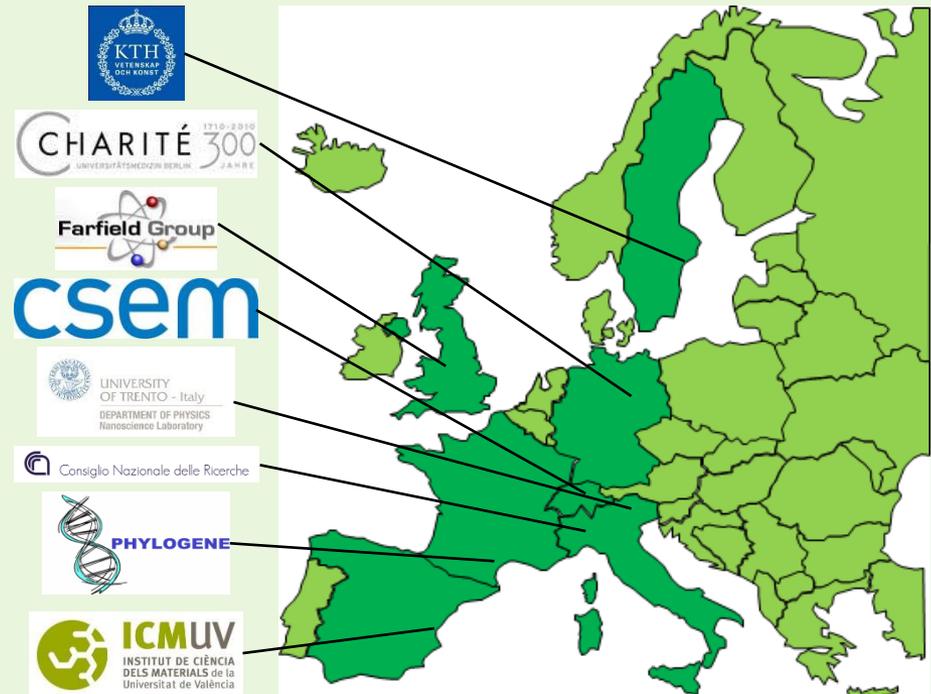


POSITIVE

A highly integrated and sensitive POrous SiLicon based lab on a chip for multiple quantitaTIVE monitoring of Food allergies at point of care.

Keywords:

Lab on a chip
Rapid cost-effective multiplexed biochip
integrated sample preparation
Microfluidic
Porous silicon
portable label-free multiallergy diagnostic
biomolecular recognition optical sensor



Contact information: www.fp7positive.eu

About food allergies

Food allergies – sensitization to food products

Life threatening:

Can provoke clinical reactions whose most severe is anaphylaxis, with respiratory and/or cardiovascular problems that might result in death.

They are common in 1-2% of adults and up to 8% of children, corresponding to a serious public health problem that affects over **15 million people in Europe** from infants to the elderly and its prevalence is increasing.



Current diagnostic technology:

Skin prick test - has its limitation.

Alternatives are blood based tests, mostly using the FEIA, RAST and ELISA techniques, usually lab-based.

Point-of-care devices exist; but only a few allergens at a time and give at best semi-quantitative determination.



