

RAPID DETECTION OF *E. coli* O157:H7 BY IFAST AND ATP BIOLUMINESCENCE ASSAY FOR WATER ANALYSIS

B. Ngamsom,¹ L. Fourie,² M.D. Tarn,¹ S. Kumar,² K. Moodley,²
K. Land,² and N. Pamme¹

¹The University of Hull, UK and

²Council for Scientific and Industrial Research, SOUTH AFRICA

ABSTRACT

The present investigation reports isolation and detection of *E. coli* O157:H7 employing a simple and portable microfluidic device based on immiscible filtration assisted by surface tension (IFAST) and adenosine triphosphate (ATP) bioluminescence assay. The device demonstrated ca. 90% *E. coli* isolation with linear responses of bioluminescence signals from isolated cells at 6 – 600 CFU mL⁻¹, suggesting great potential for point-of-need pathogenic bacteria detection for water analysis.

KEYWORDS: *E. coli* detection, Water analysis, IFAST, ATP assay

INTRODUCTION

The gold standard for detecting pathogenic bacteria for water quality control utilises membrane filtration followed by colony counting, and enzyme-based assays, e.g. Colilert®, Colisure® [1]. Such methods are expensive and require 24 h to develop results, by which point any contaminated water could have already affected water consumers. A tube-based immunomagnetic separation (IMS)/ATP assay has been reported as an inexpensive technique for detecting *E. coli* (10 - 20 CFU/100 mL), but still required 1 h [2]. IFAST employs a series of interconnected gates containing immiscible solutions through which magnetic particles, bound to target cells, can be transferred in order to separate and purify the cells from the sample matrix [3]. The aim of this study was to develop an inexpensive and portable *E. coli* detection device, based on a combination of the specificity of IFAST for rapid isolation and the sensitivity of a subsequent ATP assay (fig. 1).

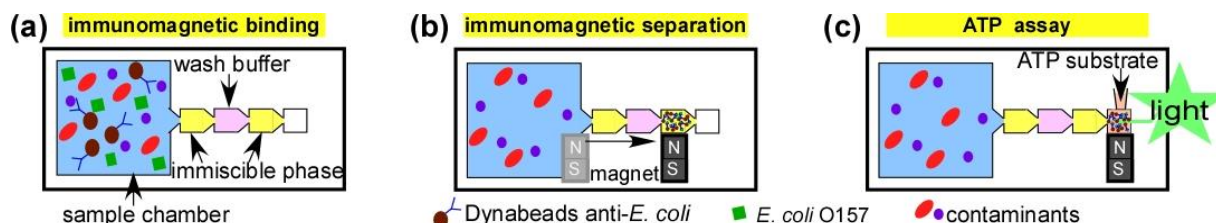


Figure 1: Principle of on-chip isolation and detection of *E. coli* O157 by IFAST and ATP assay, comprising (a) immunocapture of *E. coli* by magnetic particles in the sample chamber; (b) separation of bead-captured *E. coli* from contaminants through wash chambers; and (c) detection of captured cells via ATP assay.

EXPERIMENTAL

The IFAST chip (fig. 2a,b) was fabricated from PDMS and sealed at the bottom with an optical adhesive film. Immunocapture was performed in chamber 1 by dispersing 20 μ L magnetic particles (Dynabeads anti-*E. coli* O157, Invitrogen) in 1 mL *E. coli* O157:H7 spiked buffer with slight agitation. The bead-bound cells were isolated from the chamber 1 through chambers 2 (oil) and 3 (wash buffer) into chamber 4 (oil) by a magnet. For detection, 20 μ L ATP substrate (Promega) was added to chamber 5, followed by the captured cells from chamber 4 being pulled into the ATP substrate and the chip being placed in a detection box containing a photomultiplier tube (PMT) (fig. 2c).

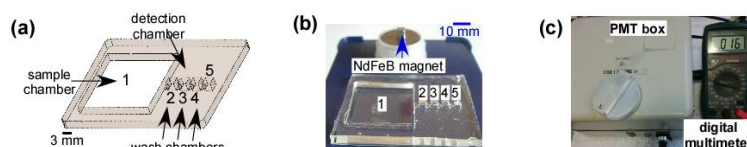


Figure 2: (a) Schematic of the device. (b) Photograph of the PDMS chip and the magnet for *E. coli* isolation. (c) The detection box containing a PMT, connected to a digital multimeter for readout.

RESULTS AND DISCUSSION

Initially, isolation of *E. coli* O157:H7 by IMS was investigated via IFAST. The optimum incubation time was obtained at 15 min (fig. 3a) and comparable capture efficiencies (83 - 90%) to the tube-based assay were obtained via microfluidic IFAST (fig. 3b), without loss in cell viability. Next, the device was tested for its ability to detect light produced during ATP assays (fig. 3c); linear responses of bioluminescence signals were observed from *E. coli* O157:H7 spiked samples (ATP content per CFU = 2.5×10^{-6} pmol; in good agreement with Tu *et al.* [4]).

