SMARTPHONE DETECTION OF *ESCHERICHIA COLI* FROM WASTEWATER UTILIZING PAPER MICROFLUIDICS

Tu San Park, Dustin K. Harshman, Christopher F. Fronczek and Jeong-Yeol Yoon
The University of Arizona, USA

ABSTRACT

Smartphone detection of *E. coli* from wastewater was successfully demonstrated using paper microfluidics. Three channels of each paper chip were pre-loaded with BSA-conjugated and anti-*E. coli*-conjugated beads with varying amount, for negative control, low- and high-range *E. coli* detection, respectively. Dipping a paper chip into wastewater (or pipetting) led *E. coli* antigens to travel through paper fibers while effectively filtering out contaminants. *E. coli* antigens caused immunoagglutination of antibody-conjugated beads (but not BSA-conjugated beads) that were still confined within the paper fibers. This immunoagglutination was quantified by evaluating Mie scattering from the digital images taken at an optimized angle and distance with a smartphone. The detection limit was single-cell-level and the total assay time was less than one minute.

KEYWORDS: Escherichia coli, Microfluidics, Paper, Particle immunoagglutination, Smartphone, Waste water

INTRODUCTION

Sensitive and specific detection of pathogens from wastewater is essential for water treatment/reuse applications. These assays should be made portable, near-real-time, and easy-to-use. Most biological assays, including antibody-based immunoassays, polymerase chain reaction, and bacterial culture, are sensitive and specific, but lengthy, difficult to perform, and requires laboratory environment [1]. Recently, portable and near-real-time methods have been emerged, which measures the turbidity or light scattering from the particulate matter in wastewater [2], or utilizes the colorimetric assays with enzymes (e.g., to measure NADH) [3]. These new methods obviously lack the necessary specificity, and often its sensitivity is inferior to the biological assays. In addition, most assays greatly suffer from the "dirty" nature of wastewater, resulting in augmented level of noise. In this work, we use 1) paper microfluidics that effectively filters the contaminants from wastewater, 2) channels pre-loaded with antibody-conjugated submicrobeads to conduct particle immunoagglutination assay, which has been proven to demonstrate single-cell-level detection [4], and 3) utilizing smartphone for sensitive angle-specific detection of Mie scatter from immunoagglutinated beads.

EXPERIMENTAL

Wastewater samples (Fig. 1a) were collected from 3 different fish ponds, mountain runoff water, and a reservoir, all in Tucson, Arizona, USA. Additionally, the samples were also incubated at 37°C for 20 hours to prepare high concentration samples. A series of *Escherichia coli* K12 solutions (10 to 10^5 CFU/mL) were prepared from lyophilized cell powder (fully incubated then serially diluted) to construct a standard curve. A 1% solution of Tween 80 was added to each diluted solution to lyse the bacterial membrane [5]. The multi-channel paper microfluidics (hereafter paper chip) was fabricated using cellulose chromatography paper and SU-8 photoresist [6].