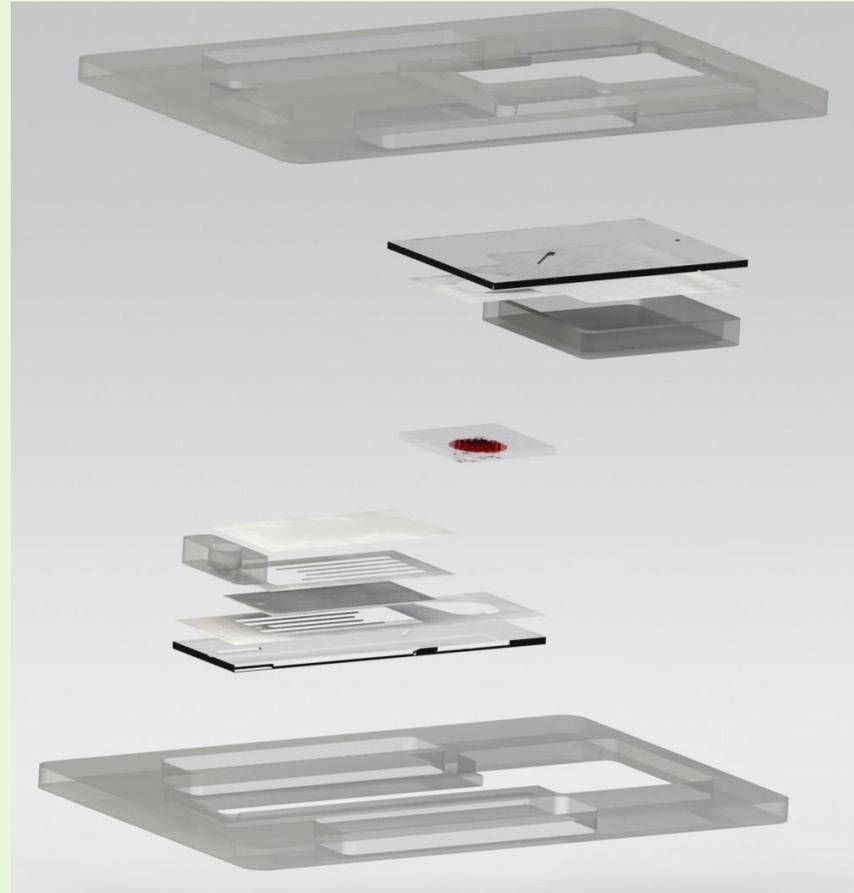
POSITIVE technology target:

100µL whole blood sample

Sensitization determination to 10 food allergies in 15'

