Talanta 170 (2017) 36–40

Contents lists available at ScienceDirect

Talanta

journal homepage: www.elsevier.com/locate/talanta

Determination of *Escherichia coli* in urine using a low-cost foil-based microfluidic device

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ARTICLE INFO

Keywords:
Low-cost microfluidics
Escherichia coli
Cultivation
Lamination

ABSTRACT

We developed a simple low-cost cultivation-based microfluidic device from office-laminator foil and Parafilm for the determination of specific microorganisms in water samples. The main goal was to obtain a device that would be portable and cheap compared to common laboratory techniques testing microorganisms. This device needs only 10 μL of a sample and can be easily used in terrain by a non-specialist. Moreover, we dealt with some technical aspects of the device fabrication such as low-cost lamination techniques and the use of different cultivation media.

1. Introduction

The starting point for low-cost microfluidic devices was in the year 2007 in the form of paper microfluidic platforms with integrated wax structures [1]. The development in this field has significantly progressed over the years and the microchips are nowadays able to analyze many analytes as e.g. glucose [2], cholesterol and lactate [3], heavy metals [4], or enzymes as transaminase [5] and galactosidase [6]. Those microchips were also used as a platform for ELISA [7] or recently they were used for monitoring interaction between ssDNA and proteins [8]. Most of these techniques are based on a chemical reaction leading to a colored product, which semi-quantitatively corresponds to the concentration of analyte. Electrochemical detection methods can be also used e.g. for determination of glucose [9] or dopamine [10]. Interestingly, another sight-evaluation of results is also possible by distance-measurement approach [11]. The most of these works are mainly done on paper-based support. However, few more low-cost microfluidics approaches are known. One of the most interesting ways is to use laminating techniques [12,13]. Here, the microchannel structures, also more complicated, can be formed by simple laminating process using common office-laminator machines.

In our paper, we addressed the problem of detection of microorganisms that can cause serious health problems. Detection of pathogenic bacteria is very important task e.g. in medicine as well as in water quality control. Common techniques for detection of bacteria include staining and microscopic observations, PCR techniques and immunoanalysis [14]. These techniques have quite high investment costs. In our best knowledge, there is only one paper dealing with detection of *E. coli* in environmental samples using paper chip with polydimethylsiloxane membrane [15] and the second with detection of *Salmonella* sp. using paper microfluidics and smartphone application [16]. However, there are many practical difficulties dealing with the use of paper as a platform for such detection, e.g. transparency or the process of injection of bacteria.

Therefore our main goal was to develop a simple “plastic” microfluidic device for the binary analysis (YES/NO answer) and with possible semi-quantitative determination of microorganisms. We used a simple office-laminator foil, which allowed lowering the costs per device up to the range of tens of USD$ cents. The idea of low-cost “plastic” microfluidic devices is based on different foundation stones than traditional PCR and DNA typing technology. They can be miniaturized, but the issue of their price and standards of operation are very complicated [17,18]. Hence, we believed that the key to lower the costs lies in the traditional microbiological procedures, where the starting point of most of them is the process of cultivation. Therefore we proposed a unique microfluidic device that consists of a cultivation zone with suitable medium, where various coliforms such as *E. coli* could be grown. The grown microbial colonies are detected under a microscope, UV light or also by sight. The simple YES/NO answer is then provided to the user. The results obtained by our device were also confirmed by parallel testing using traditional methods of microbe detection. The importance of our device in comparison with these traditional methods lies in the possibility of incorporation of the device into more sophisticated device that can be used “in terrain”, e.g. 3D printed plastic-made syringe where the device is inserted and it allows injection of bacteria as well as cultivation.

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http://dx.doi.org/10.1016/j.talanta.2017.03.089
Received 30 January 2017; Received in revised form 24 March 2017; Accepted 28 March 2017
Available online 30 March 2017

2. Experimental

2.1. Materials

Chemicals used in the experiments were as follows: Agar (A5306), ECD MUG Agar (44657), EMB Agar (70186), LB broth (L3022), LB broth with agar (L2897), lauryl sulfate broth (61749), fluorescein sodium salt (46960), all from Sigma-Aldrich (St. Louis, USA). Automatic laminator Fellowes Cosmic A2 (Fellowes, Germany), iron Tefal Primagliss 2530 (Tefal, Czech Republic), paper-cutter and squared puncher Heyda (Heyda, Czech Republic) and Rexel (ACCO, USA), optical microscope with integrated CCD camera MOTIC 102 M (Motic, Germany), incubator and shaker Mini (Labicom, Czech Republic), UV/VIS spectrophotometer Perkin-Elmer Lambda 25 (Waltham, USA), UV lamp Herolab (Labicom, Czech Republic) and UV flashlight (Ledbaterky, Czech Republic) were also used.

2.2. Microfluidic device preparation

Our devices were prepared as follows: 50×50 mm squares of Parafilm were stacked onto each other until reaching 3 mm in height; then there was made a square-shaped hole in the middle of the stack by a 26×26 mm square puncher; the stack with the hole was later on laminated in between two layers of laminating foil like a sandwich, with the upper layer also punched by the 26×26 mm square puncher; the 26×26 mm-squared-hole in the middle of the device formed a tray, that was afterwards filled with 2100 µL of required medium for the cultivation of bacteria; the medium was later covered with another layer of either stretchable foil or Parafilm to prevent drying up the medium; and finally the device was set up to the credit card-like holder by an adhesive tape (see the scheme and corresponding photographs in Fig. 1a–c). After preparation, the device was sterilized under the germicidal lamp (2×15 W, 30 min).

