

**ainia centro tecnológico** (ES) is a private, non-profit making association focusing on the agri-food and related sectors. It aims to promote R&D, increase production quality, improve competitiveness and stimulate industrial modernisation and diversification.

The **University of Southampton** (UK) works closely with business and industry. The School of Biological Sciences hosts the Environmental Healthcare Unit, recognised for the detection, tracking and physiology of biofilms and pathogens in the water and food chains, and in hospitals.

The **Dublin City University** (IE) carries out research and training for industry and has spun out several companies. The School of Biotechnology hosts the Applied Biochemistry Group, focused on the generation of antibodies and their applications in sensor-based analytical systems.

**PHOTEK Ltd** (UK) is a specialist manufacturer of vacuum based tubes and camera systems for photon detection. Its product range includes: camera systems, image intensifiers, photomultiplier tubes, streak tubes plus a range of associated electronics.

**BETELGEUX, S.L.** (ES) is a company specialised in food hygiene, bringing solutions in the following areas: cleaning and disinfection in food industries; microbiological analysis of food, water, surfaces and air; and training of food handlers and food industry staff.

**40-30** (FR) is a company with expertise in vacuum and gas systems, electronics, clean process, non destructive test and rear base deployment. Its main activity is related to the repair/refurbish of equipments and sub assemblies.

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**BIO LISME**  
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**B**♦ Betelgeux



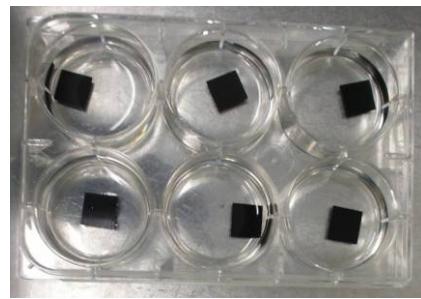


Figure 1. Six-well system for biofilm formation.

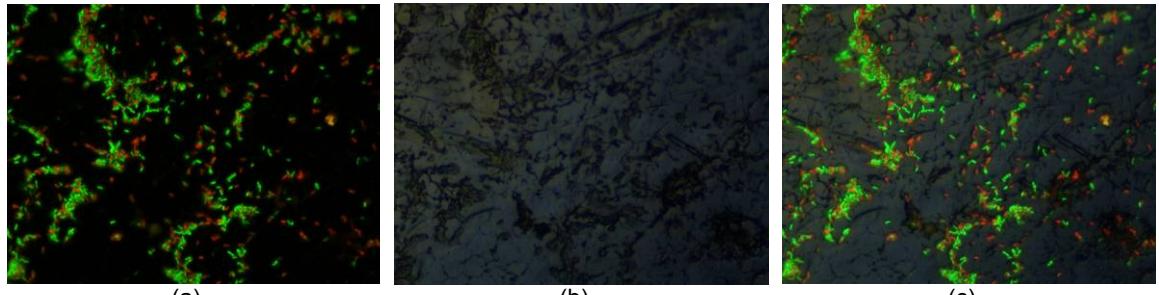


Figure 2. Observation in situ of the coupons. Magnification x1,000 of a 24 h-old biofilm of *L. monocytogenes* formed in TSB at 22°C: (a) stained with LD (combining FITC and TRITC filters), (b) under EDIC filter and (c) combination of both.

