Chapter 5

Rapid Diagnosis by Microfluidic Techniques

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Additional information is available at the end of the chapter

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Abstract

Pathogenic bacteria in an aqueous or airborne environments usually cause infectious diseases in hospital or among the general public. One critical step in the successful treatment of the pathogen-caused infections is rapid diagnosis by identifying the causative microorganisms, which helps to provide early warning of the diseases. However, current standard identification based on cell culture and traditional molecular biotechniques often depends on costly or time-consuming detection methods and equipments, which are not suitable for point-of-care tests. Microfluidic-based technique has recently drawn lots of attention, due to the advantage that it has the potential of providing a faster, more sensitive, and higher-throughput identification of causative pathogens in an automatic manner by integrating micropumps and valves to control the liquid accurately inside the chips. In this chapter, microfluidic techniques for serodiagnosis of amebiasis, allergy, and rapid analysis of airborne bacteria are described. The microfluidic chips that integrate microcolumns, protein microarray, or a staggered herringbone mixer structure with sample to answer capability have been introduced and shown to be powerful in rapid diagnosis especially in medical fields.

Keywords: microfluidics, serodiagnosis, pathogen analysis, airborne bacteria, cerebrospinal fluid

1. Introduction

For the past decade, many traditional techniques and methods of bioanalysis and diagnosis have reached the technical bottleneck. While researchers have believed the microfluidic technology is becoming a promising alternative to traditional biological techniques as it allows the operating process to complete in a small chip. Since micrometer channels can be fabricat-

ed inside a microfluidic chip with simple operating procedures, fluids, reagents, and biological samples can be controlled accurately inside the chip to perform precise analysis and diagnosis. The smaller size and easy fabrication make the microfluidic devices to have many advantages such as portability, lower sample and reagent consumption and cost, less waste, rapid mass and heat transfer, and parallel analysis abilities. Besides, the increase of automation of the operations inside the chips helps to facilitate very accurate reactions that were usually very hard to be run by traditional methods. Considering the above-mentioned advantages, the microfluidic technique has great potential application in rapid diagnostics and point-of-care detections. Furthermore, combination of microfluidics with some traditional biological techniques will offer insights toward applications in biological and medical fields.

2. Microfluidics for rapid serodiagnosis of amebiasis

Entamoeba histolytica is the causative agent of amebiasis, the clinical significance of which has been demonstrated around the world. Thus, a early diagnosis technique that is capable of rapid analysis of *Entamoeba histolytica* is urgent for the disease control and public health concerns.

A microbead-based immune analysis technique was developed by our group using an integrated microfluidic device and recombinant protein (C-Igl) prepared from an *E. coli* strain containing Gal-lectin of *Entamoeba histolytica* [1]. The novel microfluidic technique greatly reduced the processing time (<20 min compared to a couple of hours if using traditional methods and could have potential application on point-of-care diagnosis of amebiasis). Further, the chip is capable of four parallel analyses at one time, which is very promising for high-throughput analysis. Moreover, it is worth mentioning that only 20 μl of the clinical sample was required for each single assay. The low consumption of both samples and reagents makes the technique suitable for widely applications.

The microfluidic chip structure was presented in **Figure 1**, consisting a bottom control layer and a upper fluid layer. The chip can be fabricated simply by standard soft lithography using polydimethylsiloxane (PDMS) and glass slide.

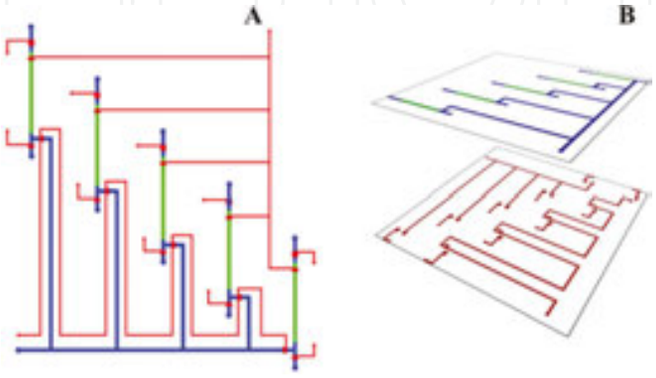

Figure 1. Structure illustration of the microfluidic chip for serodiagnosis of amebiasis [1].

