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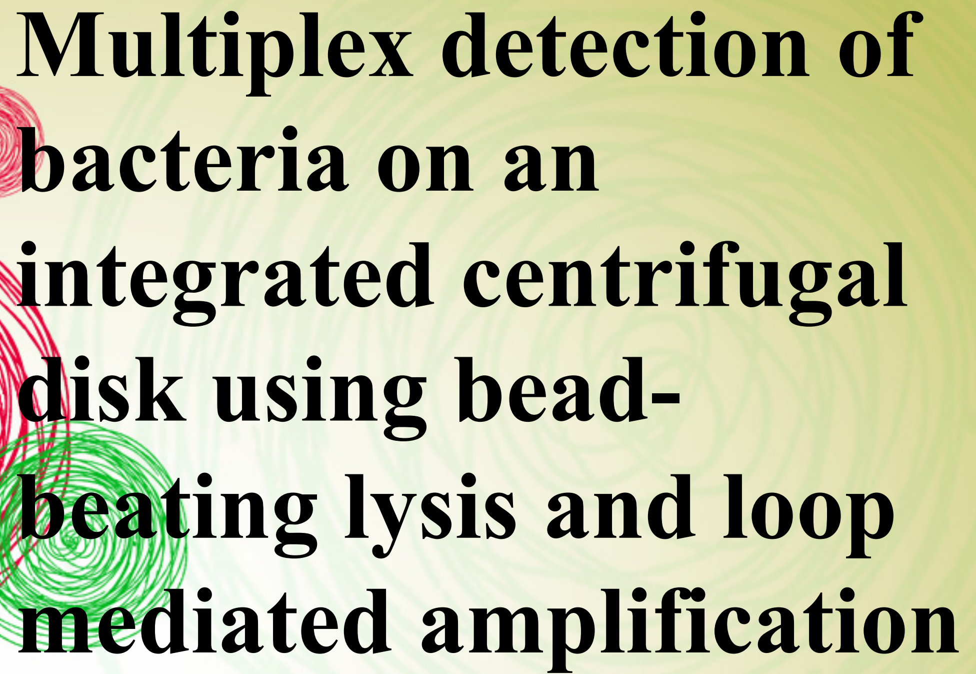

Multiplex detection of bacteria on an integrated centrifugal disk using bead-beating lysis and loop mediated amplification

Present by:

Arezoo mirzaei

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Main article

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Multiplex detection of bacteria on an integrated centrifugal disk using bead-beating lysis and loop-mediated amplification

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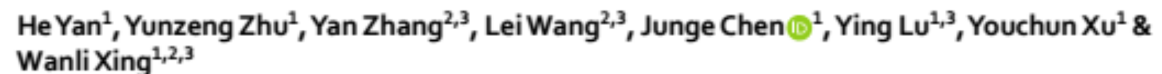
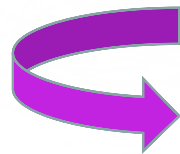
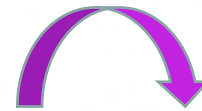

He Yan[1], Yunzeng Zhu[1], Yan Zhang[2,3], Lei Wang[2,3], Junge Chen[1], Ying Lu[1,3], Youchun Xu[1] & Wanli Xing[1,2,3]

Introduction

- ✓ Early identification of the type of pathogen bacteria is vital
- ✓ Identification and counting of bacterial pathogens in clinical samples have relied heavily on **culture-based methods**.



time-consuming



Many therapies are conducted empirically

the unnecessary use, misuse, or abuse of antimicrobials

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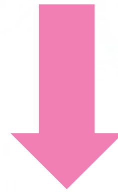
Introduction

❖ Recent advances in molecular diagnostics and microfluidic technologies have opened up new avenues for rapid detection of bacteria.



Centrifugal microfluidic chip

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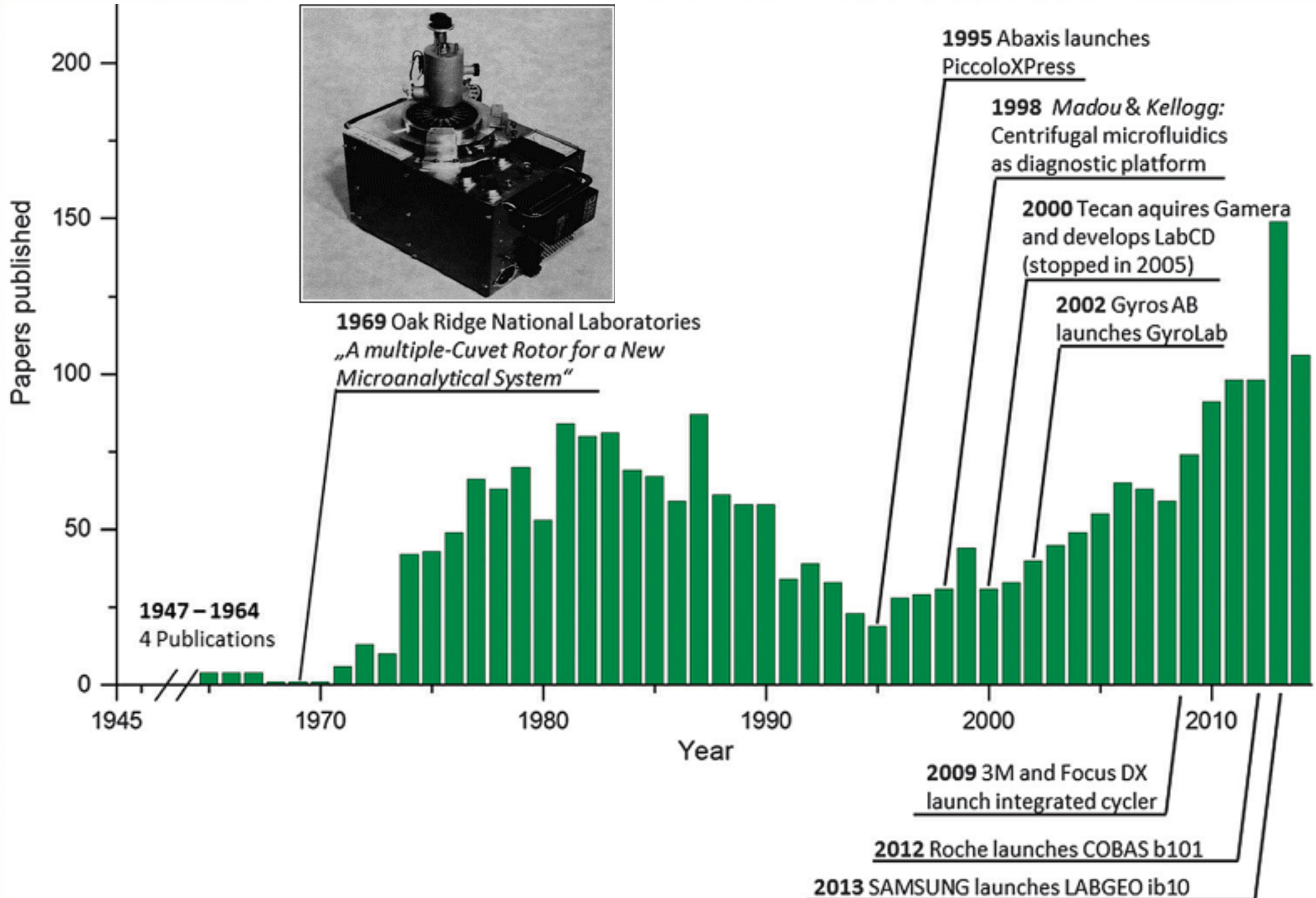
The history of centrifugal microfluidics