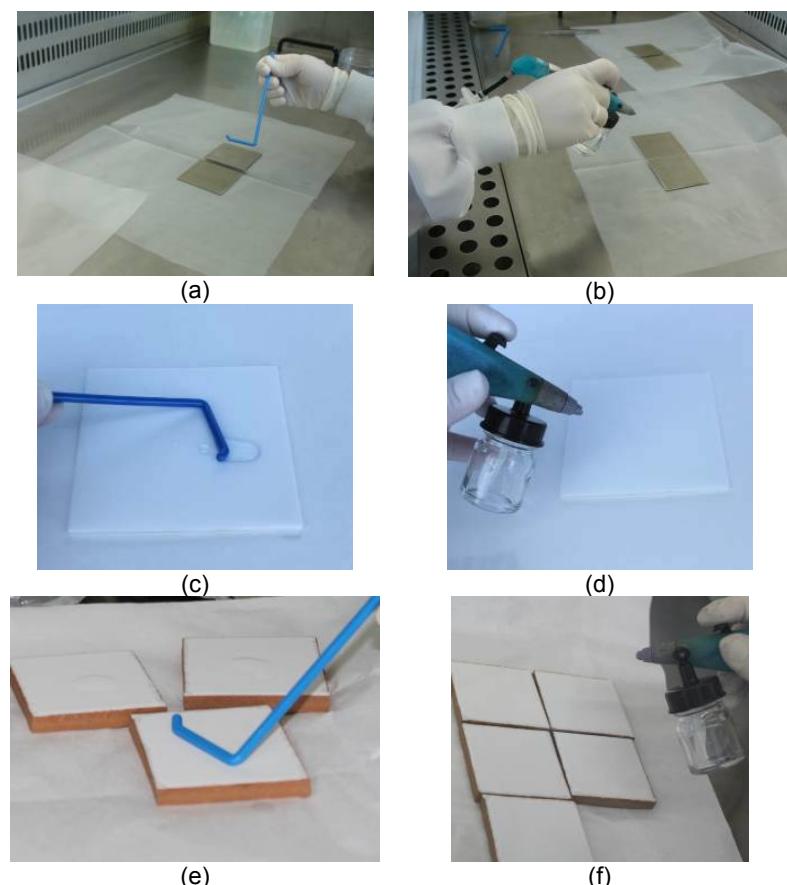


Figure 3. Inoculation of *L. monocytogenes* planktonic cells with a spreader (left column) and an aerograph (right column) on (a, b) SS, (c, d) Teflon and (e, f) ceramic surfaces.

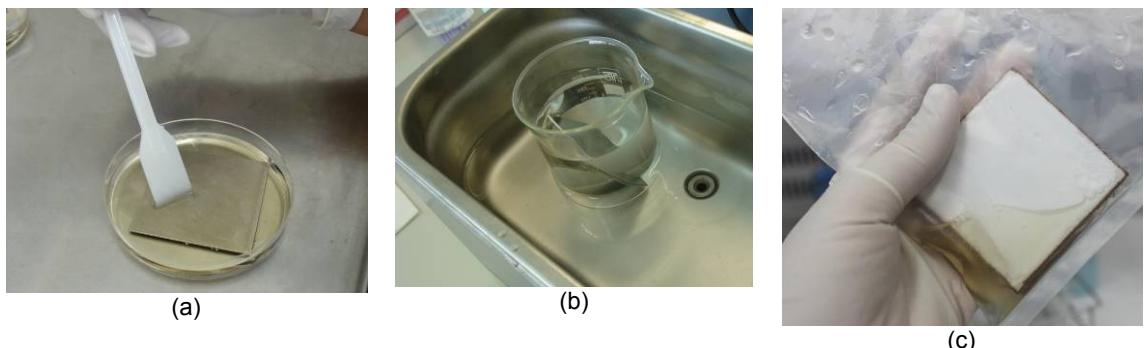


Figure 4. Recovery of *L. monocytogenes* inocula with a: (a) palette, (b) sonicator and (c) massaging.

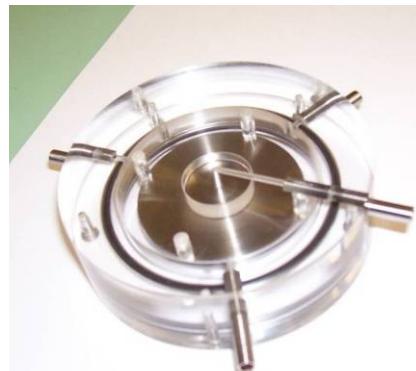


Figure 5. Radial flow cell (RFC) to assess the shear stress to detach *L. monocytogenes*.



Figure 6. First set of trials: sampling areas before (brown) and after (transparent) sampling with the prototype.