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To facilitate the on-chip immune analysis of amebiasis, the channels within the chip upper layer contained the microcolumns (**Figure 2**, green), which were built based on a sieve valve structure that can trap microspheres (C-Ig1 coated beads) so that immune reaction can be carried out within the columns by pushing the reagent and sample suspension passing through the columns, where the antigen bonds with antibody that was on the surface of the microbeads. By opening and closing the valves on both sides of the channel, reagent and wash buffer can be controlled accurately for the whole immune process.

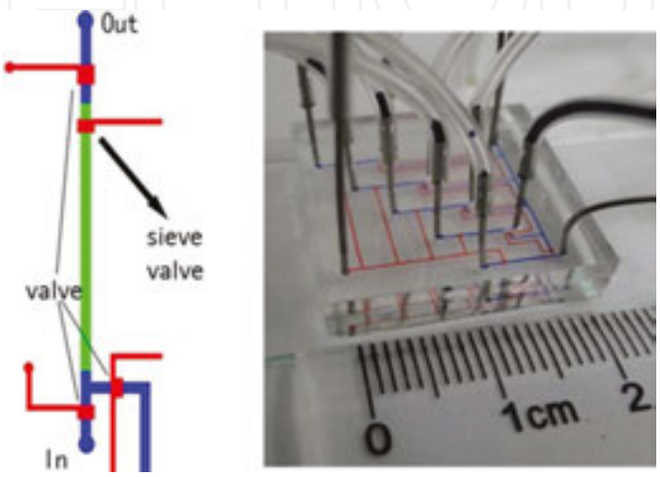

Figure 2. Microcolumn and the microfluidic chip [1].

The fluorescence signals of the immune reactions can be detected by CCD devices. **Figure 3** showed microscope photography of the column within the chip, showing great sensitivity for on-chip detection of amebiasis.

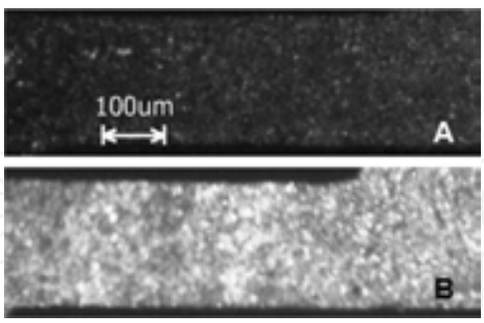

Figure 3. Fluorescence images of the microcolumns [1]. (A) Negative samples. (B) Positive samples.

2.1. Microfluidics for rapid allergy diagnosis

For allergen diagnosis, the UniCAP assay is considered the gold standard in clinical tests. However, serum-specific IgE requires separate analysis that is labor intensive and depends on complicated instruments. As a result, the time and cost expense required for the large-scale allergy survey currently employed deems the method insufficient for many applications. On-

chip allergy diagnosis provides many advantages, such as low reagent consumption, short analysis time, simple operation, environmental-friendly process, and high-throughput abilities. The microfluidics can be combined with protein microarray techniques to facilitate large-scale survey and provide a promising platform for clinical applications.

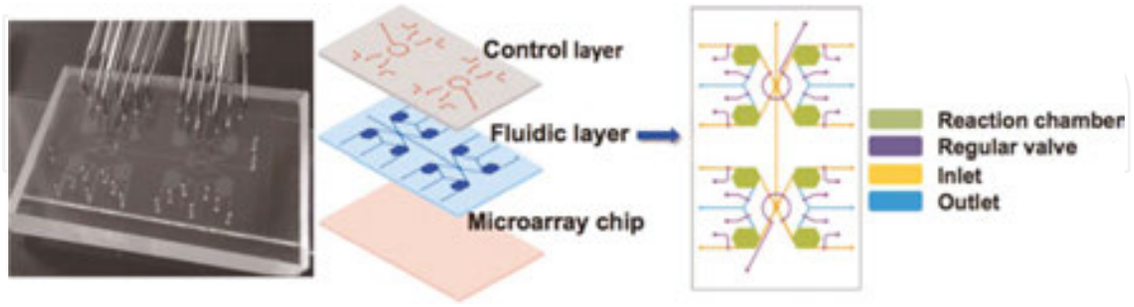

Figure 4. The microfluidic chip for high-throughput allergy diagnosis [2].

Figure 4 shows a three-layer microfluidic chip for high-throughput allergy analysis [2]. The chip was consisted of a control layer, a fluidic layer and a glass slide. Prior to the on-chip analysis, allergens were printed onto the surface of glass slide in triplicate manner using traditional protein microarray technique. The printed slide was then served as the bottom slide of the fluid layer and bonded to the PDMS layers to give a protein hybridization chip. The integrated microvalves within the control layer were used to precisely control reagents inside for the biochemical reaction process.