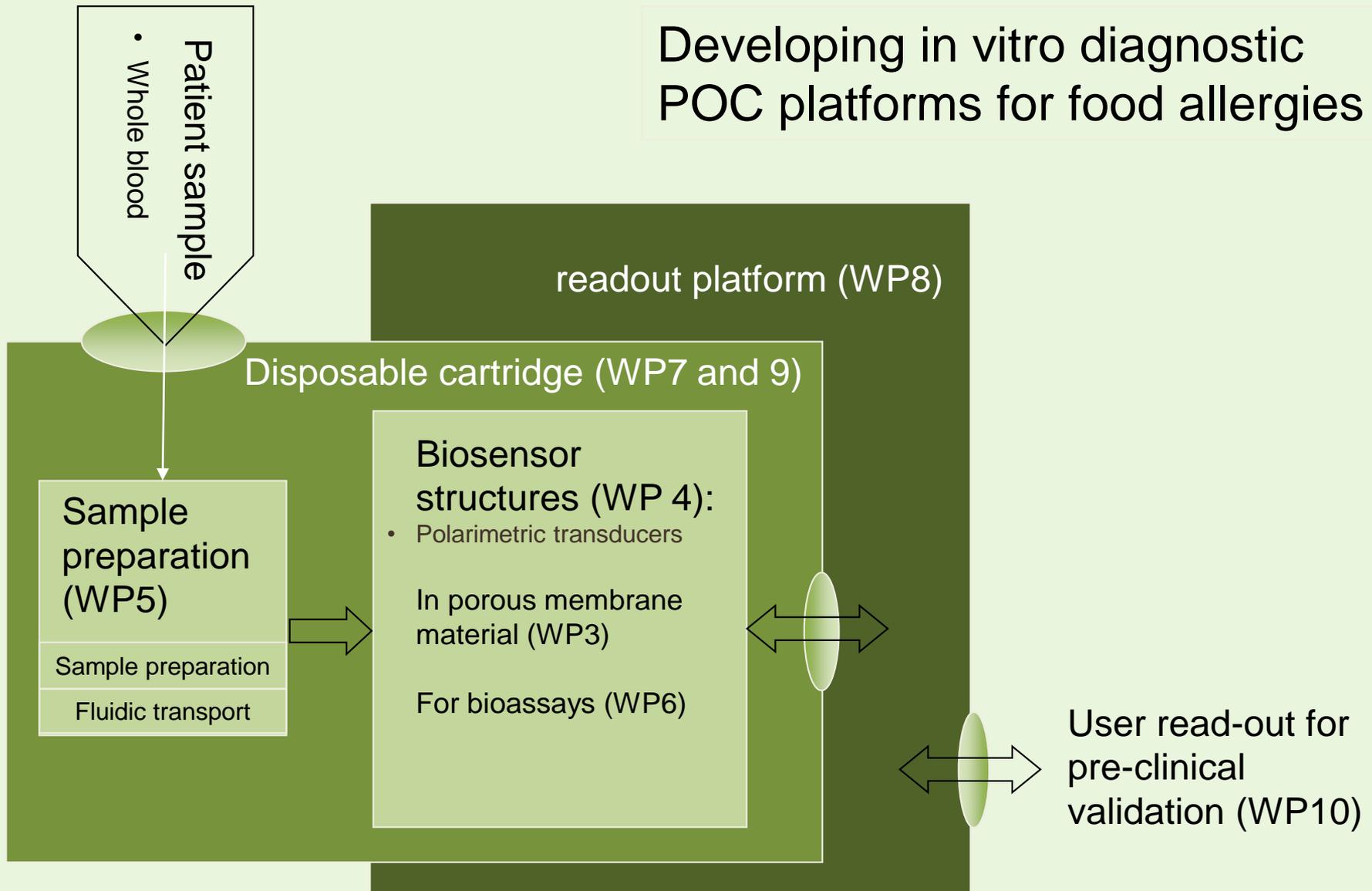
What was Our Goal

- A commercial product within two years of project conclusion.
- Innovative and commercially relevant research is ensured by the consortium's two technological companies' clear vision of what the market is and what is needed of the product if it is to be successful.
- All pediatricians should have this machine on their desk, whether they work in a hospital or in general practice.
- The companies' knowledge of the market really provides us with an excellent road map for innovation, making sure that we will be able to exploit our results fully.



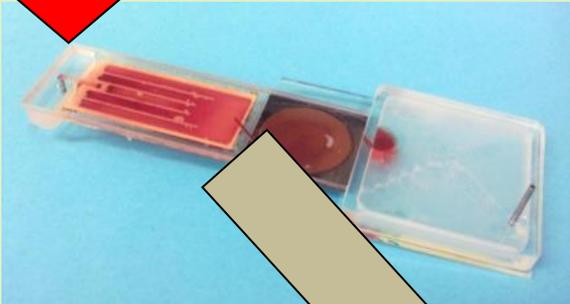
Positive diagnostic procedure

Developing in vitro diagnostic POC platforms for food allergies



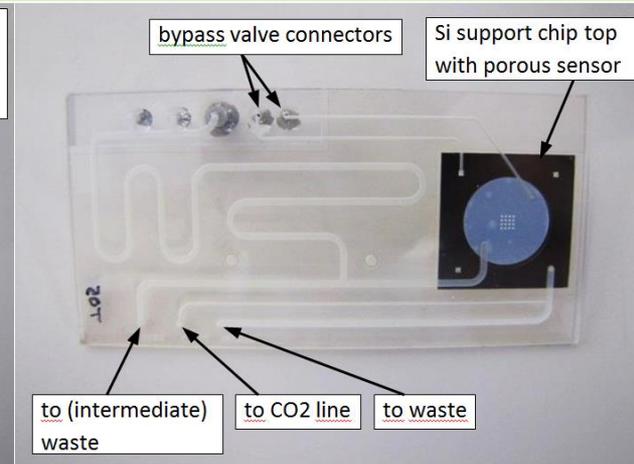
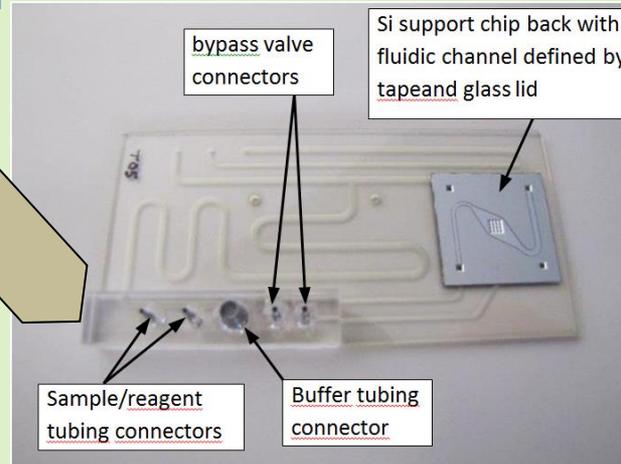
Sample preparation and transport

