Development of an Integrated Paper-based Molecular Diagnostic Platform

Manoharanehru Branavan[1*] and Wamadeva Balachandran[1]

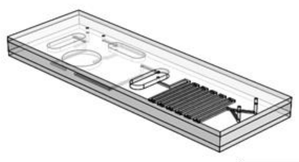
[1*] Corresponding Author: Tel: +44 (0)1895 268459 Email: eestmmb3@brunel.ac.uk

Centre for Electronic Systems Research (CESR), College of Engineering, Design and Physical Sciences, Brunel University London, Uxbridge Middlesex UB8 3PH. UK

Abstract

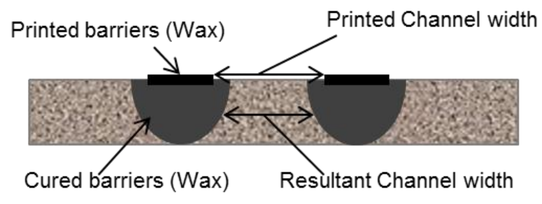
Point-of-Care testing (POCT) devices have received immense attraction due to its simplicity, ease of use and swiftness. Microfluidic paper-based analytical devices (µPADs) are directly aimed for POC testing. Molecular diagnostics result in a more specific and sensitive assay than immunoassays with much smaller window time to diagnosis. Realizing molecular diagnostics on paper-based devices is a challenge that has been little addressed. A chitosan functionalized 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 lysate-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.

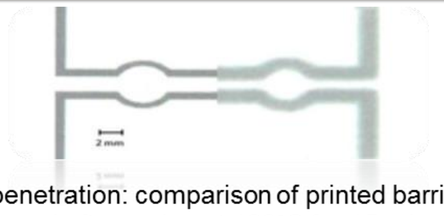
Lateral flow devices



Microfluidics

Paper-based devices

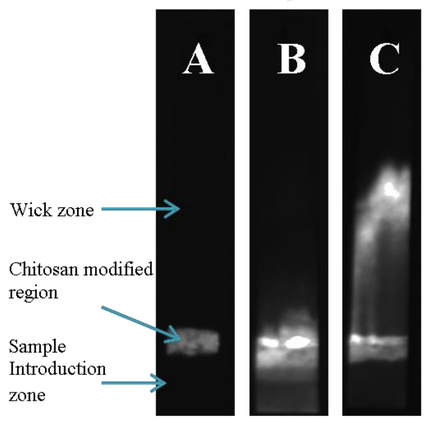




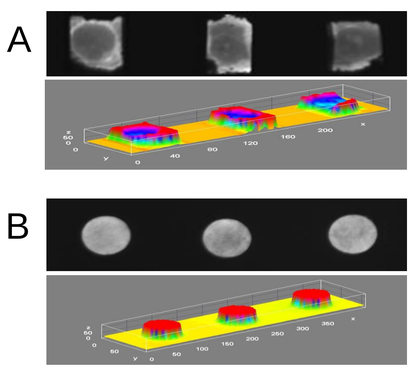
Wax penetration: comparison of printed barriers before and after curing at 120°C for 15 minutes

Modular Development of the platform

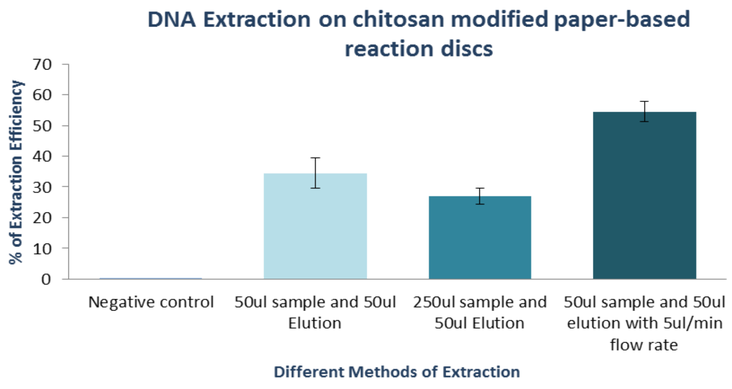
DNA extraction and purification



DNA extraction on a chitosan functionalised 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.

