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COMMUNICATION

A Microfluidic Device for Rapid Screening of *E. coli* O157:H7 Based on IFAST and ATP Bioluminescence Assay for Water Analysis

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Abstract: We present a simple microfluidic system for rapid screening of *Escherichia coli* (*E. coli*) O157:H7 employing the specificity of immunomagnetic separation (IMS) via immiscible filtration assisted by surface tension (IFAST), and the sensitivity of the subsequent adenosine triphosphate (ATP) assay by the bioluminescence luciferin/luciferase reaction. The developed device was capable of detecting *E. coli* O157:H7 from just 6 colony forming units (CFU) in 1 mL spiked buffer within 20 min. When tested with wastewater discharged effluent samples, without pre-concentration, the device demonstrated the ability to detect 10^4 CFU per mL seeded; suggesting great potential for point-of-need microbiological water quality monitoring.

E. coli is a fecal coliform bacterium among those usually present in large numbers in the intestinal flora and feces of humans and other warm-blooded animals. The pathogenic strain of *E. coli* O157:H7 is generally present at very low concentrations (<200 CFU/100 mL) in a larger heterogeneous microbial population in environmental water.[1] Detection of coliforms, specifically *E. coli*, is used to indicate microbiological water quality.[1] Several methods for detecting *E. coli* have been approved by regulatory bodies, e.g. the US Environmental Protection Agency (US EPA), and include membrane filtration followed by growth on selective M-Fc agar plates, and enzyme-based techniques, e.g. Colilert, Colisure (IDEXX Laboratories, Inc.)[1-2] Microfluidic platforms based on the enzyme-based method i.e. Mobile Water Kit (MWK),[3] and plunger-tube assembly[4] allowed field deployable *E. coli* detection in 1 h; these still rely on incubation period to increase bacteria concentration.

Rapid techniques such as polymerase-chain reaction (PCR)-based methods,[5] fluorescent *in situ* hybridization (FISH)[6] have been explored; however, these methods require expensive, specialized laboratory instrumentation and highly skilled personnel. A one-step detection of pathogenic bacteria employing immunomagnetic recognition and sample perfusion was reported; [7] based on volume change caused by bacterial growth in the fluidized chamber, and results were obtained within 2-8 h. The ATP-bioluminescence approach has been investigated as an alternative method to evaluate microbiological water quality.[8] The assay measures the bioluminescence produced during the reaction of luciferin/luciferase and molecular ATP, producing results within minutes. However, as ATP is a non-selective measure of bacterial cells, the ATP-bioluminescence assay was combined with the highly selective technique of IMS; which has been previously applied for the detection of *E. coli* in beach water.[8a] The target bacteria were selectively captured onto superparamagnetic beads functionalized with antibodies, forming bead-bacteria complexes which were subsequently separated from the matrix by a magnet. Quantitation of the captured bacterial cells was subsequently conducted using ATP bioluminescence assays. Together with pre-concentration by filtration; a detection of 20 CFU/100 mL can be achieved. This method, when used for rapid detection of *E. coli* and for enterococci enumeration in various wastewater sources, suggested a linear correlation between the results from IMS/ATP and traditional culture-based methods, with the benefit of results within 1 h rather than 24-72 h.[9] A microfluidic IMS/ATP system, so-called "3D immunomagnetic flow assay" reported 3 min detection of *Salmonella* in spiked lettuce solution after pre-concentration by incubation of 3 h.[10]

Analogous to tube-based IMS, IFAST is another microfluidic method that has been reported as a means to isolate and purify analytes of interest from complex matrices prior to detection methods.[11] Exploitation of the dominant surface tension and microfluidic geometries allowed immiscible liquids to be compartmented side-by-side, creating virtual walls.[11a-c, 12] The IFAST device consists of a series of fluidic chambers interconnected via gates. Paramagnetic particles, specifically bound to target analytes, can be transferred through these microfluidic gates by applying a moving magnetic field. Contaminants are unlikely to traverse the aqueous/oil interfaces as they remain magnetically inactive. In conventional tube-based IMS, where time and reagent consuming washing steps are required, IFAST enables rapid purification of the target analyte while also reducing costs by minimizing the use of expensive

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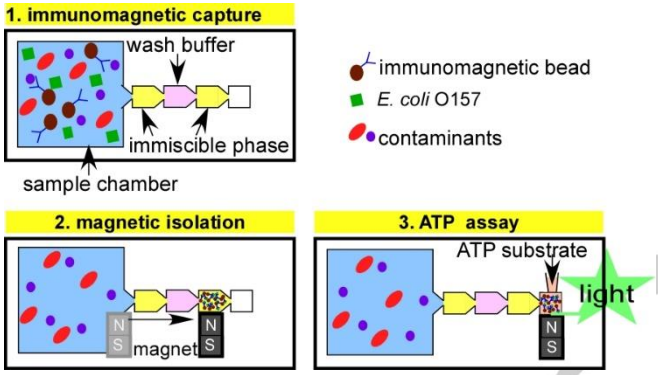
reagents through miniaturization. IFAST has been applied for concentration of nucleic acids from cell lysates,[11b, 13] breast cancer cells from whole blood,[11c] and *Helicobacter pylori* from stool samples.[11d]

In this work, the combination of the IFAST specificity and ATP assay sensitivity was exploited for the rapid detection of *E. coli* O157:H7 in contaminated water samples. The key technological approach comprises three consecutive steps (Scheme 1): (1) immunomagnetic capture of *E. coli* O157 by antibody functionalized superparamagnetic particles in the sample chamber, (2) magnetic isolation of *E. coli* O157-bound magnetic beads by applying a moving external magnetic field through immiscible liquids contained in the wash chambers and (3) rapid detection of *E. coli* O157 by ATP assay in the detection chamber using a photomultiplier tube (PMT) based detection device.