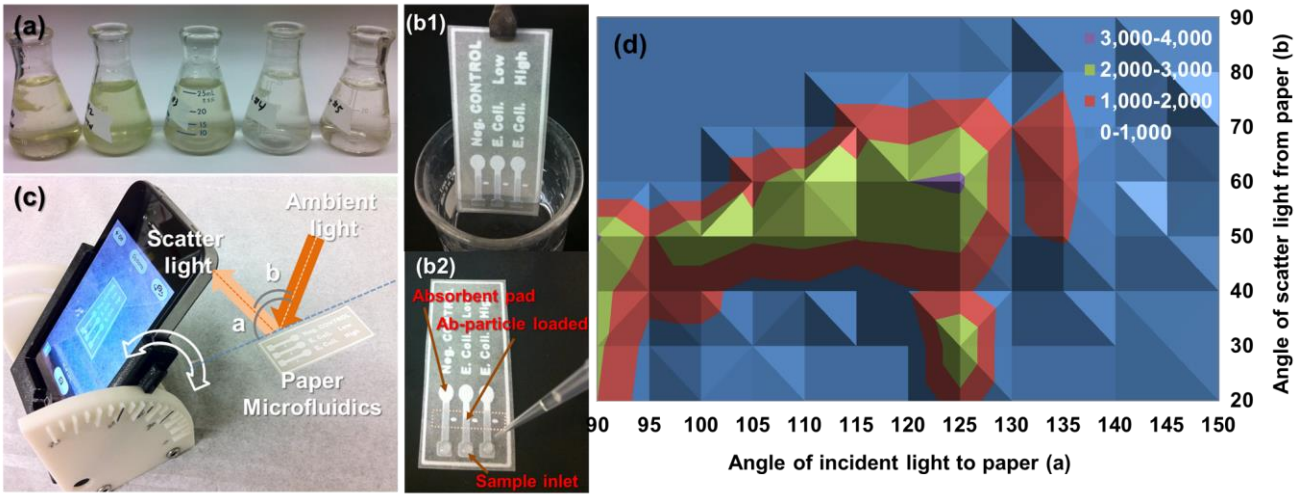

Figure 1: Collected 5 wastewater samples (from 3 different fish ponds, mountain runoff water and reservoir in Tucson, Arizona, USA) (a) were applied to the inlet of paper microfluidics by dipping (b1) or pipetting (b2). Paper filtered out large-sized molecules (soil particles, bacterial colonies, etc.) and let bacteria antigens flow through paper. Smartphone's digital camera was used for detecting light scatter intensity, arising from the particle immunoagglutination on the paper microfluidics (c). Contour plot of light scatter intensity from the particles on paper against the angles of light irradiation and scattering detection (d). The optimal angles were 125° for incident light and 60° for scatter detection from the channel of paper. (Refer to Fig. 1c).

Each channel of paper chip has sample inlet, channel, and absorbent pad (Fig. 1b). Anti-*E. coli* were conjugated to 920 nm, highly carboxylated polystyrene beads by covalent coupling [4,5] and 3.5 µl and 5.8 µl of such suspensions were applied to the detection area of each paper channel, for low- and high-range detection, respectively. BSA (bovine serum albumin) was similarly conjugated to the beads and 3.5 µl of such suspension was likewise added to the channel, as a negative control. All paper chips were dried out before use. Samples were applied to the inlets of paper chip, resulting in the capillary flow of water and subsequent filtration of contaminants (Fig. 1b). A smartphone was installed in a precisely controllable angled holder (Fig. 1c) and the paper chip was placed 9 cm horizontally away from the smartphone camera. Fluorescent lamps on the laboratory ceiling (ambient light) were the only light sources used for image acquisition from the paper chip. Images were taken using auto-exposure and auto-focus mode on the smartphone both before (background signal) and 30 seconds after (sample signal) wastewater sample was loaded. These images were analyzed using a program coded in MATLAB. The code algorithm converted the color image to a green image, recognized channel region, divided the channel area into three sections along the direction of flow, and finally calculated the average intensity of second (central) region. Each "sample signal" was divided by each "background" signal to cancel out the difference between paper chips (normalized scatter intensity or I). Same experiments were performed for a negative control channel (normalized negative control scatter intensity, or I_0). BSA-conjugated beads would not immunoagglutinate with target *E. coli*, effectively providing negative control signal. A series of I/I_0 were plotted against the concentration of *E. coli* concentration or the varying samples.

RESULTS AND DISCUSSION

Antigens of *E. coli* caused immunoagglutination of antibody-conjugated beads within paper fibers, thus increasing the effective diameter and altered morphology of beads. This change led to altered scattering characteristics (Mie regime considering the particle size of 920 nm and the visible wavelength of 400-750 nm). Through a series of experiments (Fig. 1d), the optimum angles of light irradiation and detection were found: 125° and 60° from the channel of paper, respectively, which also minimized the non-specific scatter from paper fibers [8]. A smartphone was fixed at 65 degrees (Fig. 1c) relative to a horizontal paper strip, according to the optimized Mie scatter angle (angle $a - b = 125 - 60 = 65$ degrees; from Fig. 1c).

A smartphone, mounted on a simple tilting stage, captured the images of paper microfluidics at the optimized angle, and the light scattering intensities were evaluated through the program coded in MATLAB. With varying concentrations of *E. coli*, a standard curve was constructed (Fig. 2), which corresponded very well with the Mie scatter simulation [3]. Since the curve showed a "dip" (which is purely an optical phenomenon [3]), two different concentrations of beads were used over two channels.

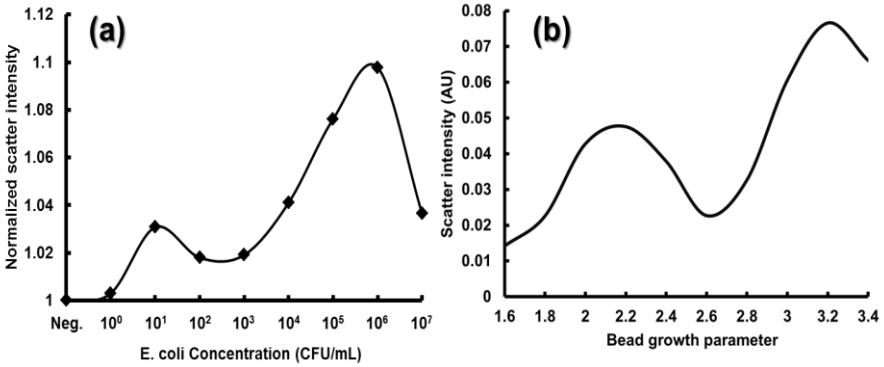

Figure 2. Normalized light scatter intensity with smartphone detection, using the serial dilutions of pure E. coli culture (a) and simulated light scatter characteristics (b) as effective bead size grows upon immunoagglutination.