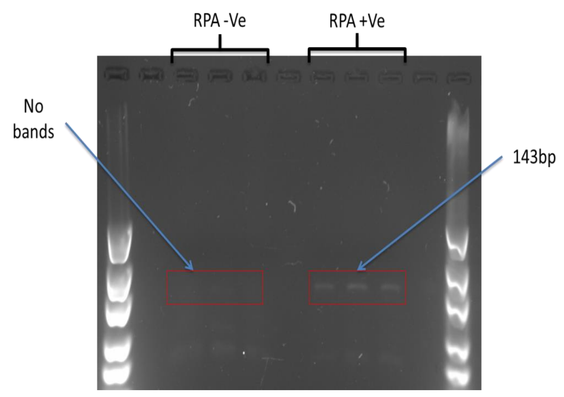


Chitosan functionalised regions of A) pipette deposited patterning of paper strips and B) Dip-coated paper reaction discs and the corresponding three dimensional topography of the chitosan modified regions.



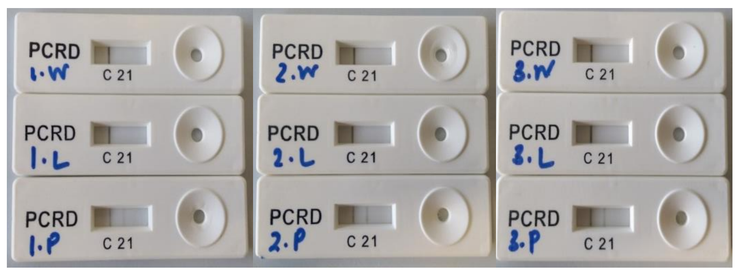
- Adjusting the flowrate increased the extraction efficiency to 57%. This can be considerably increased by fine tuning the parameters.

DNA amplification & Detection

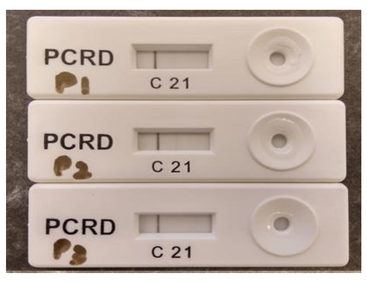


Positive control of RPA basic amplification assay on paper with end-point detection on 3% gel. Triplicate of negative controls show no amplification while only the positive control shows bands of 143 bp

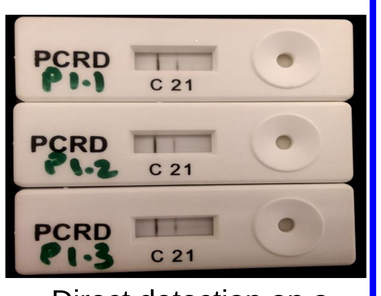
- A paper/plastic hybrid device was used to perform RPA of CT gDNA.
- To ensure the reaction only happens within the paper matrices, MgAc and sample were added to the paper and air-dried prior to the addition of master mix.



RPA of CT gDNA on paper with end-point detection on NALF devices. The plastic support that held the membranes were also washed with the LF running buffer and tested to determine amplification only proceeded within paper matrices



Direct detection on a NALF device of a negative control.



Direct detection on a NALF device of 10copies/µl CT gDNA.

Integration & Platform Development

Our approach to integrated paper-based molecular diagnostic device development allows equipment free molecular diagnostic assays in just 6 easy sequential processes

1. **Sample collected in a proprietary device under development**
2. **Cell lysis performed within the sample collection device**
3. **Lysate delivered to paper-based diagnostic device**
4. **DNA extraction, amplification and**
5. **Detection performed on paper-based device**
6. **Smartphone can be utilized to quantify NALF result**

Conclusions and Future Work

- A novel and simple method to integrate NALF based detection was determined.
- 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.
- Sample preparation device with integrated cell lysis is under development, and preliminary studies show the device can efficiently collect 4ml of first-pass urine.
- A batch of integrated paper-based molecular diagnostic devices are being manufactured at Brunel for an in-depth experimental validation of the assay protocols and device design.

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