Scheme 1. The three-step microfluidic IFAST/ATP approach enables concentration of *E. coli* O157, followed by detection via light produced by bioluminescence reaction between luciferin/luciferase and ATP released from isolated bacterial cells.

First, the ability of the IFAST chip to concentrate and purify *E. coli* O157 from spiked buffered samples (steps 1 and 2, Scheme 1), was validated against the conventional tube-based IMS, employing a PDMS microfluidic chip (Figure 1a) where 1 mL of spiked sample was mixed with functionalized magnetic beads. The bead-captured cells were then moved through the immiscible liquids for washing (Figure 1b and Section 2.2, supporting information). In previous IFAST investigations, capture and hence concentration of target molecules/cells by immunomagnetic binding was achieved by fast manipulation from an external magnetic field.[11, 13] This approach was initially employed here but resulted in only limited capture of the bacteria (<60%). To enhance the immunomagnetic binding, the magnetic beads were gently agitated by manually shaking the IFAST chip in order to ensure even dispersion of magnetic beads and improved contact with the spiked bacteria; resulting in much higher bacterial capture (>80%). Figure 1c shows the improvement in *E. coli* capture with incubation time. Comparable isolation performance (Figure 1d) and cell viability (not shown) were achieved for both the conventional tube based IMS and the IFAST system for set incubation times. Manual agitation of the device by the user was replaced by an orbital shaker (Section 2.2, supporting information); allowing improved repeatability and productivity.

The second part of the design approach exploited the sensitivity of the ATP assay to measure the light produced by the bioluminescence reaction of luciferin/luciferase and ATP from bacterial cells (step 3, Scheme 1). A custom-made battery operable PMT based detection device[14] was connected to a simple digital multimeter for readout (Figure 2a, Supporting Information). This offered a more user-friendly system compared to the conventional luminescence plate reader which could not accommodate on-chip detection and requires trained personnel to operate.

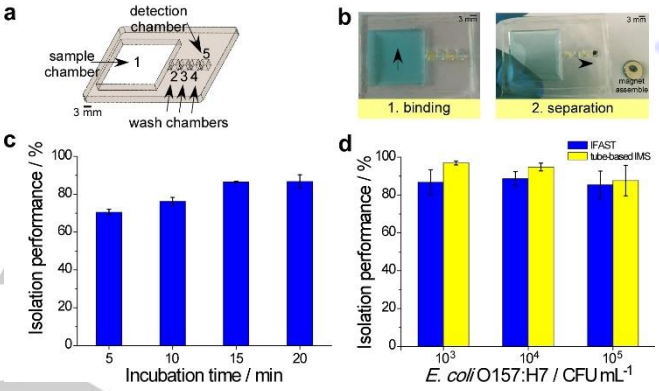


Figure 1. (a) Design of the microfluidic chip featuring a large sample chamber (26x26x4 mm^3), three wash chambers (3x3x4 mm^3) and a detection chamber (same dimensions as wash chambers), interconnected via trapezoidal microfluidic gates (3 mm to 500 μm wide, 1.5 mm long, 500 μm deep). (b) Photograph showing isolation of *E. coli* by IFAST, consisting of two consecutive steps of immunomagnetic binding and separation of isolated cells through immiscible liquids using magnet assemble. (c) Isolation performance quantified by plating revealed 15 min as an optimum incubation time required for IFAST immunomagnetic binding (n=3). (d) Isolation performance observed from IFAST and conventional tube-based IMS (n=3). (e) Similar number of viable isolated cells from IFAST and tube-based IMS.

Good linearity was observed from a logarithmic plot of ATP standard concentrations and bioluminescence responses measured by the portable device (Figure 2b). The molecular ATP released from *E. coli* O157:H7 revealed a value of 2.5×10^{-6} pmol CFU^{-1}, (Figure 2c) in good agreement with the reported value (2.0×10^{-6} pmol CFU^{-1}).[8b] This demonstrated a successful portable system for rapid *E. coli* detection (< 5 min).