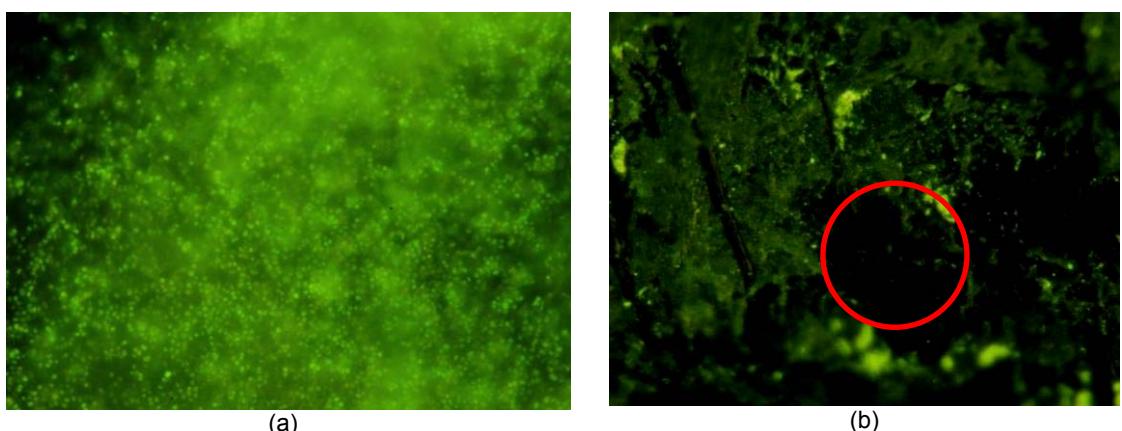
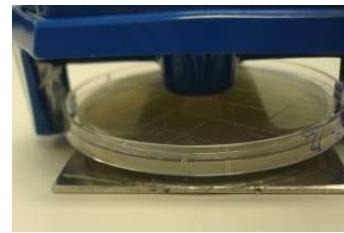


Figure 7. Second set of trials with *L. innocua* biofilms: (a) control biofilm and (b) biofilm after sampling with the prototype.



(a)



(b)

Figure 8. Conventional sampling techniques: (a) swabs and (b) Rodac contact plates.

Table 1. Comparison of BiolisSME and conventional techniques.

Sample	Conventional techniques	BOLISME technique
Planktonic cells	1.7% (using swabs) 1.9% (using contact plates)	≈26%
Biofilms	3.4% (using swabs) 1.3% (using contact plates)	>100%



Figure 9. *L. monocytogenes* cells + mAb2B3-magnetic beads.

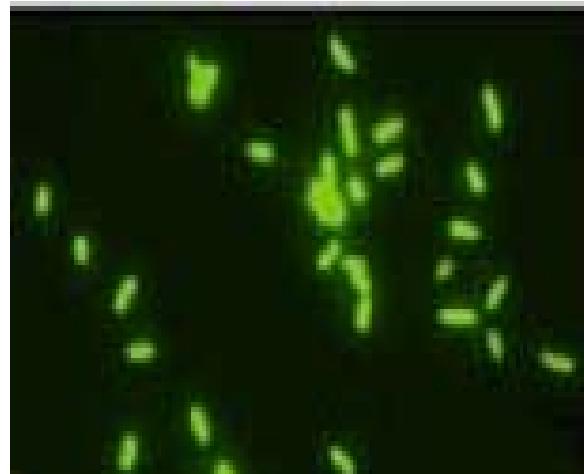


Figure 10. Fluorescence microscope image of detection of *L. monocytogenes* using mAb 2B3-fluorescent bead conjugate.

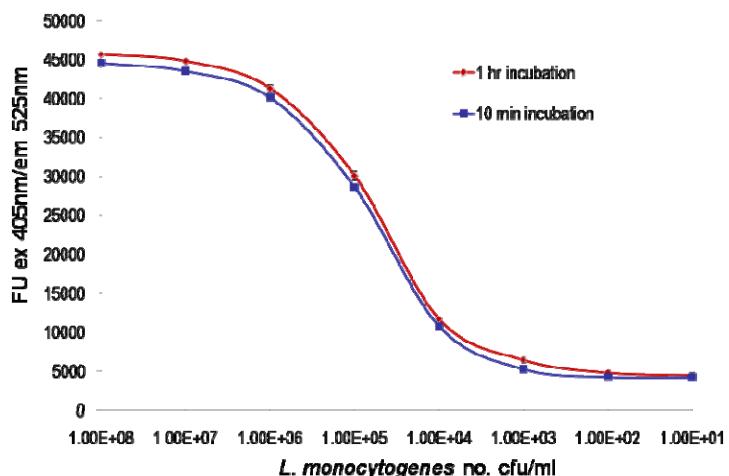


Figure 11. Sandwich FLISA showing the limit of detection of the capture and detection of *L. monocytogenes* in biofilms using mAb 2B3 with incubation times of 1 h and 10 min.