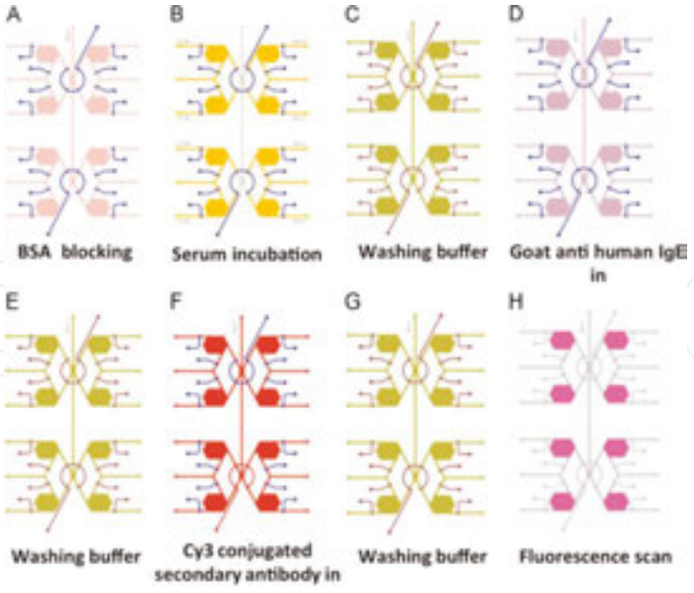

Figure 5. Operation of the microfluidic device for on-chip allergy diagnosis [2].

The operation protocol of the on-chip allergen detections of serum-specific IgE was shown in **Figure 5**. Generally, the hexagonal chambers were filled with blocking buffer containing 3%

BSA, followed by incubating the chip for 1 h at 37°C. After incubation, the eight serum samples (20 × diluted) were loaded into the eight hexagonal hybridization chambers in parallel. Meanwhile, ring-shaped valves within the control layer were closed to prevent cross-contamination. Another incubation at 37°C for 1.5 h was carried out for the bindings. After rinsing the chambers with PBS, goat anti-human IgE antibody was injected. Then, the chip was heated to 37°C for 1 h. Finally, the chip was filled with CY3-labeled secondary antibody IgG. The immune reactions were completed after incubation for 1 h at 37°C. A Luxscan-10K/A microarray scanner was involved to give the fluorescence signals.

Figure 6 shows the obtained fluorescence signals in a single assay. The signals were scanned, and the intensities were calculated for further studies. By combing microarray techniques, the microfluidic device turns into a promising platform for accurate and high-throughput IgE analysis of clinical serum samples, showing potential applications on clinical large-scale screening of allergies.

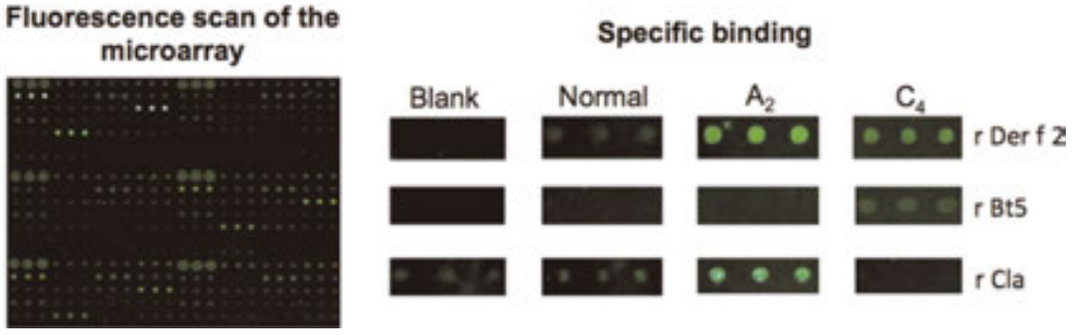

Figure 6. Fluorescence signals on the surface of the glass slide [2].

3. Microfluidics for airborne pathogen analysis (principles and methods)

Airborne pathogens are serious threat to public safety. Due to the lack of technique for rapid detecting airborne pathogens, bacterial transfer by air is very hard to prevent. Although traditional techniques such as Anderson sampler and AGI sampler have been proposed for decades, concentrations of collected pathogens cannot reach the detection limit (LOD) requirement for current bioanalytical methods. Thus, rapid analysis and early warning of airborne pathogen-related diseases is hard to be performed immediately.