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A selection of centrifugal microfluidic devices

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Centrifugal microfluidic

Benefits

- Speed
- Precision
- Increased sensitivity
- Dramatic reduction in the time

ersonnel

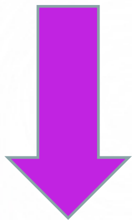
To overcoming these limitations :

integrate the different steps of analysis into a miniaturized and automated device

Integrated centrifugal disk

Ideally, a device for the detection of bacteria should perform all the steps:

- ✓ Cell lysis
- ✓ DNA extraction
- ✓ Amplification
- ✓ Detection



"sample-in to answer-out"

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The goal of this article

- Develop an **automated** and **user-friendly** device to enable multiplexed detection of bacteria **quickly** and **precisely**

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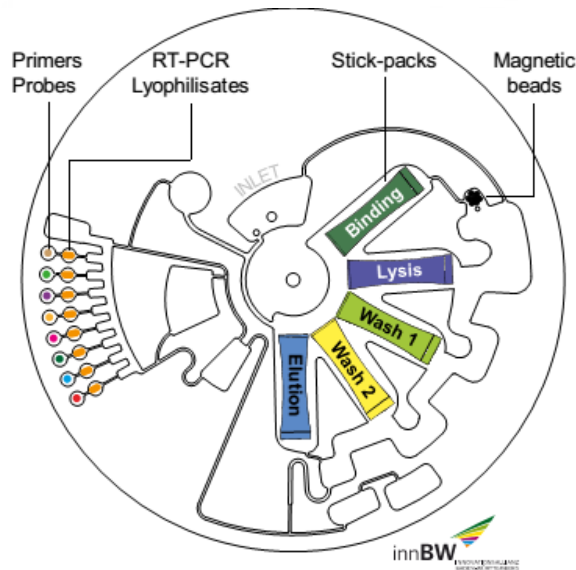
For constructing of the devise

Several considerations were taken:

1. The first consideration

Selection of the method for regulating the flow of the liquids in the chip



by pumps and valves

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Cont.

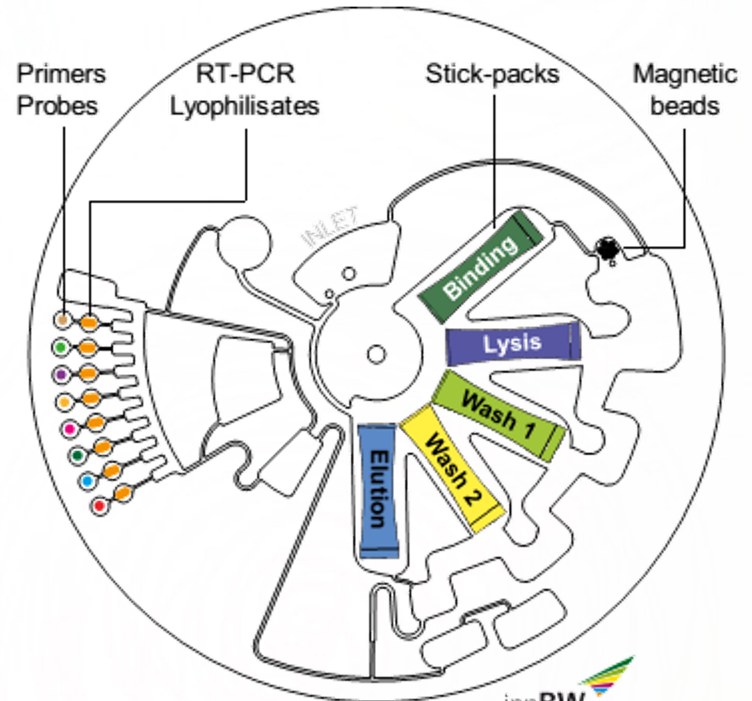
2. The second consideration

   Selection of method for the lysis of bacteria

chemical or physical methods

utilize enzymes and chaotropic salts to disrupt cell walls and cell membranes

physical lysis of cells such as mechanical lysis



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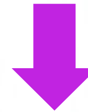
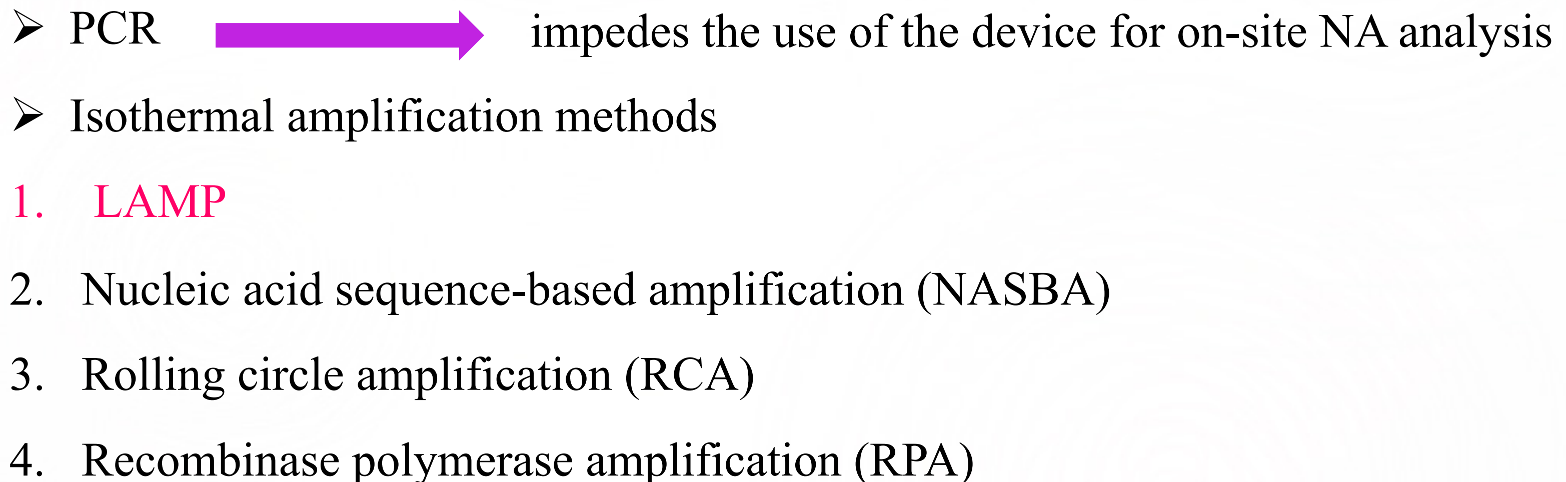
3. The third consideration