Whole blood

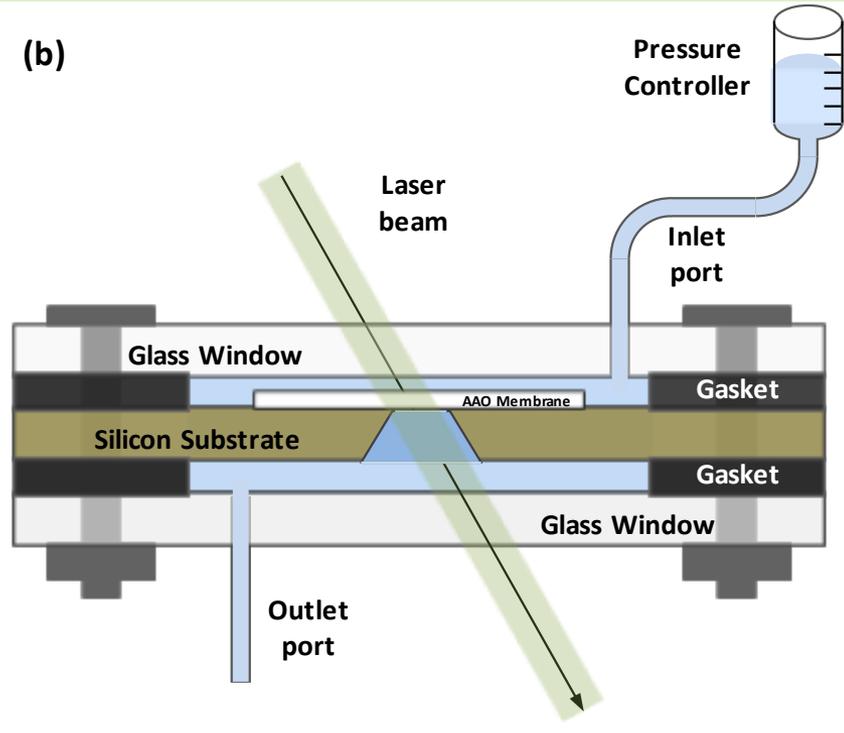
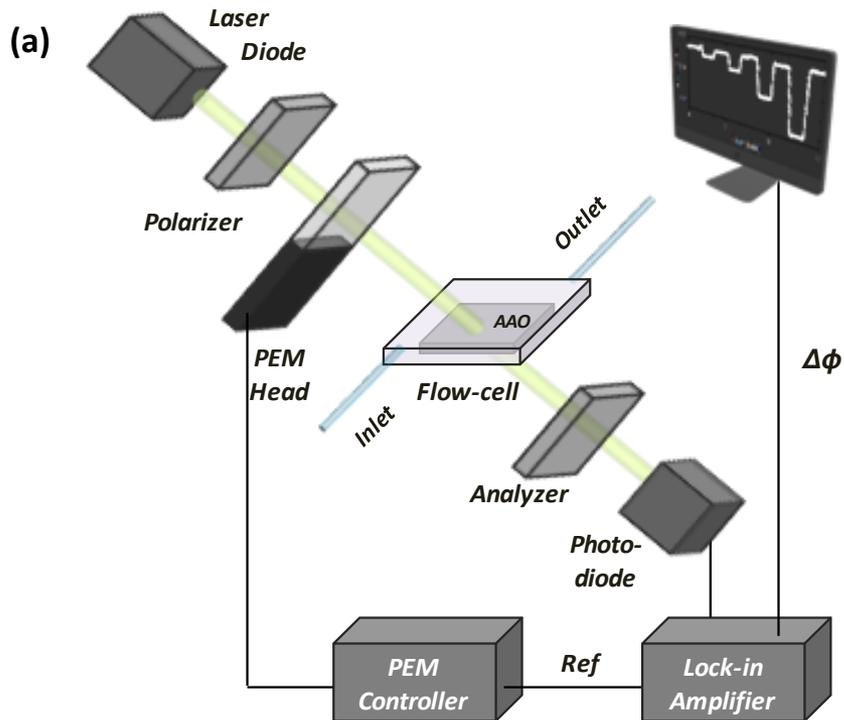