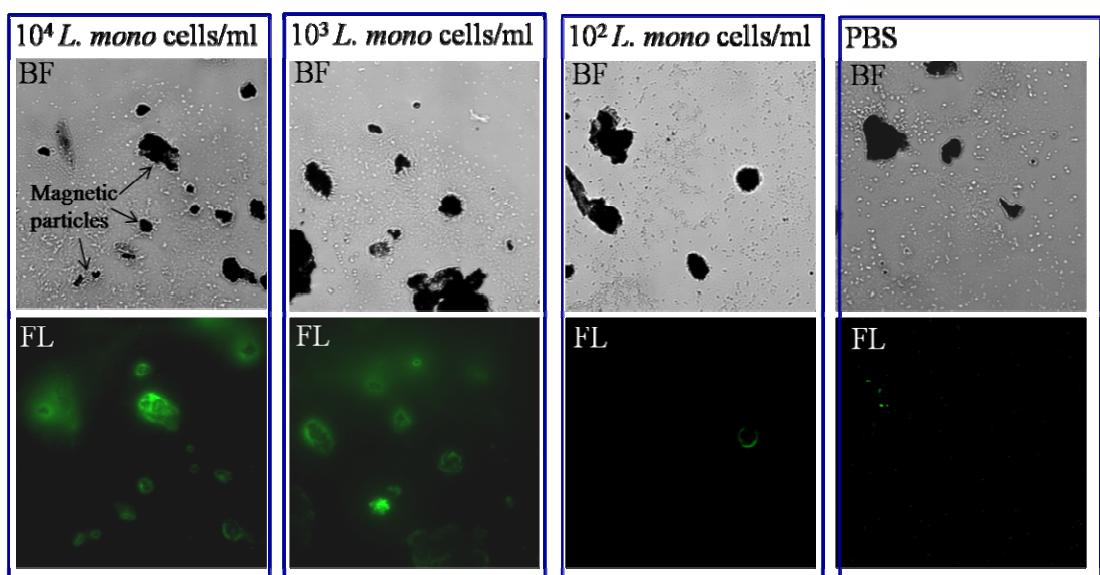


Figure 12. Brightfield (BF) and fluorescent (FL) images of capture and detection of *L. monocytogenes* cells grown in biofilms on glass slides using fluorescent microscope