   Selection of a technique for amplification of NAs





- PCR → impedes the use of the device for on-site NA analysis
- Isothermal amplification methods

1. LAMP
2. Nucleic acid sequence-based amplification (NASBA)
3. Rolling circle amplification (RCA)
4. Recombinase polymerase amplification (RPA)

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Materials and Methods

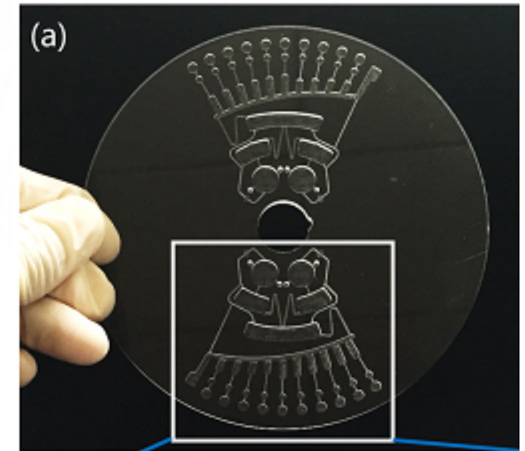
1. Fabrication and architecture of the chip

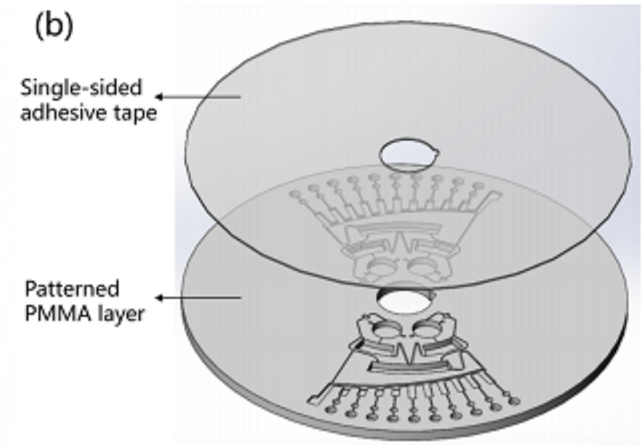
I. Lysis/clarification and storage unit

II. Mixing unit

III. Pre-distribution/reaction unit

IV. Liquid control unit





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1. Fabrication and architecture of the chip

- Polymethyl methacrylate (PMMA) was used to make the disk
- Each disk, measuring 60 mm (radius) × 3 mm (thickness)
- Disks consisted of a patterned PMMA layer and were sealed by using single-sided pressure-sensitive adhesives (PSA)

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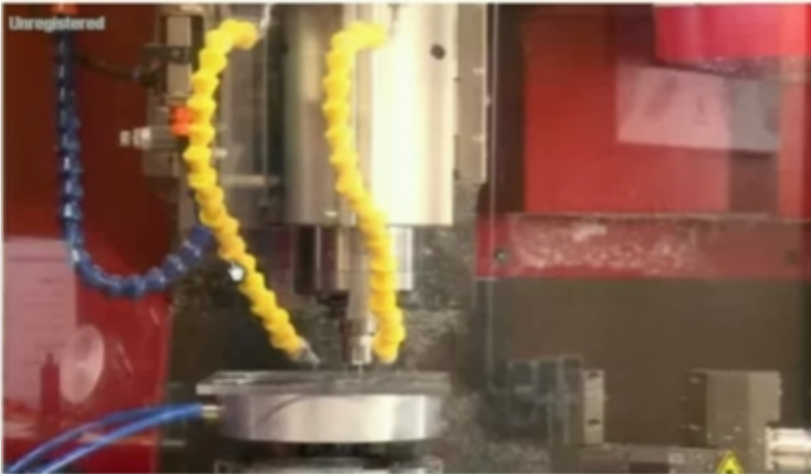
Disk Fabrication process



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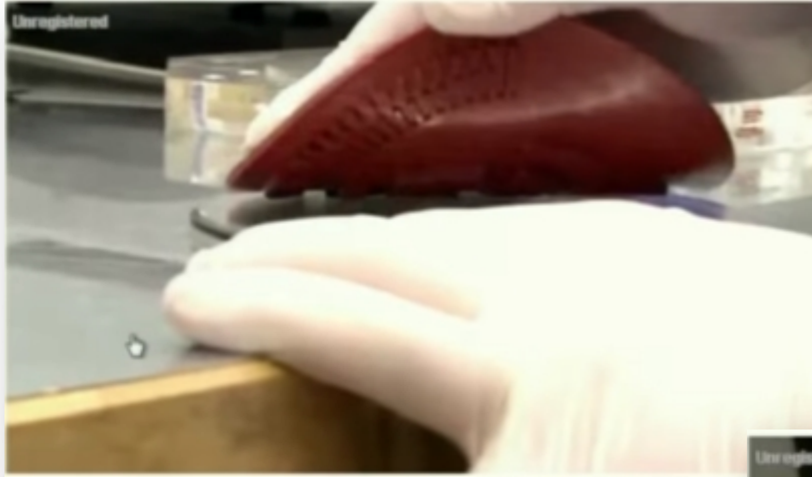

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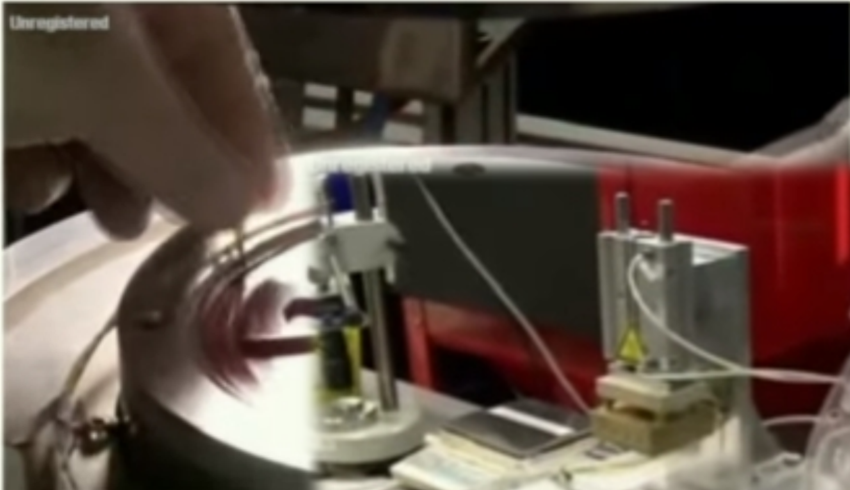