Filter mechanism for separation of serum from blood before directly entering a porous membrane

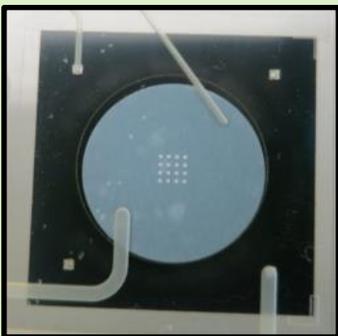
Cartridge assembly without blood filter included.



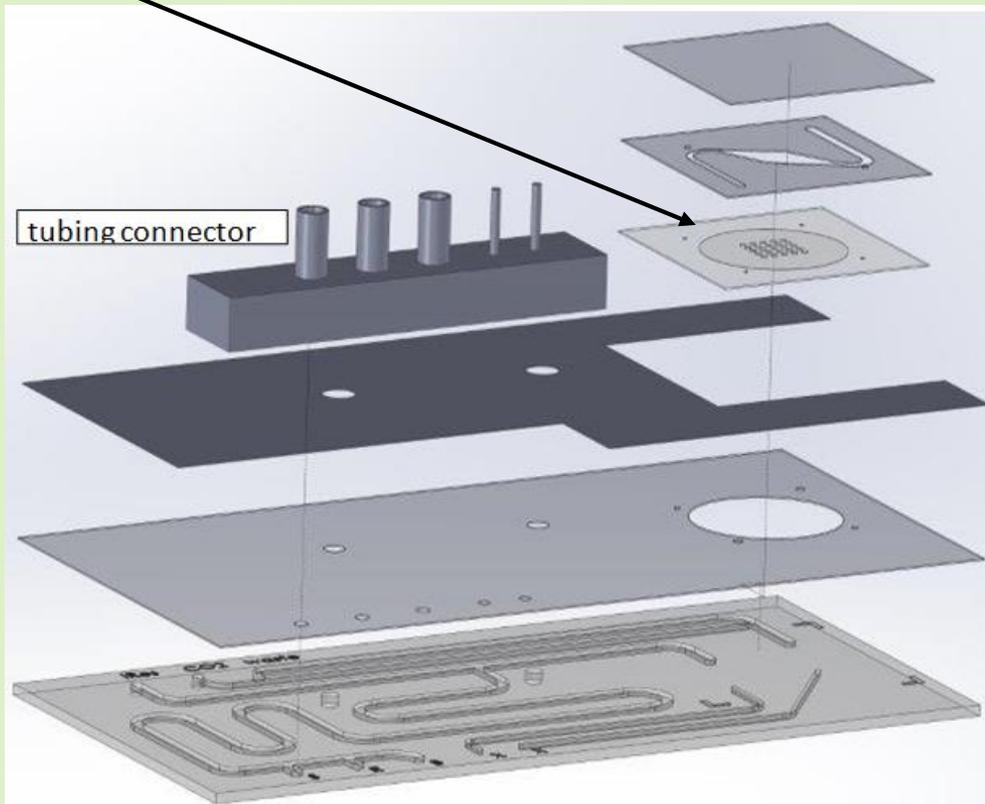
Layout of the optical polarimetric readout platform



