

# Development of Paper-Based Origami Biosensor Platform for Microorganism Monitoring

ShT-05.1-2

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The global burden of bacterial pathogens underscores the critical need for accessible and efficient diagnostic tools. While current methods offer high sensitivity, they are often economically demanding and practically complex, which particularly impacts resource-limited regions. Point-of-care (POC) approaches that meet ASSURED (Affordable, Sensitive, Specific, User-friendly, Rapid, Equipment-free, Deliverable) criteria are essential for rapid detection and intervention. Incorporating origami into paper-based biosensing platforms presents an innovative solution, offering affordability, portability, and ease of disposal. Herein, a colorimetric paper-based origami biosensing platform suitable for use in POC applications was developed. The platform was constructed via laser ablation utilizing polyvinylidene fluoride (PVDF) and cellulose membranes that constitute specific primary and secondary antibodies. In order to develop the biosensor platform, optimization of fabrication parameters and PVDF hydrophilization was carried out first. Imaging of the fabricated platforms via light microscopy, wettability analyses, protein adsorption assay, and contact angle measurements were done for characterizing throughout these optimization steps. Then, optimization of reagent amounts was carried out in terms of improving sensory characteristics, utilizing Box-Behnken experimental design. The responses generated by the biosensor in form of visible color development were then analyzed using image processing via MATLAB 2018b. The developed platform was validated against E. coli B21 strain and was calculated to have a limit of detection (LOD) of 2 CFU/mL and a dynamic working range up to 10<sup>6</sup> CFU/mL. Overall, developed biosensor platform offers a detection method that has an economic advantage compared to conventional methods, and provides rapid and sensitive results without the requirement of expertise or complex equipment.