We first attempted to used a microfluidic technique to facilitate fast enrichment of airborne pathogens. The microfluidic chip contained a long channel with staggered herringbone mixer (SHM) that was significant for airborne capture with high efficiency. Utilization of the SHM structure dates back to 2002, first proposed by Stroock et al. [3]. The structure was originally designed for mixing of the fluid flowing through microchannels and exhibited impressively high efficiency. Since the SHM possess a large number of ridges along the inner surface of the channel, as fluid flowing through the channels, laminar flow (both liquid and

gas) in the chip would be broken. Moreover, the SHM showed high efficiency of mixing fluids in chips and can be simply designed and integrated with standard microfabrication techniques. Because of its benefits, many researchers have utilized the structure in the following decade. In 2013, our group first tried using the SHM structure on airborne bacterial enrichment [4].

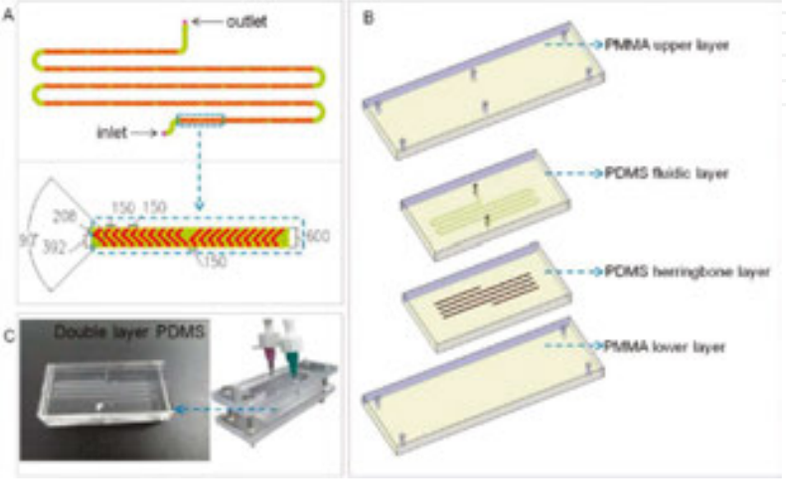

Figure 7. Structure of the microfluidic chip for airborne bacterial capture and enrichment [4].

As shown in **Figure 7**, the double-layer microfluidic chip was fabricated by standard soft lithography using PDMS [5]. The main channel inside the chip contained a standard SHM structure that has a depth of 40 μm, a width of 600 μm and a length of 17.4 cm. Inlet and outlet on each sides of the channel that have diameters of around 1.5 mm were drilled using a puncher. The chip was then clamped lightly with two polymethylmethacrylate (PMMA) plates to reduce air leakage from PDMS wall.

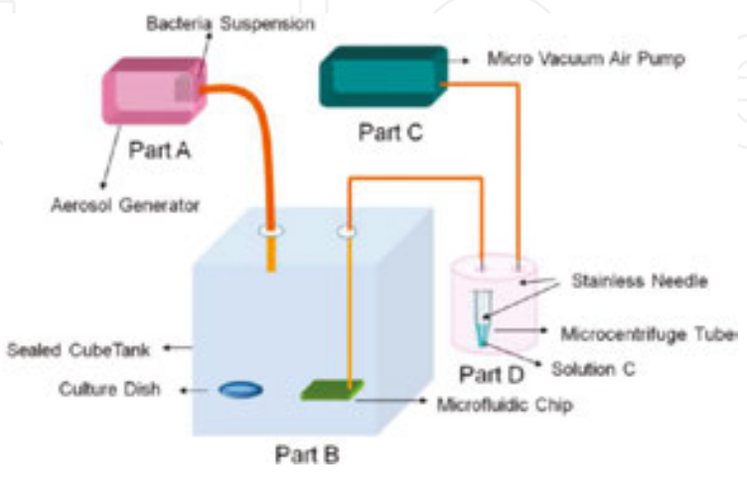

Figure 8. Illustration of the system setup [4].

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To simulate bacterial bioaerosol in the natural environment, an aerosol generator and a tank were used to make bacterial bioaerosol. **Figure 8** showed a 125-L glass tank and the setup for the enrichment assay. The microfluidic chip was connected to a microvacuum air pump (HARGRAVES) then put inside the tank. Aqueous bacterial suspension was diluted and then used to generate bioaerosol containing a series of bacterial concentrations.