2.3. Cultivation media

Media used for determination of bacteria were as follows: (i) LB Agar – containing 5 g NaCl, 10 g tryptone, 5 g yeast extract, 15 g agar – 35 g of the medium was adjusted to 1 L by water (final pH 7.2 at 25 °C); (ii) LB Agar with Fluorescein – fluorescein sodium salt was added to the first medium (LB Agar) at the final concentration of 10^{-4} mol/L; (iii) ECD MUG Agar – containing 5 g lactose, 20 g casein peptone, 5 g NaCl, 1.5 g mixture of bile salts, 4 g K_2HPO_4, 1.5 g KH_2PO_4, 15 g agar, 1 g tryptophan, 0.075 g 4-methylumbelliferyl-β-D-glucuronide – 53.1 g of the medium was adjusted to 1 L by water (final pH 7.0 at 25 °C); (iv) EMB Agar – containing 5 g NaCl, 10 g peptone, 10 g lactose, 2 g K_2HPO_4, 0.4 g eosin, 0.065 g methylene blue, 15 g agar – 42.47 g of the medium was adjusted to 1 L by water (final pH 7.0 at 25 °C); (v) LSB Agar – a medium containing 20 g casein peptone, 5 g lactose, 2.75 g K_2HPO_4, 2.75 g KH_2PO_4, 5 g NaCl, 0.1 g sodium laurylsulfate – 35.6 g of the medium was added to 15 g of agar was adjusted to 1 L by water (final pH 6.8 at 25 °C). All the experiments were done in ten replicates if not stated otherwise.

2.4. Microorganisms

Non-pathogenic *E. coli* strains were used as model organisms and they were kindly provided by the Centre of the Region Haná for Biotechnological and Agricultural Research, Palacký University in Olomouc. Stock *E. coli* culture was stored at 37 °C in LB broth. The life cycle of the *E. coli* was monitored by OD_{600} on the spectrophotometer Perkin-Elmer Lambda 25. *E. coli* was cultivated on Petri dish for comparison of data obtained from device cultivation using injection of 10 µL of the culture and cultivation for 24 h at 37 °C. Biological samples, infected urine, were kindly provided by Dr. Zahradníček from Masaryk University in Brno. All the samples including standards were considered as potentially infectious therefore safety gloves and respirator was used. All the devices were destroyed chemically by inserting them for 10 min into 5% NaClO in water. All the waste remaining from the experiments were collected and destroyed as the infectious material.

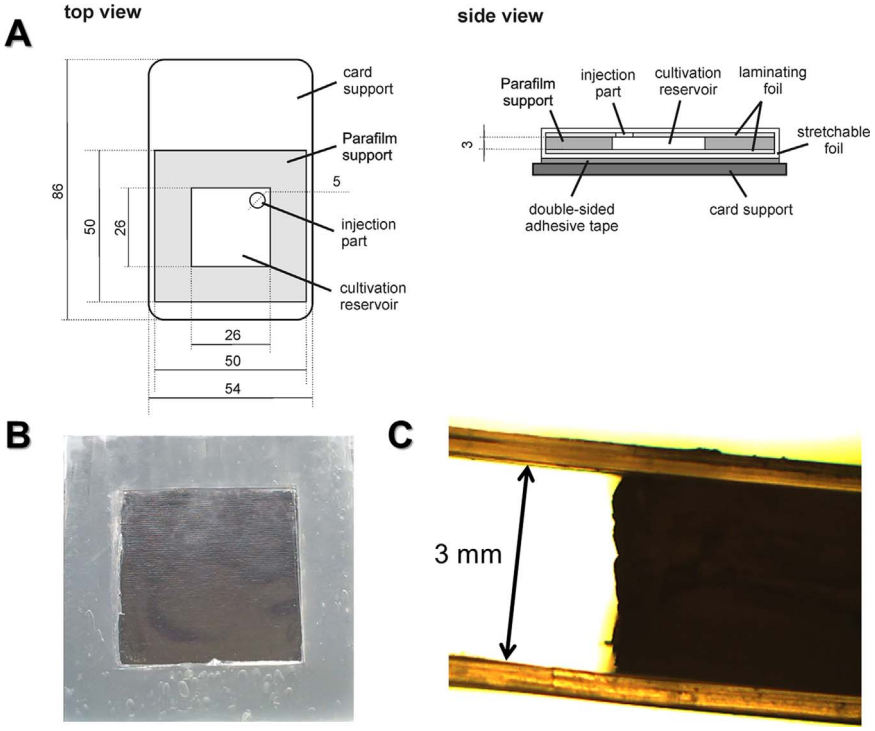

Fig. 1. Microfluidic device for *E. coli* detection. A: Scheme of the device in the credit-card format – top view and side view; B: Top view of the device without cultivation medium; C: Cross-section of the side of device with height of 3 mm (magnification 100x).