PAPER MICROFLUIDIC EXTRACTION OF BACTERIAL AND VIRAL NUCLEIC ACID FROM FIELD AND CLINICAL SAMPLES TOWARDS A DIRECT MICROTAS APPARATUS

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ABSTRACT

We investigated a rapid, paper microfluidic-based protocol for extracting nucleic acid from a variety of pathogenic samples and developed a multi-faceted paper channel to test for optimal extraction conditions. *Salmonella* Typhimuirum and Influenza A/H1N1 (seasonal) samples were loaded on the paper strips and were lysed. TE buffer was loaded for elution, and three sections of paper at specified lengths were excised for elution. The limit of detection of *S.* Typhimuirum in 10% poultry packaging liquid extracted in cellulose paper was 10^3 CFU/mL, which was significantly lower than that of 10^6 CFU/mL when extracted in nitrocellulose paper. We observed that DNA migrates through nitrocellulose at a higher rate and further than through cellulose, and larger genomic DNA separates from smaller plasmid DNA in both paper types.

KEYWORDS: Direct detection, Influenza A, Salmonella, cellulose, nitrocellulose.

INTRODUCTION

There is a need to more effectively detect common pathogens in food, water, and air. Much research has focused on early detection of pathogens while maximizing sensitivity and operation complexity. Currently, the gold standard in determining the presence of a specific pathogen is to detect its specific nucleic acid sequence. However, because extraction protocols generally yield tiny amounts of nucleic acid, amplification is necessary through polymerase chain reaction (PCR). In order to reduce assay time, various miniaturized and automated apparatuses have been created, such as microdroplet manipulation [1] and microfluidic [2] devices. In addition, cell culture has been integrated into PCR protocols in order to increase the initial nucleic acid yields, thus reducing the number of cycles necessary for pathogen detection [3]. However, these methods focus on amplification and do not account for laborious sample and assay preparations. Therefore, a more simplistic, user-friendly, and quick apparatus must be established that allows for easy and sensitive pathogen detection. Nucleic acid sequences should be targeted due to their specificity and abundance, and a simple extraction device would allow for fast sample preparation. In this study, we develop an easy-to-use paper microfluidic chip to isolate genomic nucleic acid from both *Salmonella* Typhimurium and seasonal Influenza A/H1N1 for PCR and propose a one-step direct detection apparatus using a smartphone as an optical detection system.

EXPERIMENTAL

Microfluidic channels were patterned on paper using a lithography patterning technique with UV development [4]. Cellulose and nitrocellulose strips were immersed in Su-8 negative photoresist and were UV-exposed with a mask printed on clear transparency paper. The paper chip was rinsed, and the resulting hydrophilic channel consisted of a square loading site (7 mm × 7 mm), a channel for filtration (4 mm × 30 mm) and an absorbent pad. Aliquots of cultured *S.* Typhimurium (some spiked in 10% poultry packaging) and seasonal Influenza A/H1N1 were loaded on the paper strips at a specified location (Fig. 1a), and TE buffer (pH 8.0) was loaded for lysis (Fig. 1b).

