Rapid detection of total coliform and *E.coli* in contaminated water using chemically modified microwells

Naga Siva Kumar Gunda, Selvaraj Naicker, Maryam Sadat Ghoraishi, Subir Bhattacharjee, Thomas Thundat and Sushanta K. Mitra*

Micro and Nano-scale Transport Laboratory,
Department of Mechanical Engineering,
University of Alberta,
Edmonton, Alberta, Canada, T6G 2G8.
*sushanta.mitra@ualberta.ca

Rapid detection of total *coliform* and *E.coli* in contaminated water is important to take precautionary measures to provide safe drinking water in the developing countries [1-3]. We have created a simple and easy to use method, where one can monitor the levels of total *coliform* and/or *E.coli* in contaminated water within 10 to 15 min. The present detection procedure is based on the intracellular enzyme (*β-galactosidase* (GAL) or *β-glucuronidase* (GUS)) activity of bacteria present in the contaminated water.

Total *coliform* bacteria are defined as all bacteria (including *E.coli*) possessing the enzyme *β-galactosidase*. In addition to *β-galactosidase*, most of the *E.coli* has the enzyme *β-glucuronidase* [1-3]. The presence or absence of an active *β-galactosidase* in coliform is detected by Red-Gal (substrate), which produces a characteristic red color when cleaved by *β-galactosidase* enzyme, thereby providing an easy means of distinguishing the presence or absence of coliform or *E.coli* in water. *E.coli* is identified with 4-methylumbelliferyl-β- glucuronidase (MUG) substrate. MUG is hydrolyzed, or cleaved by *β-glucuronidase* (GUS), resulting in the release of fluorigenic product 4-methylumbelliferyl (MU) [1-2]. MU can be visualized or detected by irradiation with UV light at a peak excitation of 350nm (UV) and a peak emission of ~460nm (blue).

Here, we developed a microfluidic chip containing series of micro-spots with integrated wells (MSIW) [4]. Figure 1 illustrates the schematic of microfluidic chip containing different micro-spots with integrated wells. Specially formulated enzymatic substrate solution is coated on microwells to ensure the availability of enzymatic substrate to react with coliform or *E.coli* present in contaminated water. These chemically coated microwells magnify the reaction rate due to increase in surface area, which enhances the signal intensity as well as sensitivity of the detection. These series of spots in the microfluidic chips (Fig.1) are used for simultaneous detection of different contaminated water samples at the same time as well as to confirm the test results of the same water samples by testing several times at different micro-spot of the microfluidic chip. A portable optical reader is used to measure the intensity of the developed color or fluorescence.

Specifically formulated enzymatic solutions are tested in micro-centrifuge tubes for optimizing the color/fluorescence producing conditions before applying on MSIW. Figure 2 shows the different stages of the change in color when *E.coli* reacts with specifically formulated enzymatic substrate solution in the micro-centrifuge tubes, kept at 37°C.

References:
[1] H. Sarhan and H. Foster, *Journal of Applied Microbiology*, 2008, 70 (5), 394–400.
[2] G. Pettibone, *Letters in applied microbiology*, 1992, 15 (5), 190–192.
[3] I. Gaudet, L. Florence, and R. Coleman, *Applied and environmental microbiology*, 1996, 62 (11), 4032–4035.
[4] N.S.K. Gunda, S. Naicker, M. S. Ghoraishi, S. Bhattacharjee, T. Thundat and S. K. Mitra, ICNMM2013-73037, ASME 2013 11th International Conference on Nanochannels, Microchannels, and Minichannels, 2013, Sapporo, Japan.

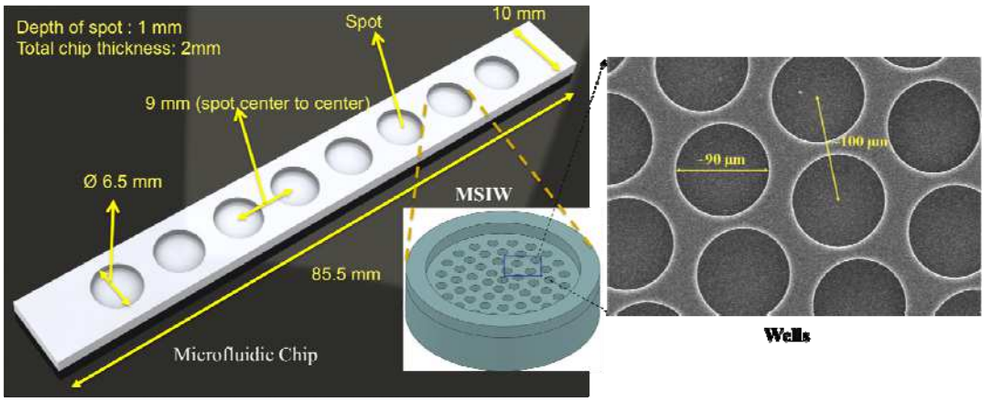
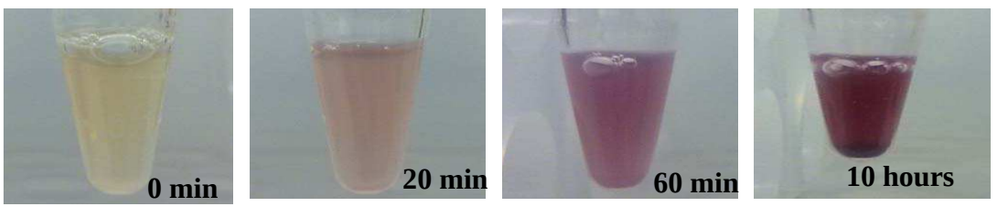


Figure 1. Microfluidic chip containing series of micro-spots with integrated wells.



(a) (b) (c) (d)

Figure 2. *E.coli* with specifically formulated enzymatic substrate solution after incubating the samples at 37°C (a) 0min (b) 20 min (c) 60 min and (d) 10 h.