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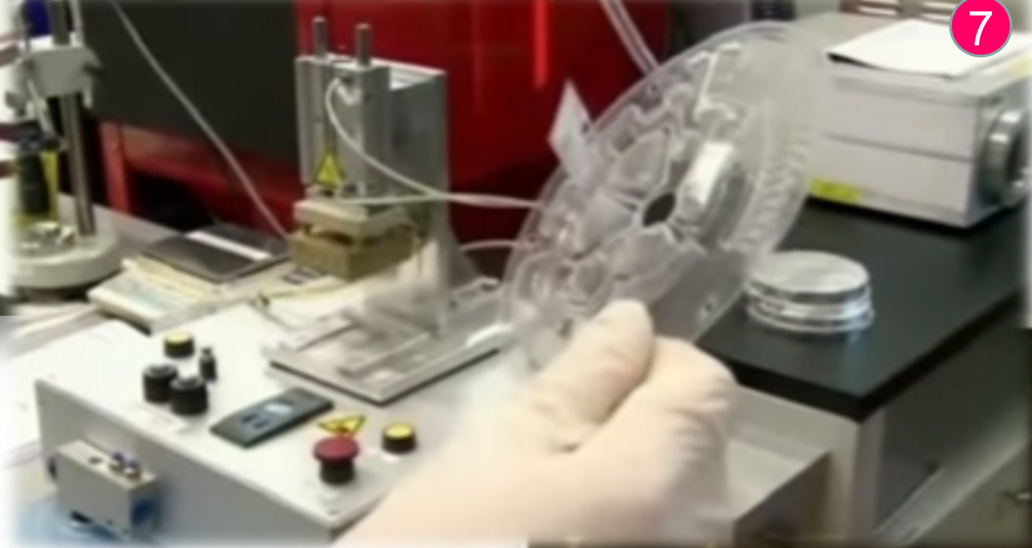

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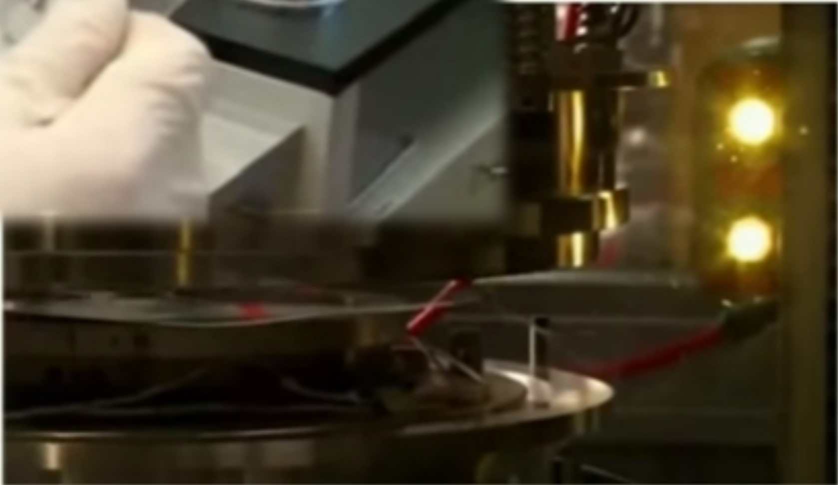

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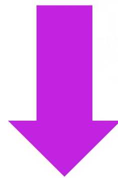
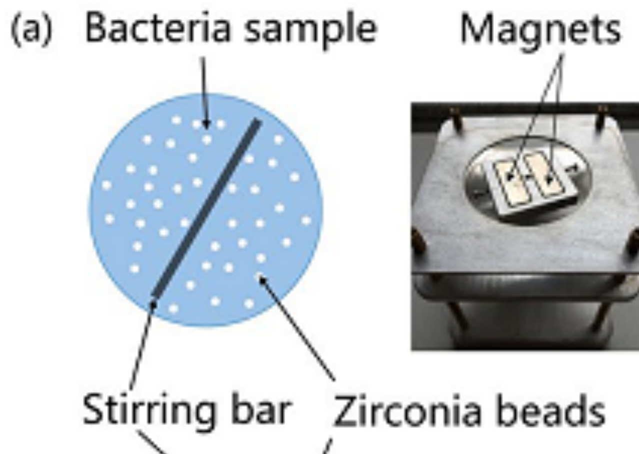
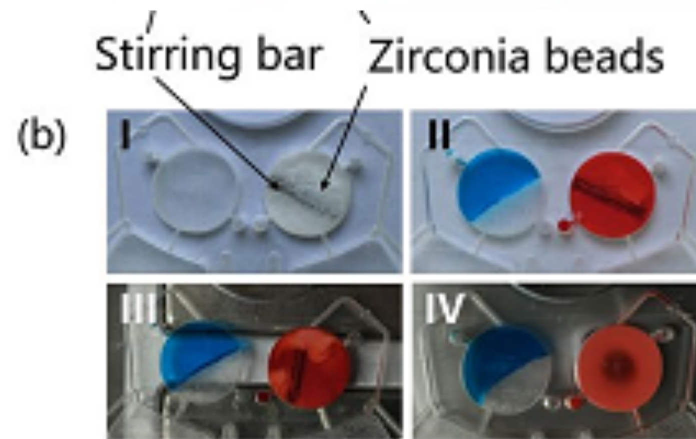
Lysis/clarification and storage unit

- The lysis chamber was circular
  - ✓ Diameter of 8 mm
  - ✓ Depth of 2 mm
  - ✓ Volume of the chamber ~100 µL



