-09-002) from the Department of Defense Chemical and Biological Defense program through the Defense Threat Reduction Agency (DTRA).

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