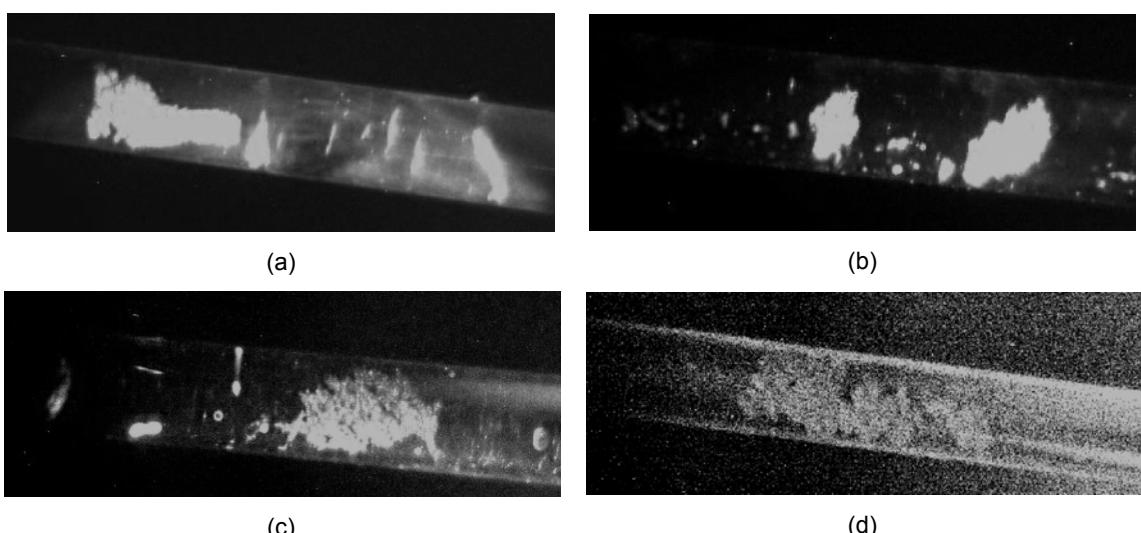


Figure 13. Images captured by means of the immunosensor corresponding to samples with concentrations of *L. monocytogenes* of : (a) 1E7, (b) 1E6, (c) 1E5 and (d) 1E4 cfu/mL.

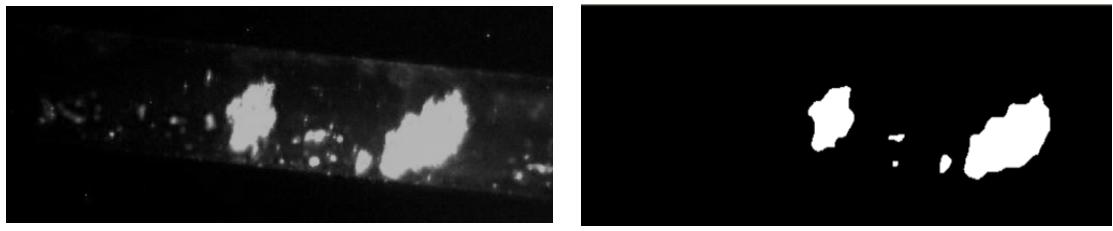


Figure 14. 1E6 *L. monocytogenes* cfu/mL sample: (a) image captured by the immunosensor and (b) screenshot of the result of the image processing.

Table 2. Results with the sampling system in a RTE food processing plant.

SAMPLING POINT	SAMPLING METHOD			
	WIPE		BOLISM	
	PLAQUE	MINIVIDAS	PLAQUE	MINIVIDAS
Slicer L22 (cured meat) - inner surface	—	—	—	—
Slicer L22 (cured meat) - outer surface	—	—	—	—
Slicer L21 (serrano ham) - inner surface	—	—	—	—
Transportation belt L21 (serrano ham)	—	—	—	—
Aluminium tray (chorizo)	—	—	—	—
Slicer L27 (Cooked ham) - outer surface	—	—	—	—
Slicer L27 (cooked ham) - inner surface	—	—	—	—
Transportation belt L27 (cooked ham) - lower side	—	—	+	+
Slicer L27 (cooked ham) - meat landing zone	—	—	—	—
Floor next to drain	—	—	—	—

## BIOLISME

Food-borne infections, caused by the consumption of foodstuff contaminated with bacteria, viruses or toxins, keep at the top of the list of diseases affecting people every year. This is a public health problem, critical Europe-wide and world-wide, and not only for consumers, but also for the whole food chain.

*Listeria monocytogenes* is one of the pathogen microorganisms that cause great concern nowadays. This bacterium is the causing agent of listeriosis, a potentially lethal disease in high risk populations [newborns, pregnant women and immune-compromised people]. Avoiding the proliferation of this microorganism involves keeping a strict environmental cleanliness and disinfection, specially when considering its ability to grow in biofilms.

Sampling of the product and of working environment allows evaluating the compliance with HACCP programmes and Good Manufacture and Hygiene Practices. Nevertheless, current methods for the detection of *L. monocytogenes* in food products are not always reliable, such as: low recovery rates of the microorganism with the sampling methods; long duration and low sensitivity of the detection techniques; and need of special equipment and skilled staff. As a consequence, companies must wait several days since samples are taken until they receive the analytical results, having a risk of getting false negatives.

The objective of BIOLISME is to develop a system to monitor the levels of *L. monocytogenes* in food products. With this new system, companies will be able to carry out the determinations by themselves, analysing more samples *in situ* and in a quick, simple and reliable manner.