Consisted of a **magnetizable stirring bar** of 7 mm length and zirconia beads





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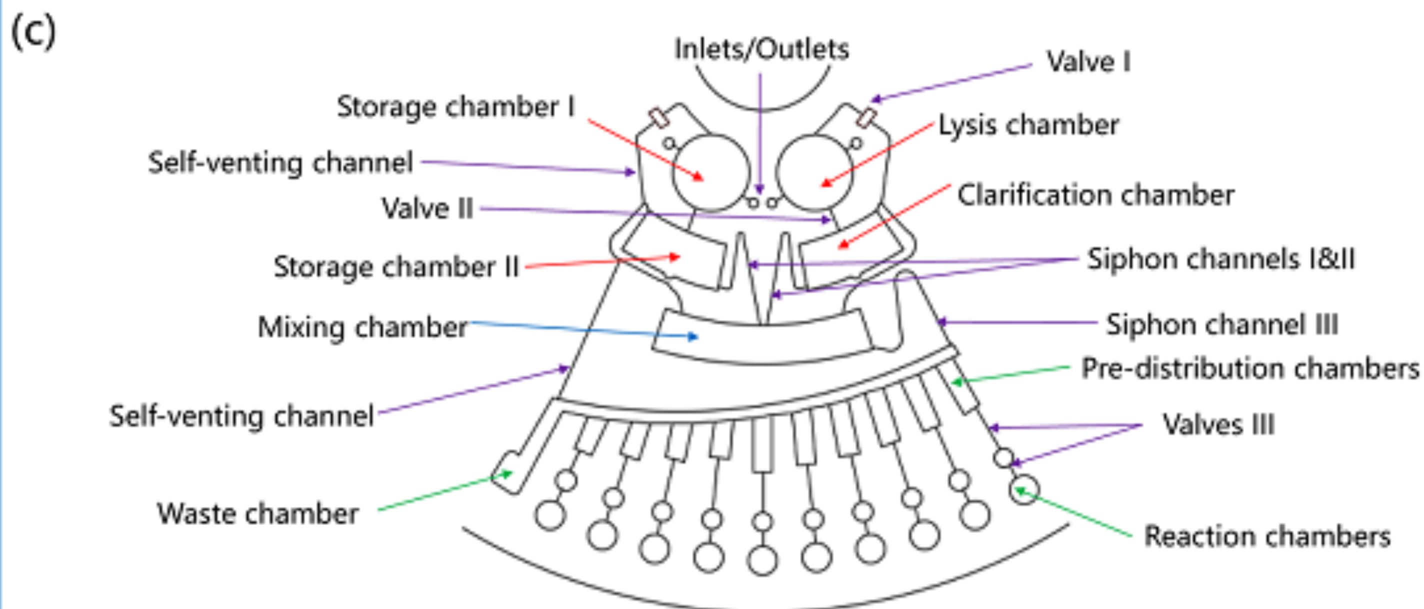
Clarification chamber

After lysis, **the lysate** was transferred to the **clarification chamber** via **high-speed centrifugation**. Cell debris precipitated during the process.

The transfer of the LAMP master mix from storage chamber I to storage chamber II occurred simultaneously.



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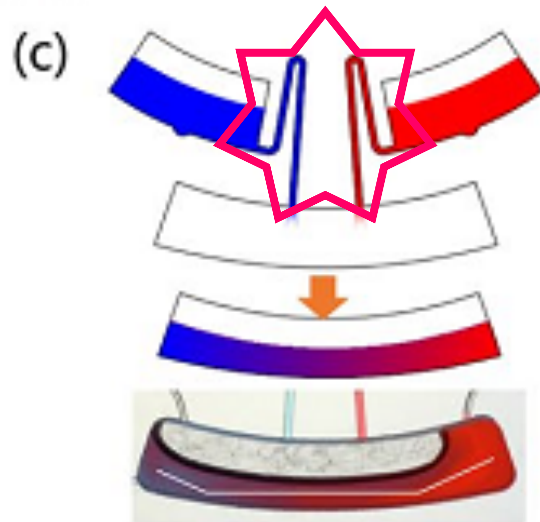
Mixing unit

- Homogeneous mixing of the LAMP master mix with the lysate is important for the success of the amplification reaction
- The **position of the two siphon channels** I and II affected the mixing of the two solutions



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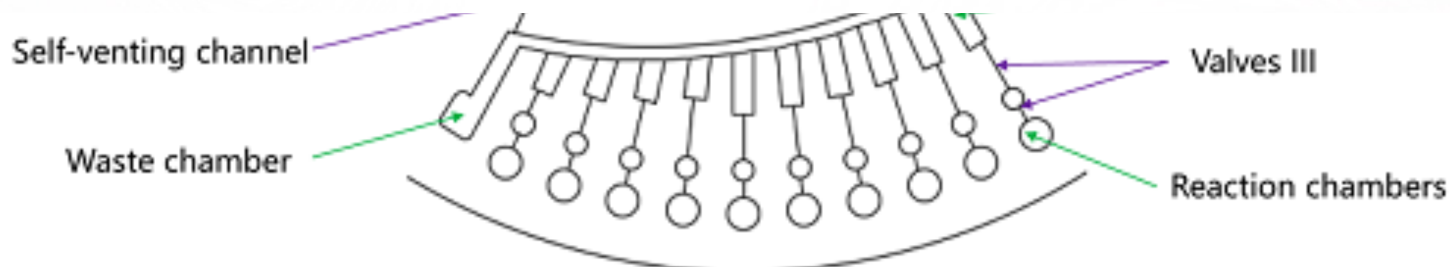
Pre-distribution/reaction unit

- After mixing, the mixture of the lysate and LAMP master mix was introduced into each reaction chamber
- The volume of the reaction chamber was **10 µl**, and specific primer pairs were preloaded into each well and dried under room temperature before sealing the chip.
- The mixture was aliquoted into 10 pre-distribution chambers before it was centrifuged into the reaction chambers



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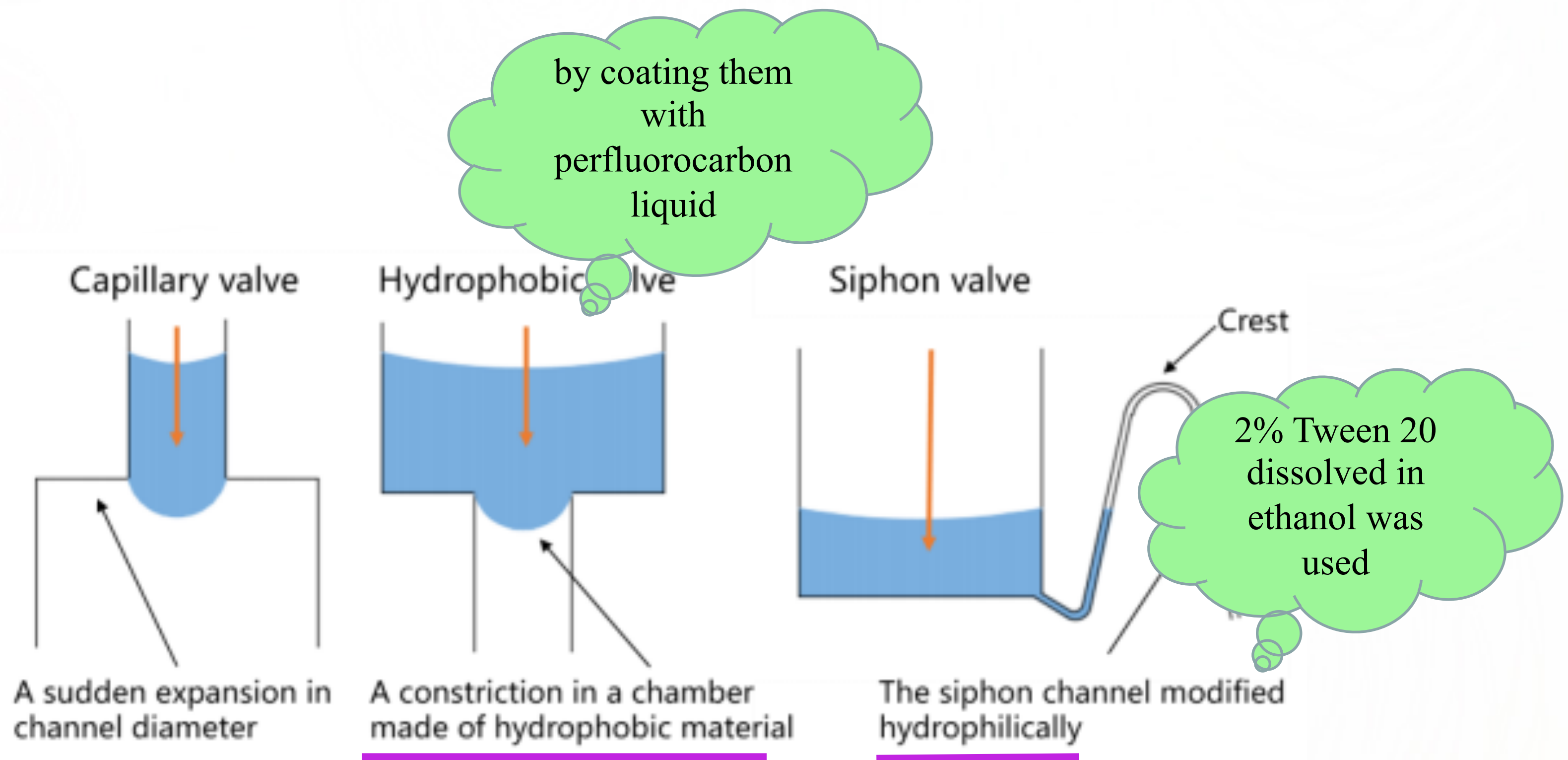
Liquid control unit