The flow rate of the microvacuum can be adjusted from 1.0 to 12.0 mL min^{-1}. By drawing the air into the chip, the aerosol passed through the channel very fast. The pumping time can be set from 10 to60 min. The SHM structure helped to break the laminar flow inside the channel, which increased the probability of collision of the airborne bacteria with the inner surface of the channels, so that bacterial cells in the aerosol would hit and attach to the inner surface of the channel wall so as to collect the airborne bacteria.

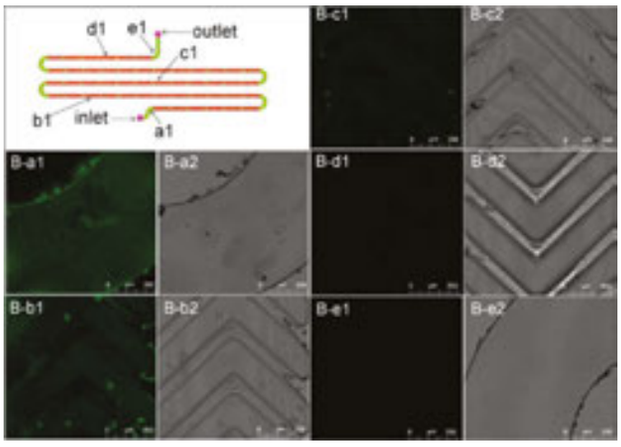

Figure 9. Microscopy photography of fluorescent *E. coli* cells along the channel [4].

To evaluate the enrichment efficiency, GFP-labeled bacteria were used to make the bioaerosol. **Figure 9** showed the fluorescence photography of GFP-labeled *E. coli* cells inside the chip channel after the collection step. Strong fluorescence signals were observed in the front side of the channel, indicating the chip can capture the bacteria with very high efficiency. Along the channel, the GFP signal intensity showed a gradual decrease, indicating that the channel length was enough for airborne bacterial capture with no cells leaking from the chip. Technically, the SHM channel can be build short (around 10–15 cm), which is believed to be long enough for the bacterial capture.

After the enrichment step, a couple of microliters of aqueous media (or lysis buffer) was loaded into the chip. The SHM channel was flushed with ddH_2O. The washed bacterial cells were then moved directly into another microfluidic chip using a micropump for immune or PCR analysis or to bacteria counting using dilution plate or cell culture for further studies.

3.1. Microfluidics for rapid analysis of airborne bacteria (*Escherichia coli*, *Enterococcus faecalis*, *Klebsiella pneumoniae*, *Enterococcus faecalis*, *Staphylococcus aureus and Citrobacter koseri*)

Polymerase chain reaction (PCR) has been applied to the detection of bacteria for decades. However, PCR often requires DNA purification process which is time-consuming and expensive devices to provide thermal cycles of the reactions to carry out. The defects make the technique not suitable for rapid detecting applications in field. For rapid detections (point-of-care tests) of airborne *Escherichia coli*, *Enterococcus faecalis*, *Klebsiella pneumoniae*, *Enterococcus faecalis*, *Staphylococcus aureus and Citrobacter koseri*, chip collection followed by on-chip PCR analysis is a very promising way due to the high sensitivity and selectivity of the assay.

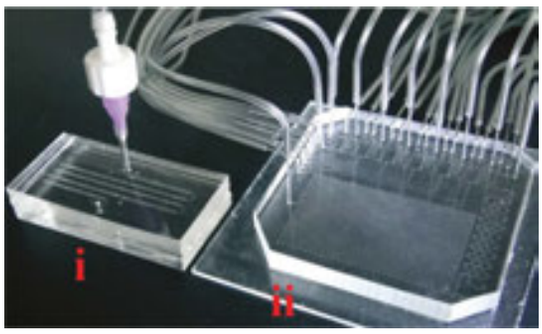

Figure 10. The airborne bacterial enrichment and PCR system [6].

A microfluidic system comprising an airborne bacterial enrichment chip (**Figure 10**, i) and a high-throughput PCR chip (**Figure 10**, ii) is shown in **Figure 10** [6]. The rectangular chip has a length of 5.5 cm and a width of 5.5 cm. The height of the channels inside the chip is 30 μm. To facilitate sample and reaction mixtures inlet and outlet, holes of 650 μm diameter were punched in the chip. The heating process (95 and 55°C) for PCR was provided by two polyimide heating membranes that were assembled beneath the chip.

The 2.5 μl PCR reagent consisting PCR primers for each bacterial target, 0.13 μl bacterial cell lysis products, 0.125 μg BSA, 0.5U ExTaq, 1 × buffer, and 2.5 mM dNTP.