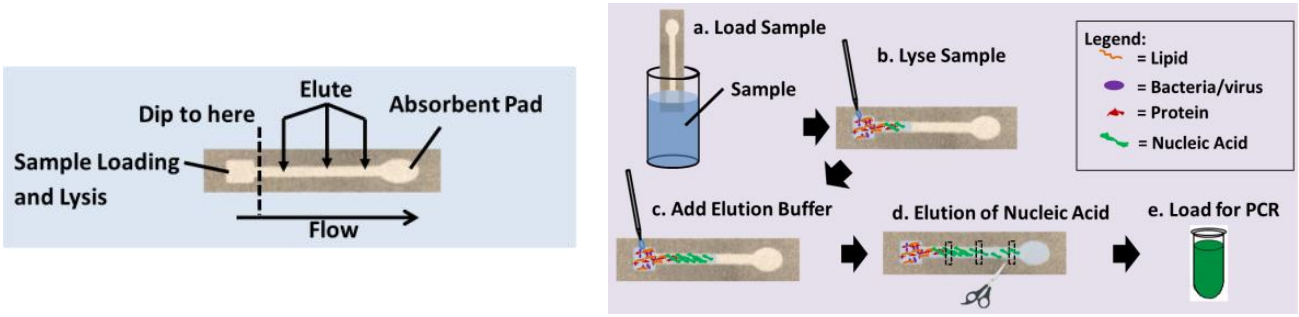

Figure 1. Left: patterned cellulose channel using modified lithography. Elution regions are equally spaced. Right: overall sample preparation process: (a) load sample on paper; (b) add TE lysis buffer and incubate for 5 minutes; (c) add TE elution buffer; (d) elute nucleic acid for PCR from three regions by cutting the paper; (e) load nucleic acid in pre-mixed solution of PCR reaction. The flow shows theoretical separation of various components within paper.

Following a 5 minute incubation period, TE buffer was loaded for elution (Fig. 1c), and three sections of paper at specified lengths were excised for elution (Fig. 1d). 1 mL syringes were used to elute DNA and RNA from this paper. For viral samples, cDNA was synthesized from eluted RNA using a high capacity RT kit with random hexamer primers. For PCR, bacterial genomic DNA and viral cDNA were amplified using 2X Promega Green Master Mix, and PCR was

run for 35 cycles. The following primer sets were used to target the *S.* Typhimurium genome: sal201F (5'-CGGGCCTCTTGCCATCAGGTG-3') and sal597R (5'-CACATCCGACTTGACAGACCG-3'). The H1N1 M gene was targeted with the following specific primer sets: MP-39-67For (5'-CCMAGGTCGAAACGTAYGTTCTCTCTATC-3') and MP-183-153Rev (5'-TGACAGRATYGGTCTTGTCTTTAGCCAYTCCA -3'). Standard gel electrophoresis with ethidium bromide staining was used for amplicon determination. A power supply (120 V and 0.1 A) provided voltage, and the gel was stained and imaged in a UV gel documentation system.

RESULTS AND DISCUSSION

Experimental data showed that the limit of detection of *S.* Typhimuirum in 10% poultry packaging liquid extracted in cellulose paper was 10^3 CFU/mL (Fig. 2d), which is significantly lower than that of 10^6 CFU/mL when extracted in nitrocellulose paper (Fig. 2a). In addition, we observed that DNA migrates through nitrocellulose at a higher rate and further than through cellulose (Fig. 3a,b), and it is known that nucleic acid binds more specifically to cellulose than to nitrocellulose [5]. We were also able to extract concentrated influenza virus from both paper types (Fig. 3c).

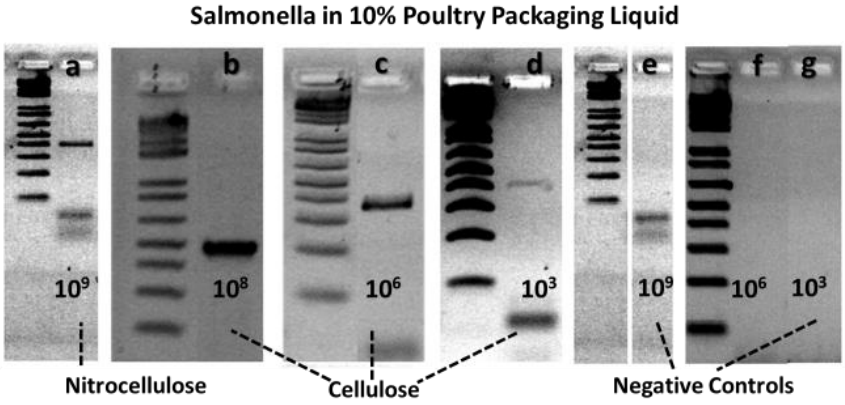

Figure 2. Gel electrophoresis showing amplicons from various concentrations (in CFU/mL) of S. Typhimurium in 10% poultry packaging liquid on nitrocellulose and cellulose paper strips. Parallel tests run in centrifugation tubes, with no extraction, served as negative controls. Expected product length was 400 bp.