- In addition to centrifugal force, liquid control was enabled by capillary/hydrophobic valves, siphon channels, and self-venting channels



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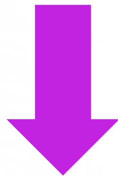
2. Cell culture and counting

- *E. coli, B. subtilis, Salmonella typhimurium, S. aureus*

were grown in 20 mL LB broth, at 37 °C for 12 h

- *Streptococcus uberis* and *Streptococcus dysgalactiae* were cultured and counted using BHI medium



A small portion of the culture was diluted to an appropriate concentration with water, and the cell counts were estimated by plating diluted cultures on nutrient agar plates

different concentrations of diluted cultures for the actual on-chip experiments

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Reagents and devices

- The genomic DNAs of bacteria

  were extracted by chemical methods using a TIANamp Bacteria DNA Kit

- LAMP mastermix and bovine serum albumin

- Four to six primers for LAMP reaction

- The gel and chip images were obtained and processed using a gel imager

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Preparation of bacterial samples for analysis

- *S. aureus* and *S. typhimurium* were both spiked into human serum at varying final concentrations (10^5 , 10^4, 10^3 CFU/ml) to mimic clinical sample

- Sterile syringe filters were used to isolate bacteria from the serum

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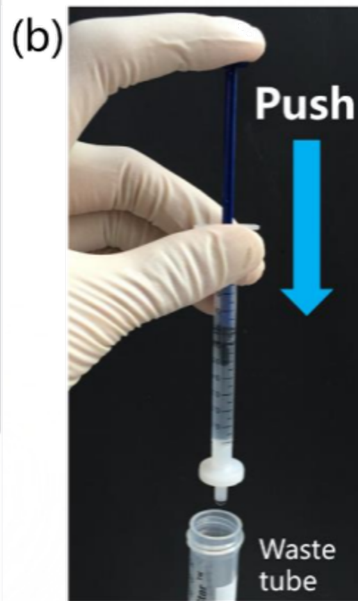
Cont.

1. 200 µL serum was first diluted with 800 µL water

2. Diluted serum sample was pushed through a filter and bacteria were captured on it.



3. The filter was washed by water.

4. The syringe was pulled slowly and pure water was drew up and through the filter to suspend bacteria.

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Results

- Optimization of parameters for lysis of bacteria

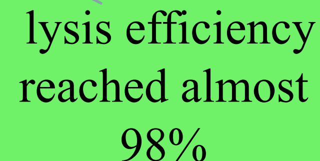
Three parameters, were varied to determine the optimal conditions for cell lysis:

1. **Stirrer voltage** high lysis efficiency was obtained at **4 V**
2. **Bead quantity** **0.2 g** beads gave the best lysis efficiency

Having optimized the voltage and the amount of beads

Determine the optimal time for lysis of six different types of bacterial cells. Lysis efficiency: (N original− N alive)/N original

3. **Lysis time** **3 min** as the time for lysis



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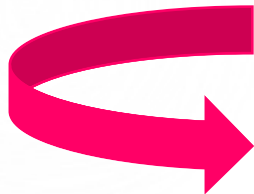
Results

- The results of the bacterial cell lysis experiments demonstrated that the on-chip bead-beating procedure adopted by us was highly efficient in lysing both Gram-negative (Eco, Sty) as well as Gram-positive bacteria (Bsu, Sau, Sub, and Sdy).

- At the end of 0.5 min, the efficiency of lysis were for Eco (Gram-negative) cells 90.2%, Bsu(83.3%), Sau(64.6%), and Sub(76.8%).