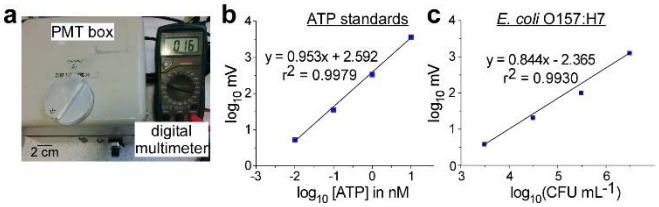


Figure 2. (a) The portable detection device for detection of light produced during bioluminescence ATP assays. (b) Logarithmic plots between luminescence responses (mV) and ATP from standard solutions and molecular ATP released from *E. coli* O157 (n=3, s.d. < 1%).

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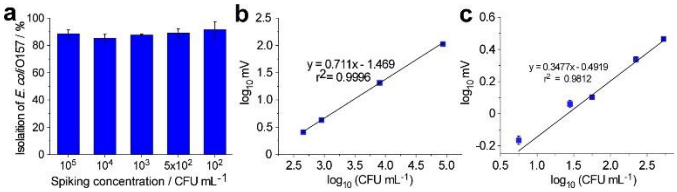

Figure 3. IFAST/ATP assays for rapid detection of *E. coli* O157 from seeded buffer employing the developed microfluidic system. (a) Isolation performance (n=3). (b) Excellent linearity obtained from bioluminescence responses of isolated *E. coli* (n=3, s.d. <1%). (c) By improving the light collection optics, the system was capable of detecting lower concentrations (6-600 CFU mL^{-1}) (n=3).

In the combined IFAST/ATP experiment (Figure S3, Supporting Information), bead-bacteria complexes formed via immunomagnetic reaction resulted in high *E. coli* isolation (≥ 90%, Figure 3a) and bioluminescence responses from the isolated cells demonstrated excellent linearity (r^2 = 0.9996) and repeatability (s.d. < 1%), from 500-10^5 CFU mL^{-1} in spiked buffer samples (Figure 3b). To improve the detection sensitivity, a mirror was placed opposite the PMT element to increase the light collected and hence increase the output signal.[14] This allowed lower concentrations of *E. coli* (as low as 6 CFU mL^{-1}) to be detected in spiked buffer samples (Figure 3c).

As further proof of principle, the system was applied to treated urban wastewater effluents (collected from a wastewater treatment plant in South Africa) spiked with *E. coli* O157: H7. Immunomagnetic capture with selective culturing on Sorbitol MacConkey agar plates showed good isolation of *E. coli* O157:H7 (Figure 4a: ≥ 96%). Similar data was obtained when bead-bound bacteria was cultured on selective and non-selective agar. Only a few non-O157:H7 strains (≤ 5%) were in differential media); an indication of highly specific binding. Although Dynabeads anti-*E. coli* O157 are designed to specifically target O157 strains; antigenically similar organisms, e.g. *E. hermanii* and *Serratia liquifaciens*, can cross-react and non-specifically bind, albeit to a very limited extent.[15] A linear luminescence response was obtained from 10^4 CFU mL^{-1} (Figure 4b) for the *E. coli* O157:H7 isolated from effluent samples. This limit of detection was achieved for those effluent samples where the overall background ATP reading was low as a result of adequate wastewater treatment. In cases of inefficient wastewater treatment, this limit of detection was lost as a result of interference from background ATP. This could be improved by redesigning the chip to enhance the buffer washing step. The current set-up requires minimal hands-on time, 2 min chip preparation including sample and reagent loading, and 1 min chip placement within detection box for ATP assay. Importantly, the results can be obtained in a single device within 20 min (16 min bacteria isolation, < 5 min detection) without off-chip pre-treatment.

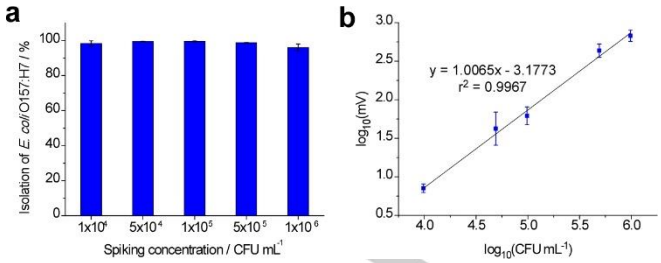

Figure 4. IFAST/ATP assays for rapid detection of *E. coli* O157:H7 from spiked wastewater effluents. (a) Isolation performance (n=3). (b) Luminescence signals detected from isolated *E. coli* O157:H7 from spiked effluents by the developed device (n=3).

In summary, a portable and user-friendly microfluidic system for the detection of *E. coli* O157:H7 in water samples was developed. To our knowledge, this is the first time an integrated system where bacterial isolation and detection are combined in one device, enabling 10^4 CFU *E. coli* O157:H7 to be detected from 1 mL of contaminated urban wastewater effluents within 20 min. The system is inexpensive, involves no off-chip pre-concentration, and requires minimal reagents and only semi-skilled personnel. Further development could yield a protocol capable of real-time monitoring of microbiological water quality, suitable for point-of-need use. The versatility of functionalized magnetic particles, coupled with appropriate assay chemistry, would also benefit other applications, e.g. rapid screening of pathogenic bacteria in food or clinical samples.

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Keywords: water chemistry • IFAST • luminescence • ATP assays • *E. coli* O157:H7

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