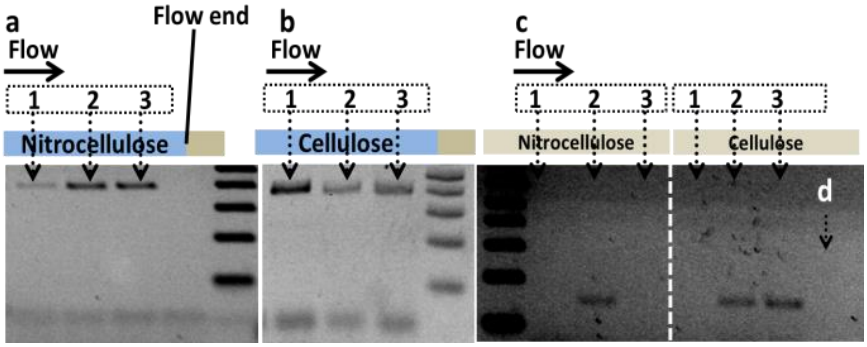

Figure 3. Gels showing amplicons from 10^6 CFU/mL S. Typhimurium in 10% poultry packaging liquid extracted from nitrocellulose (a) and cellulose (b) strips. These experiments illustrates the movement of large target DNA (4.86 Mbp) was faster through nitrocellulose compared to cellulose. Similarly, (c) shows the clean amplicon of seasonal Influenza A viral RNA extracted in both paper types, showing similar trend. Number 1-3 correspond to elution location on the paper strips, and (d) is the negative control for the virus. Expected product size of the viral RNA product was 165 bp.

We determined that larger genomic DNA separates from smaller plasmid DNA in both paper types (Fig. 4).We hypothesize that nitrocellulose is more effective in isolating larger nucleic acid, at the cost of detection limit, while cellulose is more effective in isolating smaller targets.

Given our findings, we designed a multiplexed, sensitive, rapid multimodal paper microfluidic platform to selectively extract specific DNA and RNA from various samples, which can be combined with microdrop or microfluidic PCR device, towards a field-deployable assay (Fig. 5). Direct detection of pathogens can be attempted from paper microfluidics, using a smartphone, through targeting the abundant rRNA within the pathogens.

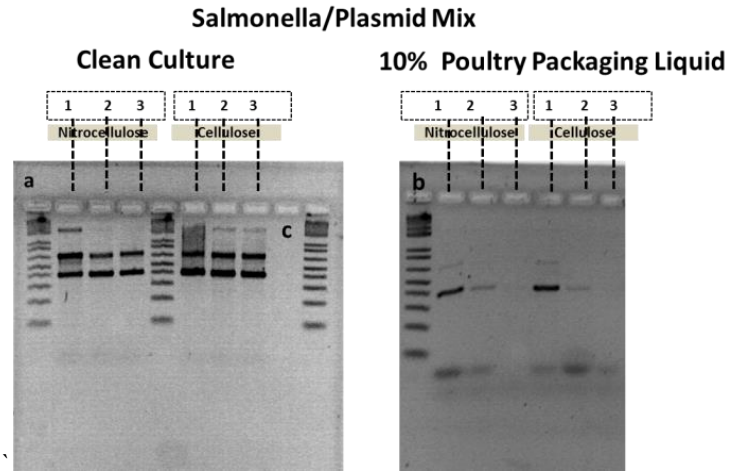

Figure 4. Experiments demonstrating the separation of large bacterial genomic DNA (S. Typhimurium, 4.86 Mbp) and small plasmid DNA (1.517 kbp) in cellulose and nitrocellulose paper. Gels show amplicons of mixture in clean culture (a) and in 10% poultry packaging liquid (b). Expected E. coli amplicon size was 400 bp for Salmonella and 1.2 kbp for plasmid, and the gels show an unexpected band at 600 bp. (c) shows a negative control.