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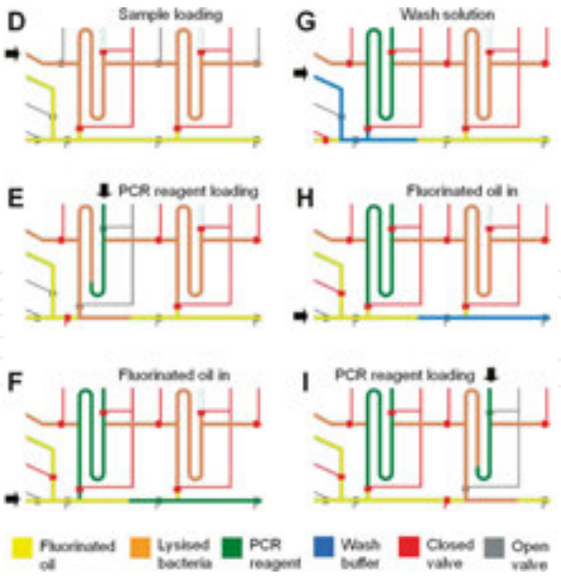

Figure 11. Schematic operating procedures of the high-throughput PCR chip [6].

Figure 11 shows the operating procedures of the PCR chip. After airborne sample collections, the bacterial cell lysis products were washed and injected into the S-shaped sample-loading channels. Next, the channels was isolated via increasing the pressure in the valves, and then the reaction mixtures were loaded into the chip using a micropump, followed by loading fluorinated oil to push the PCR plug to moved inside the channels. To prevent contamination, buffer was loaded to wash the channels inside the chip. Then, fluorinated oil was injected again to push the washing plug to move along the channels. Finally, the following group of reaction mixtures was loaded into an adjacent sample-loading channel. Thus, the six PCRs can be carried out continuously in the chip by repeating the described steps.

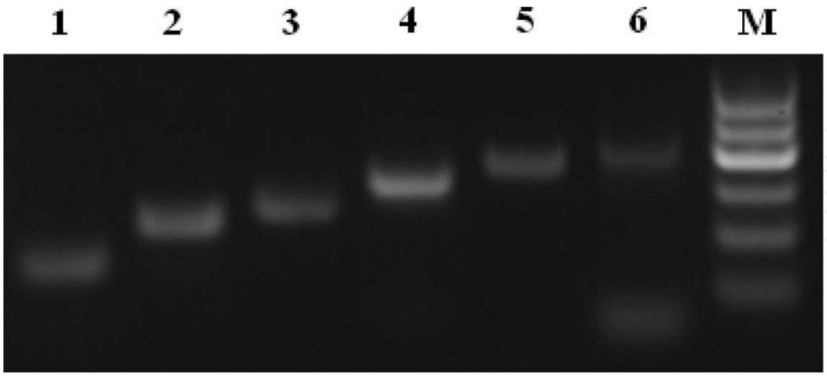

Figure 12. Agarose gel electrophoresis image of the on-chip PCR products [6].

After pushing the reaction mixture plug pass through the temperature zones alternately, the amplified DNA fragments were collected and detected using agarose gel electrophoresis. **Figure 12** shows the PCR produces of airborne (*Escherichia coli*, *Enterococcus faecalis*, *Klebsiella pneumoniae*, *Enterococcus faecalis*, *Staphylococcus aureus, and Citrobacter koseri*).

3.2. Microfluidics for rapid analysis of airborne *Mycobacterium tuberculosis*

Mycobacterium tuberculosis is considered as the most common cause of tuberculosis infections. The selective analysis of *Mycobacterium tuberculosis* depends on bacteriological immune techniques, while the reagents are expensive and skilled workers are required to run the tests using traditional methods. On the other hand, on-chip immune analysis of *Mycobacterium tuberculosis* has advantages of lower reagent consumption, automatic operation, low detection limit, and shorter assay time.

An integrated microfluidic device for rapid enrichment analysis of airborne *Mycobacterium tuberculosis* was shown in **Figure 13** [7].

The airborne bacterial enrichment part (**Figure 13**, i), as described previously, consisted a SHM structure that facilitates capturing of the airborne *Mycobacterium tuberculosis* in bioaerosol. The following analysis steps of *Mycobacterium tuberculosis* depend on a high-throughput double-layer chip containing control and fluidic layers (**Figure 13**, ii). A standard soft-lithography was used to fabricate PDMS based microfluidic chips.