This system will bring a number of benefits, such as:

- Higher recovery rate of microorganisms.
- Improved detection levels of the microorganism.
- Integration of the steps of the analysis (sampling, sample processing and detection).
- Use of a user semi-automated system, minimum handling.
- Cutback in the duration and cost per analysis.
- And also:
- Increase in food safety levels for consumers.
- Competitive advantage for end user companies.
- New business opportunities for companies in related sectors.

BIOLISME [FP7-SME-2008-232007] is a 2-year project with a budget of 1,319,337 €.

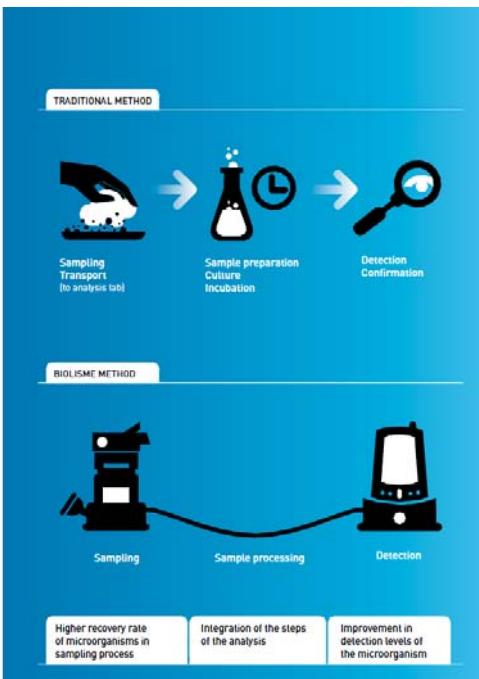


Figure 15. English version of the BioliSME leaflet.



## Co-operation profile details from Enterprise Europe SEIMED

11 ES 23C7 3NSI - Testing of new instruments for detection of Listeria monocytogenes on surfaces in contact with food.  
**Technology collaboration OFFER**

### Abstract

Listeria monocytogenes has been one of the greatest safety concerns for food industry in the last decades. A Spanish SME has developed two instruments that improve the performance of the methods used nowadays for sampling and detection of the bacterium on surfaces. The SME seeks industrial partners for a technical cooperation agreement with the purpose of testing and adapting the instruments to the requirements of different sectors.

### Description

Listeria monocytogenes has been one of the greatest safety concerns for food industry because it can cause listeriosis. This is a rare but potentially lethal foodborne infection which mainly affects the elderly, pregnant women and newborns, among others. The analysis of processing environments is an effective way to identify and prevent the presence of the pathogen in final products. However, it has been implemented by sampling and detection methods with several limitations.

A Spanish SME has developed two prototypes that improve some aspects of current methods, namely recovery rates and length of the analysis. A sampling unit was developed to recover microorganisms from surfaces in contact with food, in particular L. monocytogenes. Moreover, a detection unit was developed to estimate the concentration of the bacterium in environmental samples.

The SME seeks companies interested in testing the prototypes in their premises. The tests will involve identifying flat surfaces where contamination by L. monocytogenes may occur. Samples will be analysed by the prototypes developed by the SME and by conventional techniques. As a result, the companies will have a first hand demonstration of the prototypes' performance and will also receive a report with the results of the tests.

#### Innovative Aspects:

- \* Higher recovery rates of microorganisms adhered to surfaces with the sampling unit in comparison to conventional techniques.
- \* On-site detection of the bacterium in environmental samples in 1 h with the detection unit.
- \* Easy interfacing between both units.

### Target partner expertise sought:

- Type of partner sought: Industry.
- Specific area of activity of the partner: food processing companies concerned by the risk of environmental contamination by L. monocytogenes. Some targeted sectors are: meat, cheese, fish, vegetables, beverages and milk derivates, among others.
- Task to be performed by the partner sought: Testing of new applications of the prototypes.

### Key information:

Country of origin: Spain

Figure 16. Technology offer of the BioliSME project in the Enterprise Europe Network database.



Figure 17. Promotional flyer for BioliSME workshop.