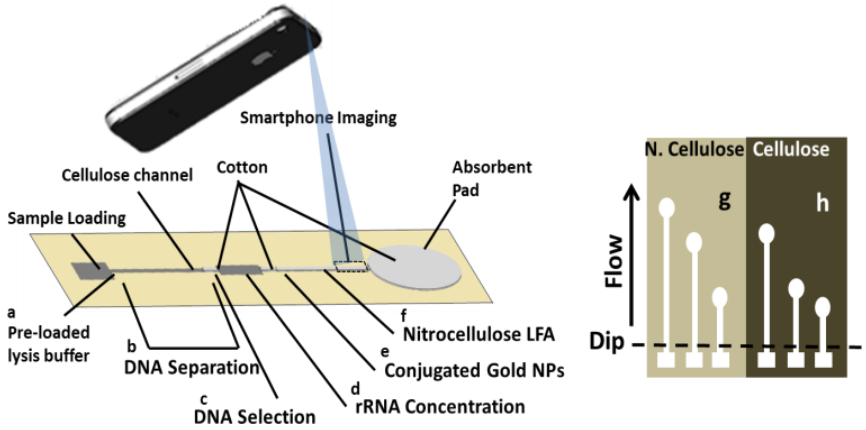

Figure 5. A microTAS paper microfluidic device for the direct detection of bacterial and viral genetic material. Samples will be loaded as shown and introduced to pre-loaded lysis buffers (a). Once the nucleic acid is isolated from other proteins (b), it will be selectively filtered in a cotton membrane containing specialized ligands (c). From there, the target nucleic acid will be denatured to reveal prevalent rRNA, which will be concentration at a centralized cellulose pad (d). The rRNA will then bind oligonucleotide-bound gold nanoparticles in a filtration cotton pad (e) and will enter a nitrocellulose lateral flow assay (LFA) (f). Downstream, control and test bands will be visible, and a smartphone in a cassette will measure the band intensity at an optimal angle. (g and h) show a possible schematic of a chip containing six channels, one segment nitrocellulose and the other cellulose, to best determine extraction conditions for a single sample.

REFERENCES:

1. D.J. You, P.L. Tran, H.J. Kwon, D. Patel and J.Y. Yoon, *Very quick reverse transcription polymerase chain reaction for detecting 2009 H1N1 influenza A using wire-guide droplet manipulations*, Faraday Discuss., 149, 159-170 (2011).
2. M.U. Kopp, A.J. de Mello and A. Manz, *Chemical amplification: continuous-flow PCR on a chip*, Science, 280, 1046-1048 (1998).
3. D. Jonas, M. Speck, F.D. Daschner, and H. Grundmann, *Rapid PCR-Based Identification of Methicillin-Resistant Staphylococcus aureus from Screening Swabs*, J. Clin. Microbiol., 40, 1821-1823 (2002).
4. A.W. Martinez, S.T. Philips, E. Carrilho, S.W. Thomas, H. Sindi and G.M. Whitesides, *Simple telemedicine for developing regions: Camera phones and paper-based microfluidic devices for real-time, off-site diagnosis*, Anal. Chem. 80, 3699-3707 (2008).
5. V. Munster, A. Wallensten, B. Olsen, G.F. Rimmelzwaan, A.D.M.E. Osterhaus, and R.A.M. Fouchier, *Influenza A Virus Surveillance in Wild Birds*, In *Avian Influenza: Prevention and Control*; Schrijver, R.S., Koch, G., Eds.; Springer: Dordrecht, The Netherlands, pp. 25–30 (2005).

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