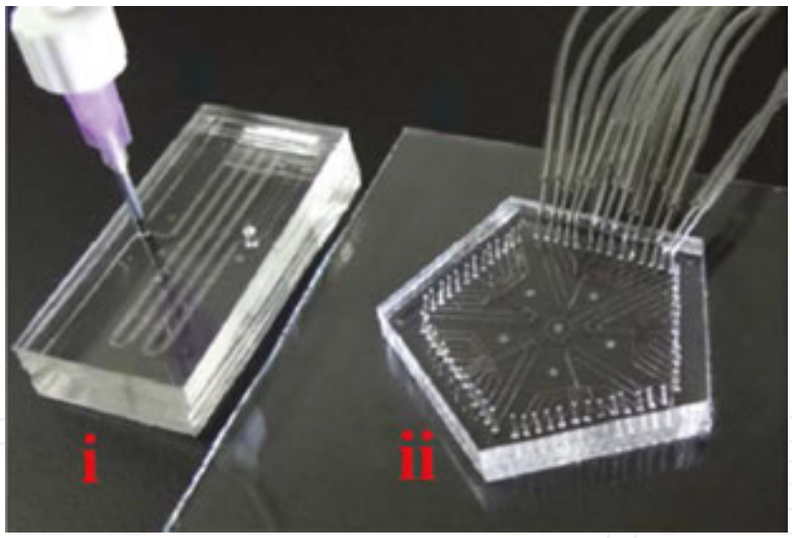

Figure 13. The airborne *Mycobacterium tuberculosis* enrichment analysis chips [7].

On-chip immune analysis targeted on the specific protein Ag85B of *Mycobacterium tuberculosis*. **Figure 14** shows the operations for the on-chip analysis. Sieve valves were built within the channels to form microcolumns [8]. Microspheres pre-coating with protein A and rabbit polyclonal antibody were loaded to work as substrates for capturing the Ag85B. Monoclonal antibody and FITC-conjugated secondary antibody were sequentially injected into the microcolumns to show the signals.

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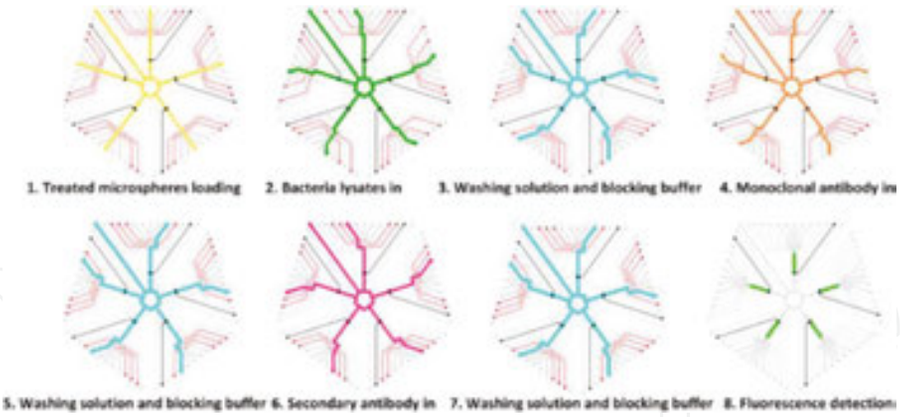

Figure 14. Schematic diagram of operation procedures of the on-chip immune analysis [7].

3.3. Microfluidics for rapid analysis of bacteria in cerebrospinal fluid

For rapid analysis of bacteria in aqueous samples, a promising method is loop-mediated isothermal amplification (LAMP). It is a novel isothermal nucleic acid analysis technique exhibiting high analytical sensitivity with less assay time. The results generated from LAMP amplification are visible to the naked eyes, which makes it a convenient method for rapid diagnostic. On-chip LAMP can be utilized for diagnosis of pathogen infections in cerebrospinal fluid (such like staphylococcus spp.). The reactions require only a couple of microliters of sample and no sophisticated instrument. Plus the aqueous samples can be directly used without a DNA extraction process, providing a robust approach for bacterial detections in field.

Figure 15 shows a three-layer microfluidic chip for cerebrospinal fluid diagnostic [9]. The chip was fabricated from PDMS, where the upper layer controlled the liquid flow within the middle fluid transport layer. To perform four analytical reactions targeting on four different samples, four microchannels for LAMP reactions were fabricated in parallel in the bottom layer of the chip.