Supports the fact that Gram-positive bacteria have sturdier cell walls

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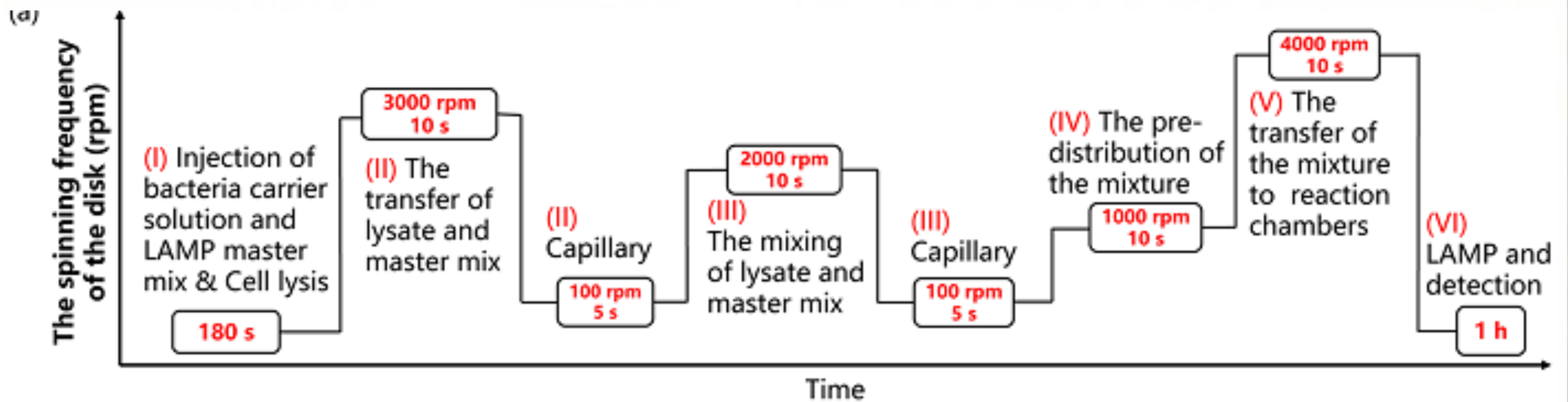
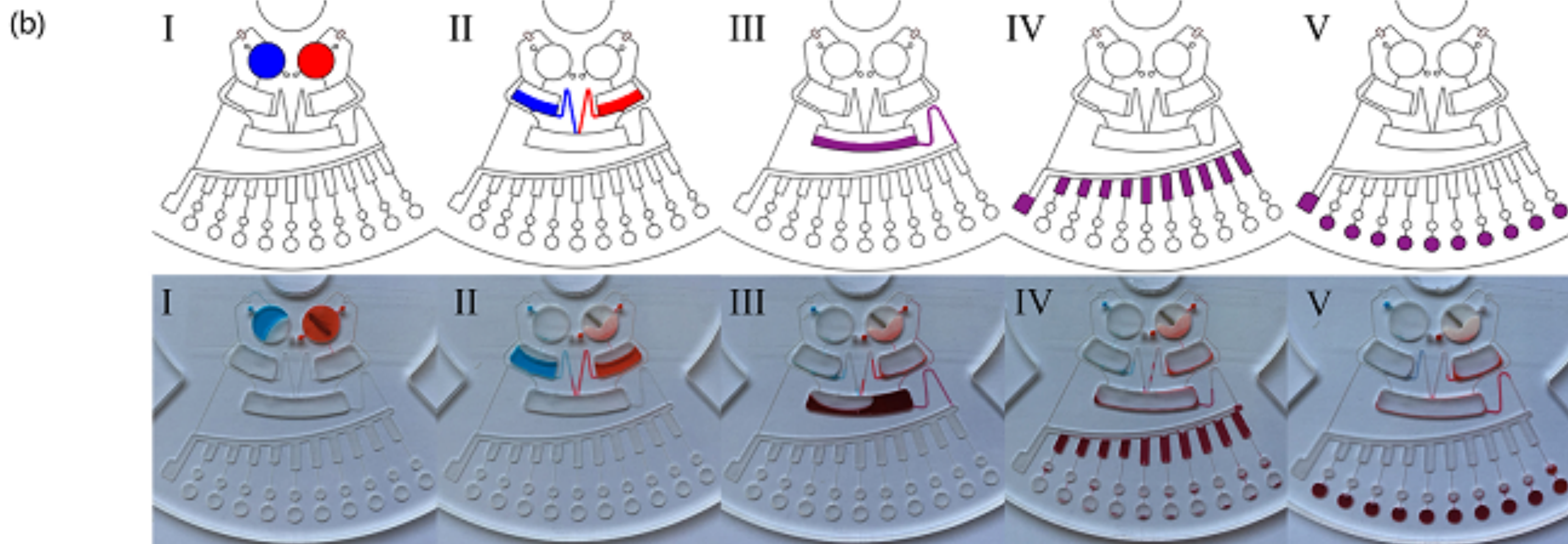
Workflow of the chip





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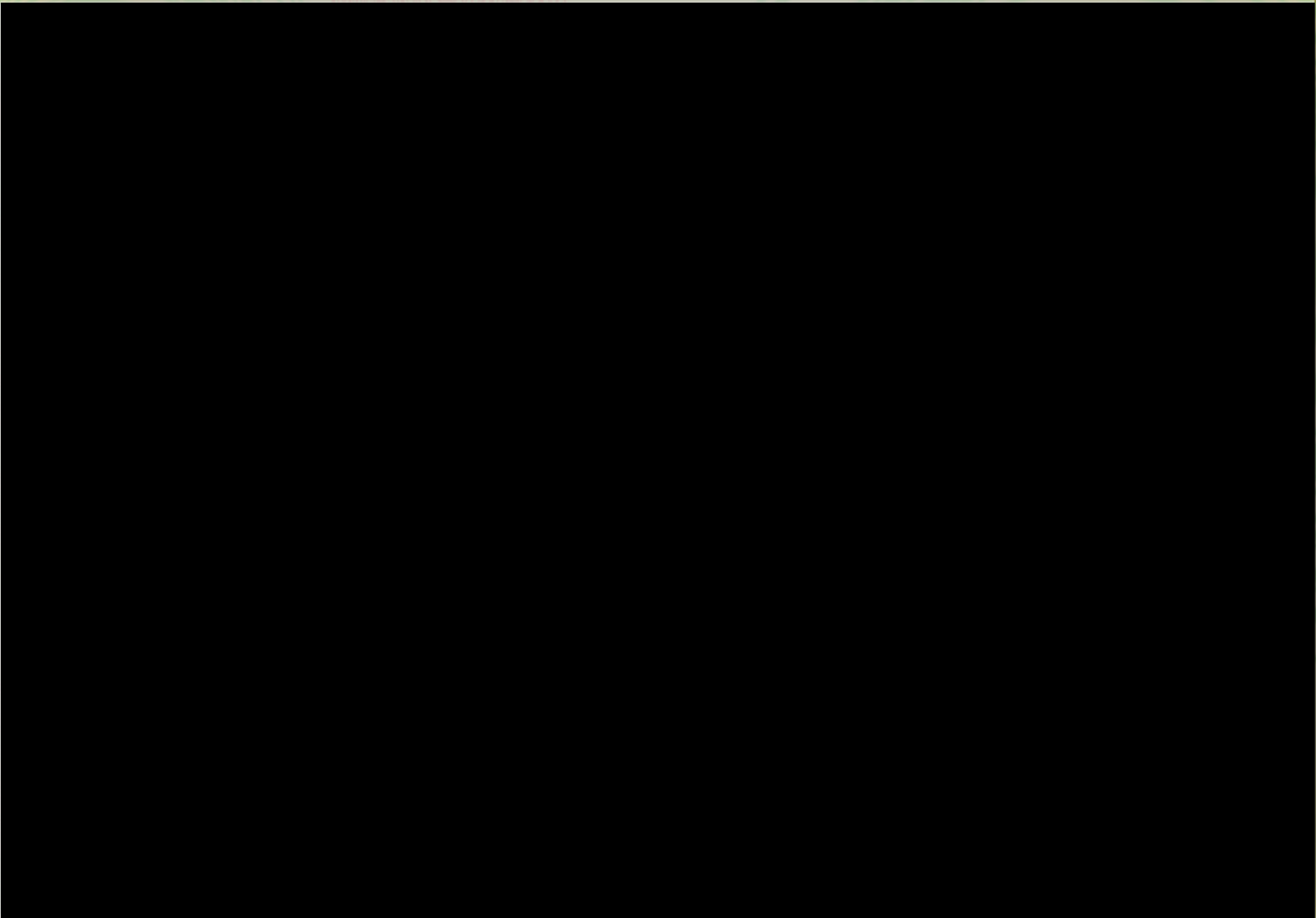

