Integrated Approach for Low-Cost and Sensitive Multiplex Pathogen Detection on 3D Paper-Based Microfluidic Devices

Alejandra Valadez  
Department of Chemistry, University of Texas at El Paso, avvaladez@miners.utep.edu

Huan Hu  
Department of Chemistry, Shantou University, 09hhu@stu.edu.cn

XiuJun James Li  
Department of Chemistry, University of Texas at El Paso, xli4@utep.edu

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Integrated Approach for Low-Cost and Sensitive Multiplex Pathogen Detection on 3D Paper-Based Microfluidic Devices

Alejandra Valadez, Huan Hu, and XiuJun James Li

Recently, pathogenic microorganisms have aroused wide public concern for their potential to cause serious diseases in humans, animals and agricultural crops. Although DNA microarrays are commonly used for pathogen detection, the approach is sophisticated, time-consuming and requires expensive equipment, limiting its application in resource-poor settings. Herein we present a novel, low-cost, paper-based microfluidic approach for multiplexed DNA detection of Giardia Lamblia, S. aureus, and Entamoeba, by “on-chip” isothermal DNA amplification and hybridization. Paper-based microfluidics offers a novel, sensitive and low-cost alternative for Point-of-Care Diagnostics for the Developing Countries. The project described utilizes Loop-mediated Isothermal Amplification (LAMP), a novel isothermal DNA amplification method using six primers to amplify a target DNA sequence specific to each pathogen. ssDNA probes covalently-immobilized at three separate detection zones then bind their complementary CY3-labeled amplification products. Currently, the flow speed in our paper-based device has been optimized to reach the detection zones quickly after amplification and with as little as 15 µl of reagents. The integration of both the amplification and hybridization steps in one multiplexed paper device will allow for on-site testing of pathogens with minimal device fabrication costs, less reagent consumption, no need for expensive laboratory equipment, and minimal expertise requirements to retrieve highly-sensitive genetic testing. As such, our paper-based microfluidic device will potentially bring sophisticated DNA testing to people in regions with limited healthcare infrastructure and adequate laboratory facilities, but with a high demand for in situ diagnosis and treatment.

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