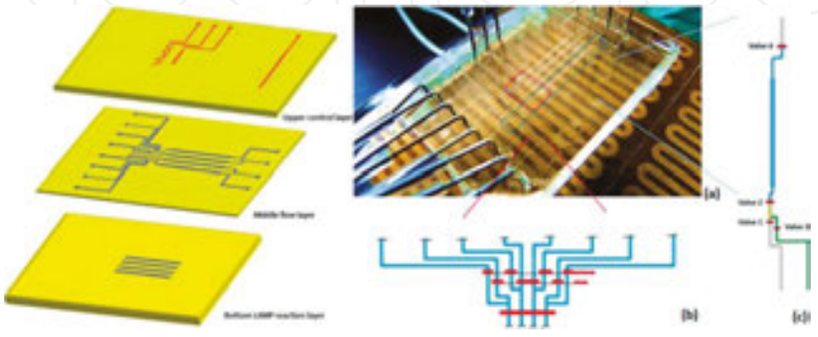

Figure 15. Schematic diagram of the microfluidic chip for LAMP analysis [9].

As shown in **Figure 16**, each unit consists of one sample-loading channel (width 200 μm, depth 30 μm), one lysis chamber (width 200 μm, depth 30 μm), and one amplification chamber (width 400 μm, depth 330 μm). In the microfluidic chip, the channels and chambers were opened and closed by oil valves (**Figure 16**, valve 1–4).

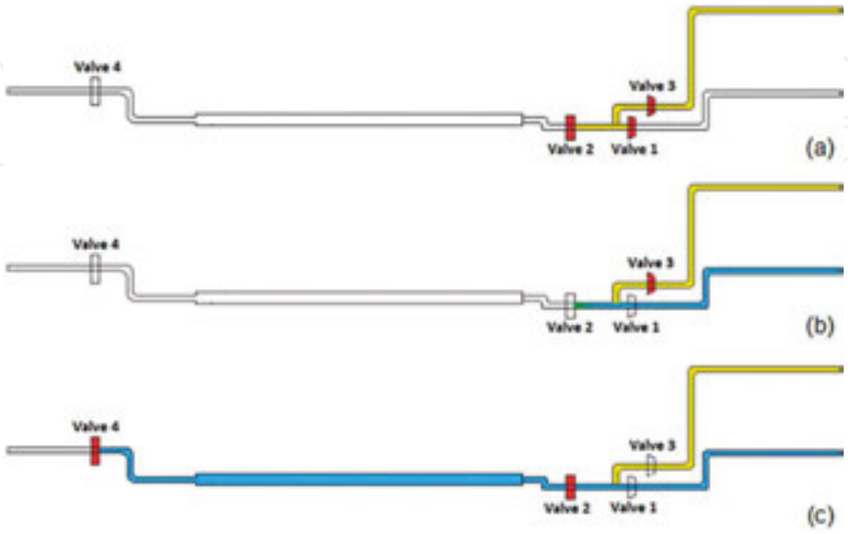

Figure 16. Operation of the microfluidic device for on-chip LAMP [9].

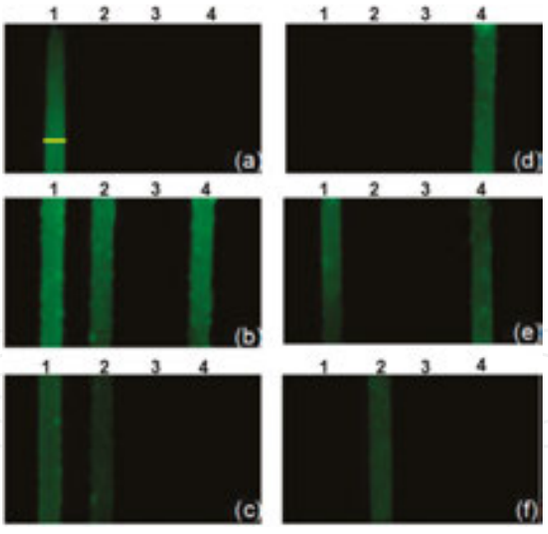

Figure 17. Fluorescence images of LAMP amplifications of clinical cerebrospinal fluid samples [9].

The suspension of aqueous sample mixed with lysis buffer was injected into the channels by a micropump. Valves 1, 2, and 3 were closed to seal the reaction chamber. Then, the chip was heated to 70°C for 30 min as that bacteria in cerebrospinal fluid can be lysed to release DNA. Subsequently, valves 1 and 2 were open. LAMP reagents were injected into the amplification chamber with valve 3 closed. Next, valves 2 and 4 were closed, following by heating the

chip to 65°C for 40 min for LAMP amplification. Result signal can be detected under 365 nm UV (**Figure 17**).

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