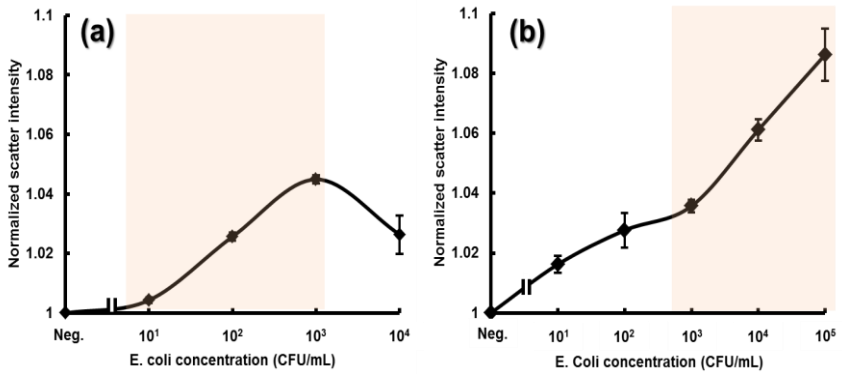

Figure 3: Standard curves for detecting E. coli using smartphone and paper microfluidics from low- (a) and high-range (b) concentrations. Each channel contained different amount of antibody conjugated beads.

A standard curve was constructed with varying concentrations of *E. coli* (Fig. 3). Different amount of particle in each channel widened the linear range of the low concentration area (Fig. 3a) or the high concentration area (Fig. 3b), effectively creating a standard curve from 0 to 10^5 CFU/mL. The lowest detection limit was 10 CFU/mL or single-cell-level considering the sample volume of 7 μL. These small error bars can be attributed to the fact that the light scatter intensities were averaged over a substantial area through image detection.

Using this standard curve, *E. coli* concentrations were evaluated for wastewater samples, and compared with the plate counting results using LB and MacConkey agar plates, showing excellent agreement (Fig. 4a). Smartphone detection of *E. coli* was successfully demonstrated with multi-channel paper microfluidics. The cellulose fibers in paper served excellent medium to filter out "big" molecules such as soil particles, floating matters, and algae in wastewater that may cause signal error on detection. This smartphone-based method has a potential for on-site, near-real-time, and user friendly assay for waterborne pathogens (Fig. 4b).

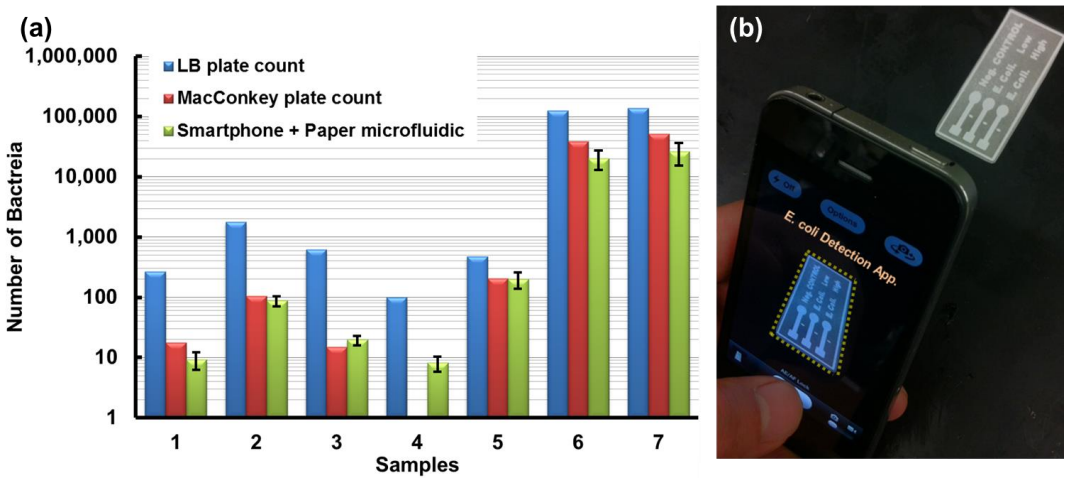

Figure 4: Results of plate counting (LB and MacConkey agar plates) and the smartphone + paper microfluidics assay for 7 different wastewater samples (a). Hypothetical smartphone application displaying a trapezoid for positioning the smartphone at the optimized angle and distance while photographing the paper microfluidics (b). From the captured images, the application can locate detection zones, evaluate the light scatter intensity, and convert it into E. coli concentration using the pre-stored calibration curve.

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CONTACT

Jeong-Yeol Yoon, +1-520-621-3587 or jyyoon@email.arizona.edu