Porous alumina sensor within the cartridge



Functionalised porous alumina membranes in a micromachined silicon chip support structure



glass cover

double sided adhesive tape with fluidic channel

Si-support chip with porous alumina membrane fixed to it

polymer protection

Double-sided adhesive tape

glass plate with fluidic

tubing connector

Positive Progress

Highlights of technology developed in Positive:

- After switching to porous alumina membranes the project progressed rapidly and following a 6 month extension in its final 18 months we have been able to demonstrate:
- Real time measurements for concentrations of IgG down to 33.7ng/ml (225pM), with a noise floor of 3.7ng/ml (25pM) and a good reproducibility, bound to Anti β -LG which is attached to the β -lactoglobulin immobilized on the functionalized porous alumina membrane for a total assay time below one hour for sample volumes (Anti β -LG) < 100 μ l. Capture efficiency was <70%.
- Serum flow through the mounted membranes with a very large binding response through physisorption and also flow through functionalized and β -LG spotted porous alumina chips for which a new mounting chip with a smaller spot size was developed.
- Chips can be multiply spotted with allergen and these multiple allergens are active on planar devices, both in array format as a fluorescent assay and in sequential experiments on a Refractive index sensitive device. One allergen has also been spotted and shown to be active on the porous POSITIVE device and used for a full concentration dependent assay.
- The multiplexed instrument showed very well its suitability for simultaneous multi-spot phase change measurements with linear response to different saline concentrations, so far down to a 4% solution or 10mrad.

Positive Innovation From Start to End

Although resources have not permitted us develop an instrument that meets all of the required end-user specifications within the time frame of the project whilst endeavoring to do so several interesting technologies or novel application of existing technologies have been developed and/or demonstrated including:

1. Combination of OSTE(+)¹ with copolymer. The method aims at improving and simplifying the batch back-end processing of microarrays and create microfluidic cells. The Biosticker is aimed to be a plug-in for existing microarray platforms to enable faster protein assays and DNA hybridizations through mass transport optimization. (KTH, CNR)
2. A micro-well platform enabling simultaneous flow through and optical inspection. This unique technology has applications in single cell studies, where the response of individual cells trapped in the micro-wells to stimulants supplied in the flow stream can be followed by microscopy in real-time. (KTH, CSEM, UVEG)
3. A high performance sensor chip thermal control system that has already been implemented in optical instrumentation in over a dozen international University and industrial research laboratories. (Farfield)

¹ Mercene Labs AB is a spin-off Company from KTH commercializing OSTE, which was developed during FP7 InTopSens and FP7 Positive, for device fabrication by customers.

Positive Innovation From Start to End

4. A module developed for blood filtering that enables several 100 μ l of whole blood to be filtered and plasma to be generated for subsequent analysis. This will find uses in lab on chip applications which require alternatives for plasma extraction from whole blood samples which is currently done in dedicated laboratories by centrifugation. (CSEM)
5. A module² for sequential actuation of a set of fluids through a microfluidic cartridge, which also enables priming of the cartridge with CO₂ and avoids the introduction of air plugs between the different fluids (CSEM).
6. A fluorescence based milk and egg allergen microarray for detection of specific IgE and IgG with sensitivity and reproducibility comparable to the commercially available ImmunoCAP ISAC from Thermo Fisher. (C-UB, CNR)

² CSEM is working on a demonstrator of a compact, stand-alone pressure driven fluid handling module and it is intended to have this ready for SLAS 2015 in Washington DC to present to the lab automation and instrumentation community. CSEM is also implementing such a module in two currently running projects, one for food quality monitoring and one for 3D cell tissue generation for pharma research.

Read more about the project at www.fp7positive.eu