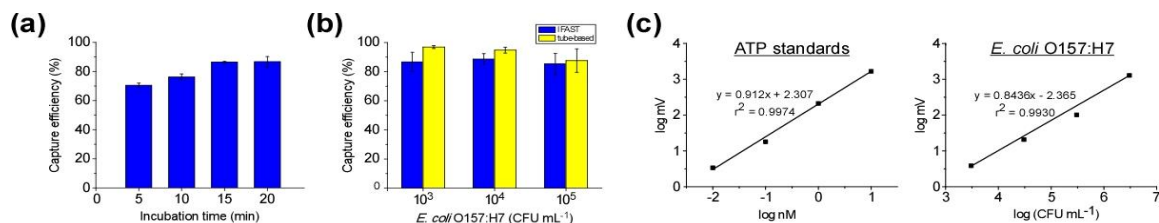


Figure 3: (a) Effect of incubation time on the immunocapture of *E. coli* O157:H7. (b) Capture of *E. coli* O157:H7; IFAST vs tube-based assays. (c) Bioluminescence signals observed from the PMT detection box for ATP standards (left) and *E. coli* O157 standards (right) ($n=3$).

Finally, the combination of on-chip isolation of *E. coli* and detection of isolated cells was explored (fig. 4a); within 20 min operation, the current setup showed 88 - 93% isolation of *E. coli* from spiked samples (fig. 4b) and a linear response of bioluminescence signals of 6 - 600 CFU mL⁻¹.

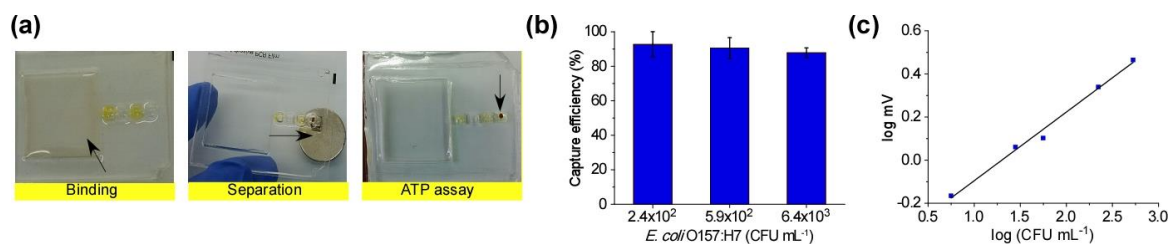


Figure 4: (a) On-chip immunomagnetic binding and separation via IFAST, followed by detection of *E. coli* by ATP assay. (b) Capture efficiencies and (c) bioluminescence responses obtained from *E. coli* spiked samples.

CONCLUSION

A simple microfluidic setup for fast determination of *E. coli* O157:H7 was developed and tested with spiked samples, demonstrating a process in which cell detection can be achieved from small sample volumes within 20 min. Being highly sensitive, with a detection limit of 6 CFU mL⁻¹, the present setup shows great potential for rapid pathogenic bacteria detection for water quality monitoring.

ACKNOWLEDGEMENTS

The authors thank Prof. G. M. Greenway, Dr. A. Iles, Mrs. C. Murphy, and Mr. N. Parkin for their technical support. The Newton Fund is greatly acknowledged for financial support.

REFERENCES

- [1] J. Olstadt, J.J. Schauer, J. Standridge, and S. Kluender, "A Comparison of Ten USEPA Approved Total Coliforms/*E. coli* Tests," *J. Water Health*, 05.2, 267-282, 2007.
- [2] J. Lee and R.A. Deininger, "Detection of *E. coli* in Beach Water within 1 Hour Using Immunomagnetic Separation and ATP Bioluminescence," *Luminescence*, 19, 31-36, 2004.
- [3] S.M. Berry, L.N. Strotman, J.D. Kueck, E.T. Alarid, and D.J. Beebe, "Purification of Cell Subpopulations via Immiscible Filtration Assisted by Surface Tension (IFAST)," *Biomed. Microdevices*, 13, 1033-1042, 2011.
- [4] S.-I. Tu, D. Patterson, J. Uknalis, and P. Irwin, "Detection of *Escherichia coli* O157:H7 Using Immunomagnetic Capture and Luciferin-Luciferase ATP Measurement," *Food Res. Inter.*, 33, 375-380, 2000.

CONTACT

* N.Pamme; phone: +44 (0)1482 465027, n.pamme@hull.ac.uk