5h *Salmonella* detection in milk samples

Protocol and methodology developed within LoveFood2Market EU project

Introduction

LF2M methodology relies on the use of advanced micro-nano-bio technologies and components for the rapid and simple detection of *Salmonella* Typhimurium strain in milk. It consists of 3 steps and an unprecedented time-to-analysis of 5 h, including the pre-enrichment step.

Materials needed per test

- Magnetic particles pre-functionalized with anti-*Salmonella* Abs (50 μl of 0.5 mg in PBS)
- LAMP amplification mix (25 μl) and 0.1% Triton (10 μl)
- Solution A: surface blocking polymer (50 μl of PLL-PEG)
- LB medium (25 ml) and PBS buffer (500 μl)
- 1 falcon (50 ml) & 1 Eppendorf tube (1 ml)
- 1 plastic card (to run 4 tests)
- 1 acoustic biochip (to test 4 tests)

Instrumentation

- Electronic & peristaltic pumps units (+ PC)
- Docking station
- Magnet integrated in a falcon/Eppendorf base

Protocol*

Step 1: Pre-enrichment (4 h)

- Mix 25 ml milk with 25 ml of LB and 0.5 mg (50 μl solution) of functionalized magnetic beads in the falcon tube and Incubate at 37^{0}C for 4 hours under shaking.

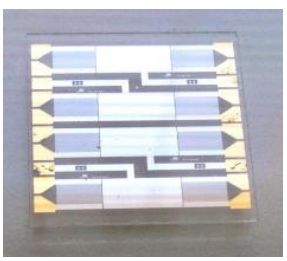
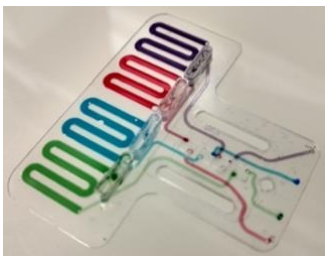
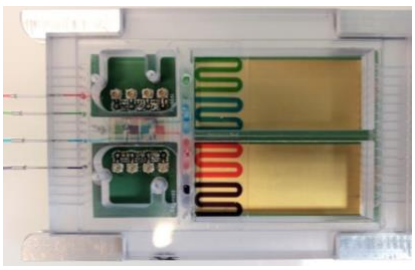
Step 2: Washing and cell-lysis (30 min)

- Place the falcon on a magnetic separator (5 min), discard the supernatant and dilute the magnetic particles in 500 μl PBS buffer; transfer the suspension in an Eppendorf tube, discard supernatant and add 10 μl of Triton-X solution. Incubate at RT for 10 min (or at 98^{o}C for 1 min), then add 15 μl of the LAMP mix.

Step 3: Amplification and detection (30 min)

- Place the acoustic biochip and plastic card (A) inside the docking station (B) and pipette manually 20 μl of solution A (pLL-g-PEG) in the test channel.
- Load the 15 μl of the LAMP mix and press start.
- Results are displayed after ½ h in the PC under experiment.dat file.

*The protocol can be used for the detection of **1-10 *Salmonella* cells in 25ml of** milk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