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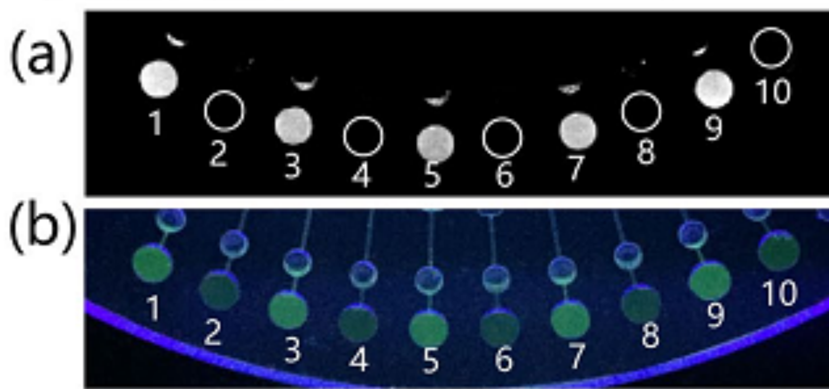
Testing for cross-contamination

- Evaporation of liquids was inevitable during the heating step at 65 °C, raising the possibility of liquids seeping into adjacent wells.

- To determine if the evaporation led to cross-contamination of primers, we preloaded primer pairs for Sty in odd-numbered reaction chambers and primer pairs for Sau in even-numbered reaction chambers.

- Only Sty cells were added to the lysis sample



✓ Strong signals in the

No cross-contamination

✓

numbered chambers

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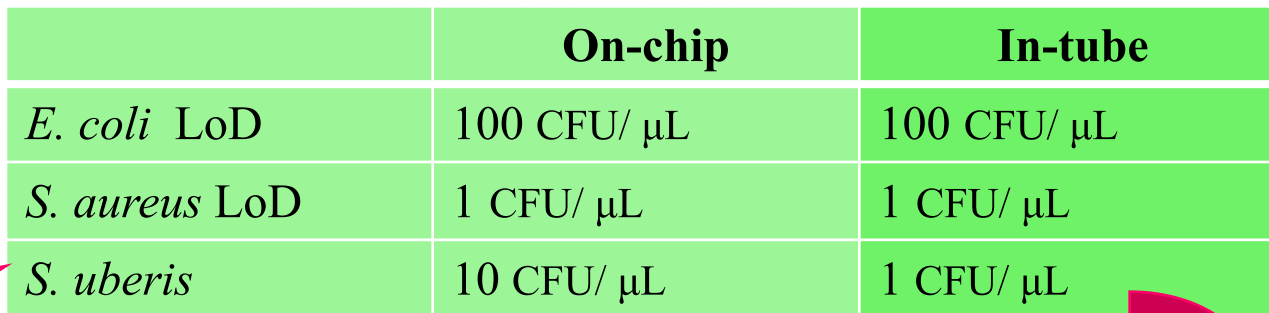
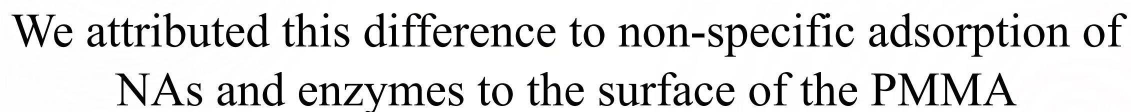
LoD for different kinds of bacteria

- Limit of Detection : LoD
- The minimum concentration of bacteria that resulted in a detectable electrophoresis band



	On-chip	In-tube
E. coli LoD	100 CFU/ µL	100 CFU/ µL
S. aureus LoD	1 CFU/ µL	1 CFU/ µL
S. uberis	10 CFU/ µL	1 CFU/ µL



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Specificity of multiplexed detection of bacteria by the chip

- Ideally, a chip should be able to detect different types of pathogens rapidly and accurately

Twelve combinations of bacteria spiked in water were prepared as samples to test the performance of this chip in study.

- Results of experiments indicating that the integrated chip was robust and the chip was able to accurately identify bacteria

- The current chip design could be upgraded to detect more pathogens in separate reaction chambers on a single chip

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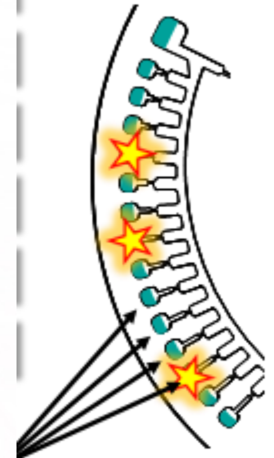
Real-time detection of bacteria in serum samples

- The fluorescence intensities were monitored by real-time fluorescence scanning

The sensitivity of this assay could be further increased by:

- Using a larger volume of serum
- A smaller volume of elution buffer
- Enhancing the enrichment factor
- Optimizing the LAMP primer pairs

Avoid false negative results



PCR chambers with primers/probes pre-loaded to specifically amplify DNA fragments of interest

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Discussion

- ✓ This method of detection is rapid, highly sensitive and accurate.
- ✓ Operations like mechanical lysis using beads, clarification of cellular debris as well as LAMP have been integrated seamlessly and are performed in an automated mode on the chip
- ✓ Different types of bacteria could be detected accurately

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Cont.

The chip displayed the following advantages:

1. It was kept stationary during lysis, which simplified the use of valves and, therefore, was easy to fabricate and operate

2. The time of duration of the entire workflow (~70 min) this chip has an advantage over others for use in emergency situations

3. The amplification results could be detected using an imager, a real-time fluorescence monitor and by the naked-eye

4. No cross-contamination between adjacent chambers, reducing the risk of false positives

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Cont.

- Current efforts are focused on enhancing the utility of our on-chip analysis method for the detection of bacteria from other kinds of samples such as urine, sputum and river water. Clinical samples usually include blood or sputum, which need to be processed before they can be analyzed on the chip.

- Efforts are underway to integrate the step of separation of serum or plasma from whole blood, the liquefaction of sputum and other bacteria capture methods on the chip itself.

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Summary

- The chip constructed in this study was robust and user-friendly
- Accomplish multiplexed detection of bacteria rapidly and accurately
- Steps like lysis of bacteria, clarification and LAMP were integrated and could be successfully automated on the chip
- A powerful tool in clinical diagnostics for multiplexed detection of bacteria from different samples

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