Development of an Integrated Paper-based Molecular Diagnostic Platform

Manoharaneru Branavan1 and Wamadeva Balachandran1

1 Corresponding Author: Tel: +44 (0)1895 268459 Email: eestmmmb@brunel.ac.uk
Centre for Electronic Systems Research (CESR), College of Engineering, Design and Physical Sciences, Brunel University London, Uxbridge Middlesex UB8 3PH, UK

Abstract

Point-of-Care testing (POCT) devices have received immense attraction due to its simplicity, ease of use and swiftness. Microfluidic paper-based analytical devices (μPADs) are directly aimed for POC testing. Molecular diagnostics result in a more specific and sensitive assay than immunoassays with much smaller window time to diagnosis. Realizing molecular diagnostics on paper-based devices is a challenge that has been little addressed. A chitosan functionalyzed paper-based nucleic acid extraction method, isothermal amplification of Chlamydia trachomatis on paper-based devices, and endpoint detection on a nucleic acid lateral flow test strips are presented here. In addition, a proprietary integrated paper-based diagnostic device developed to perform lystsae-in-to-answer-out in less than an hour with a limit of detection of 0.2 copies/µL and ongoing studies to realise a complete system suitable for resource limited settings are also presented. The integrated device is capable of performing affordable, sensitive and specific, and equipment free molecular diagnostic assays in just 6 easy sequential processes.

DNA extraction and purification

DNA extraction on a chitosan functionalyzed μPAD (paper strip). A) Negative control B) FAM tagged DNA sample (pH 5.0) captured onto the chitosan modified region C) Elution of DNA sample with the introduction of pH 9.0 Tris/KCl buffer.

Positive control of RPA basic amplification assay on paper with end-point detection on 3% gel. Triplicate of negative controls show no amplification while only the positive control shows bands of 143 bp

DNA amplification & Detection

A) A novel and simple method to integrate NALF based detection was determined.
B) 0.2 copies of CT gDNA/µL of spiked buffer sample was extracted on a chitosan membrane, amplified and detected on an NALF to establish proof-of-principle of the integrated paper-based molecular diagnostic device being developed at Brunel University, London.

Integration & Platform Development

Conclusions and Future Work

Acknowledgements:
The authors would like to thank STI2 for funding the eX2 project, grant number G30010388

References: