INTEGRATION OF COLORIMETRIC LOOP-MEDIATED ISOTHERMAL AMPLIFICATION (LAMP) IN MINIATURIZED DEVICE FOR THE DETECTION OF FOODBORNE PATHOGENS

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Introduction

With increasing pressure in the food supply chain, the product are coming to the market faster, leading to less time to control and ensure their safety. For this reason, faster methodologies, allowing the detection of different pathogens at the same time and an in-situ analysis performed by unspecialized technicians need to be developed to follow this intense production. In Europe, more than 23 million people fall ill from eating contaminated food every year, resulting in 4654 deaths and more than 400,000 disability-adjusted life years [1] Miniature devices have the advantage to automatized the analysis and reduce the costs [2]. The integration of colorimetric isothermal amplification technique in a miniaturized device, combine the reliable and sensitive detection of DNA-based detection with a naked-eye detection in an easy to use system. In this study the development of a LAMP reaction in a miniature systems was achieved for the simultaneous detection of *Salmonella* spp., *E. coli* O157 and *L. monocytogenes*. Two different devices were tested, a microfluidic channels composed by 8 capillarity-driven microchannels and a different alternative with an integrated heating system where silicone tubing are placed to performed the reaction. The two devices were compared analysing pure culture of the targeted pathogens, where the silicon tubing system was chosen for its higher sensitivity. The final methodology combining a short enrichment of 7 hours with the colorimetric LAMP reaction integrated in the miniaturized device was then evaluate for the analysis of different type of milk samples (UHT, Fresh and Raw).

Materials & Methods

Food analysis with improved short enrichment

Enrichment
25 mL sample + 25 mL TSB
↓
37 °C, 7 h
↓

Sample treatment
Centrifugation
↓
Protease treatment (Alcalase/ Neutrase)
↓
37 °C, 10 min
↓
Centrifugation
↓
Washing 2X (PBS + surfactant)
↓

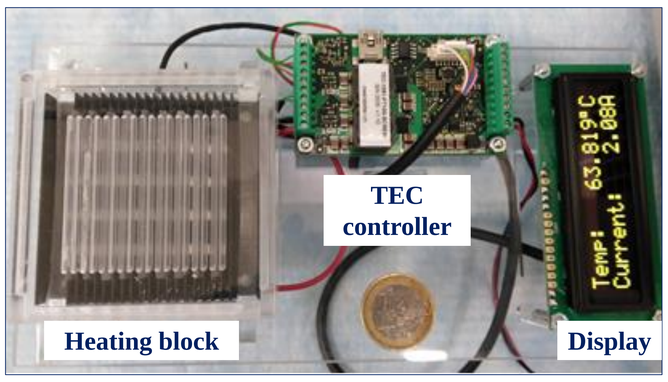
DNA extraction
Enzymatic lysis (Lysozyme/ Achromopeptidase)
↓
Protein degradation (Guanidine Thiocyanate)
↓
DNA precipitation (Ethanol)
↓

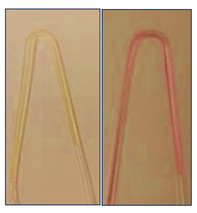
DNA amplification
Colorimetric LAMP

L. monocytogenes (*plcA*) — *Salmonella* spp. (*invA*) — *E. Coli* O157 (*rfbE*)

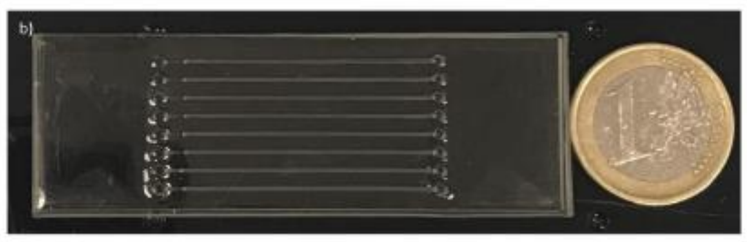
Miniaturized devices

Integrated heating system
where silicone tubing are placed to performed the reaction.





Microfluidic device composed by 8 capillarity-driven microchannels
To be incubated in a conventional laboratory incubator



Results

Comparison between miniaturize device and conventional approaches

Bacteria target	Methodology	LoD DNA (ng/ µL)	LoD Bacteria (cfu/ mL)
L. monocytogenes	qPCR	0.022	6.91×10^4
	Thermocycler	0.022	6.91×10^6
	Tubing device	0.22	6.91×10^7
	Microfluidic channels	-*	-*
E. coli O157	qPCR	0.0148	3.27×10^4
	Thermocycler	0.0148	3.27×10^5
	Tubing device	0.148	3.27×10^5
	Microfluidic channels	1.48	3.27×10^6
Salmonella spp.	qPCR	0.0193	1.28×10^4
	Thermocycler	0.0193	1.28×10^5
	Tubing device	0.0193	1.28×10^6
	Microfluidic channels	-*	1.28×10^7

*Not possible to determine the value, as the initial DNA extract (not diluted) gave a negative result

Tubing device chosen for final methodology
Due to the poor performance of the microfluidic channels

Final methodology performance
Milk samples analysis

	LoD_{50}*	LoD_{95}*
L. monocytogenes	32.5	140.6
Salmonella spp.	3.4	14.7
E. coli O157	3.9	16.7

"*" cfu/ 25 mL.

- **Complete analysis in 9 hour instead of 7 days for the three target pathogens in multiplex.**
- **This approach shows a real advantage for the food industry, reducing the time of analysis, and allowing to automatize the procedure.**

References

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