A PAPER-POLYMER CENTRIFUGAL DEVICE FOR LOW-COST SAMPLE PRE-CONCENTRATION AND COLORIMETRIC LATERAL FLOW ASSAY ENHANCEMENT

M.S. Wiederoder[1,2], S. Smith[1], P. Madzivhandila[1], D. Mager[3], K. Moodley[1], D.L. DeVoe[2], and K.J. Land[1*]

1Council for Scientific and Industrial Research, Pretoria, SOUTH AFRICA and
2University of Maryland, College Park, USA
3 Karlsruhe Institute of Technology, GERMANY

ABSTRACT

This study describes a novel hybrid paper-polymer centrifugal microfluidic device for pre-concentration of *E. coli* and lateral flow immunoassay enhancement for water quality verification. The device balances rotational centrifugal force with the capillary force of paper to manipulate fluid flow. The device functions as a low-cost centrifuge to concentrate bacteria before supernatant removal by paper inserts. In addition, the paper acts as a colorimetric lateral flow immunoassay where sample pre-concentration and rotational speed manipulation enhance optical signal. The techniques yield a 10× reduction in detection limit over a similar manual, dipstick-based assay.

KEYWORDS: Lateral Flow Immunoassay, Centrifugal Microfluidics, Paper Microfluidics

INTRODUCTION

Paper-polymer hybrid centrifugal microfluidic devices manipulate fluid flow by balancing rotational centrifugal force with capillary forces of paper inserts for applications such as chromatography [1], plasma extraction and analysis [2], and timing valves [3]. Here we demonstrate a hybrid device that employs rotational centrifugal force for bacteria concentration before rotational speed reduction facilitates supernatant removal via capillary force by paper inserts for low-cost centrifugation. The study also demonstrates lateral flow immunoassay optical signal enhancement through pre-concentration of bacteria with gold nanoparticle immunoconjugates (AuNPs) and rotational speed variation to increase sample residence time. While Kim et al. demonstrated paper based DNA detection in a centrifugal device [4], this is the first known immunoassay demonstrated.

EXPERIMENTAL

The constructed device consists of three polycarbonate (PC) layers, two interstitial layers of pressure sensitive adhesive (PSA), Whatman 1 paper inserts, and absorbent cellulose pads. The PC is milled, the PSA and paper are laser cut, and the cellulose is manually diced before pressing layers together with a roller (Fig. 1). The paper inserts are 1 mm wide at the fluid interface to remove supernatant while preserving a concentrated droplet that is mixed and resuspended by axial fluid motion during deceleration. *E. coli* dilutions spun at 6000 rpm in a commercial centrifuge or the disc device were enumerated on agar (Fig. 2). The effect of pre-concentration and rotational speed on lateral flow assay performance with Whatman 1 paper assays was also examined. Dilutions of *E. coli* O157:H7 with AuNPs were pre-concentrated using a commercial centrifuge, incubated, and resuspended in buffer. Next Whatman paper lateral flow devices (Fig. 3A) were dipped into this solution and a control incubated without centrifugation, dipped in a rinsing buffer, and imaged with a

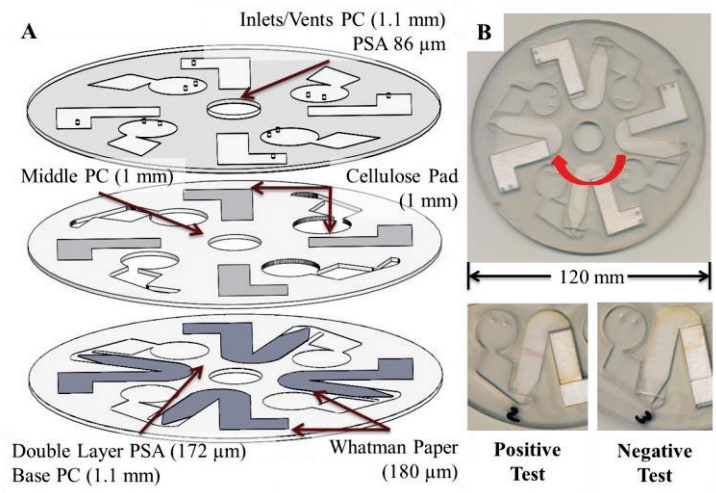
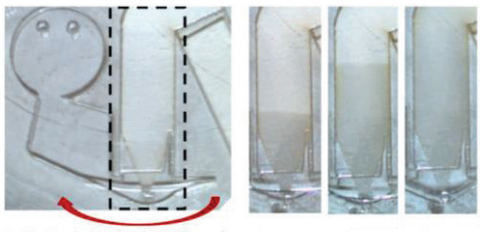


Figure 1: (A)Hybrid device consists of polycarbonate (PC) and pressure sensitive adhesive (PSA). (B) Completed device shown with positive and negative colorimetric lateral flow immunoassays.

commercial reader to determine relative absorbance of the test line. To examine rotational speed effects a 10^7 CFU/mL sample was incubated with AuNPs for 10 min, introduced to the paper assay from 0 to 600 rpm, rinsed with buffer, imaged on a flatbed scanner, and analyzed with Image J to determine relative absorbance (Fig. 3B).



Concentration (CFU/mL)	Capture Efficiency		Concentration Factor	
	Centrifuge	*Hybrid Device*	*Centrifuge*	*Hybrid Device*
10^8	43.4%	28.5%	7.10	5.09
10^7	39.1%	24.7%	6.66	4.62

Figure 2: E. coli concentration procedure on disc with live bacteria capture efficiency and concentration factor using commercial centrifuge or hybrid device.

RESULTS AND DISCUSSION

Live bacteria capture efficiency was 39-43% with a commercial centrifuge and 25-29% in the hybrid device with a bacteria concentration increase of 6-7× and 4-5× respectively in the collected droplet (Fig. 2). This suggests similar performance, with bacteria loss in the hybrid device likely due to absorption into the paper during initial acceleration and incomplete fluid extraction.

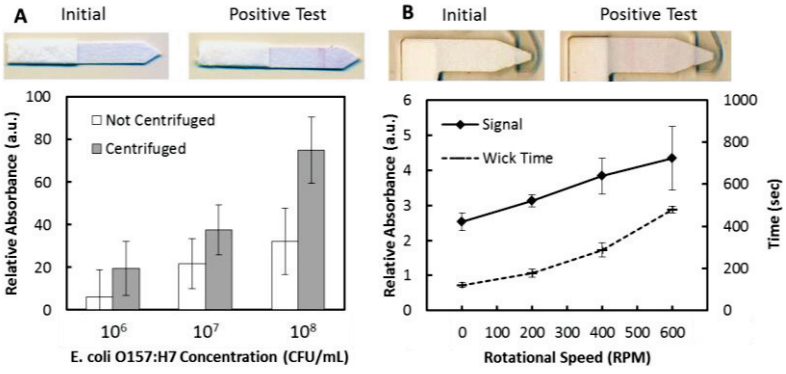


Figure 3: (A) The peak absorbance of lateral flow immunoassays for E. coli O157:H7 with and without pre-concentration. (B) Effect of rotation speed on wicking time and relative absorbance. Initial and positive test shown for each study with n=3 and error bars ± 1 SD.

For dilutions of *E. coli* O157:H7 pre-concentrated with AuNPs the detection limit was 10^6 CFU/mL, a 10× reduction over the non-centrifuged sample with a greater average signal for each dilution tested (Fig. 3A). Pre-concentration improves binding efficiency to increase AuNP capture and signal amplitude. As rotational speed increased during sample infusion into the paper the wicking time increased (Fig. 3B). The flow rate reduction increased the residence time of bacteria-AuNP aggregates on the test line resulting in more captured species and a greater signal.

CONCLUSION

The results demonstrate novel applications of paper-polymer hybrid centrifugal devices for low-cost centrifugation and colorimetric lateral flow assay enhancement through pre-concentration and sample residence time increase. Future work will focus on optimizing assay parameters and reagent integration to achieve low-cost *E. coli* detection for water quality testing in resource limited settings.

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CONTACT

* K.J. Land; phone: +27-12-841-3065; KLand@csir.co.za