Final activity report: Mass produced Optical Diagnostic Labcards based on Micro and Nano SU8 Layers

OPTOLABCARD

EU STREP PROJECT (N.016727)

Summary

This report describes how nine partners from six different countries across Europe were working together in a EU project focused on the development of a point of care system. This system uses disposable Lab on a Chips (LOCs) that carry out the complete assay from sample preparation to result interpretation of raw samples. The LOC is embedded and easy to replace using a packaging capsule. The OPTOLABCARD (www.optolabcard.com) has extended and tested the use of a thick photoresit (SU-8) as a structural material to manufacture LOCs by lamination. This project produced several examples where SU-8 microfluidic circuitry revealed itself as a viable material for several applications, such as the integration on chip of a nucleic acid amplification method that includes sample concentration, nucleic acid extraction, Polymerase Chain Reaction-PCR amplification and optical detection of *Salmonella spp.* using clinical samples and *Camplylobacter spp.* using chicken farm samples. The developed system was validated according to an ISO 16140:2003 standard and using crude sample.

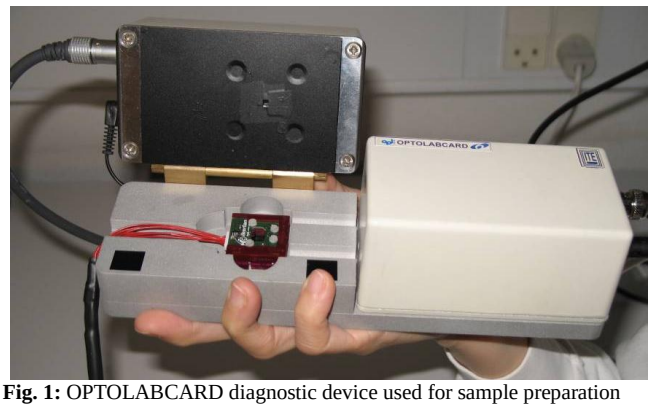

Fig. 1: OPTOLABCARD diagnostic device used for sample preparation and detection od Salmonella spp. and Campylobacter spp.

Contractors

N	Participant name	Country
1	IKERLAN S. Coop.	SPAIN
2	GAIKER	SPAIN
3	DTU Nanotech, Technical University of Denmark	DENMARK
4	Danish Institute for Food and Veterinary Research	DENMARK
5	Institute of Electron Technology	POLAND
6	EV Group	AUSTRIA
7	MICRORESIST micro resist technology, GmbH	GERMANY
8	SILEX MICROSYSTEMS	SWEDEN
9	BIOEF (Hospital de Donosti)	SPAIN

Fig. 2: Contractor list

Introduction

There is an urgent need to provide portable diagnostic tools to ensure rapid, affordable and simple analysis in many scenarios of our society (hospitals, airports, doctor's practice, roadside police controls, natural environment). The advance of Micro Electronic Mechanical System (MEMS) delivered some progress in this field. The 90´s was a decade of incredible microfabrication process developments and transducing mechanism characterisation. However, few of these processes have been transferred successfully into portable biological applications, mainly because of the lack of sample preparation integration and the difficulty to mass-produce the devices reliably. Currently, portable devices are based on low specific immunochromatographic strips or low sensitive electrochemical detection systems, whereas desktop systems are sensitive and automatic but bulky, heavy and expensive. Hence, owing to the new advances in dry film lamination and its synergy with Information Communication Technologies, it is now possible to prioritise and combine automatic sample preparation with low cost manufacture. This combination will creates an intelligent and portable system across many sectors for efficient health treatment, high productivity food processing and secure environment monitoring.

This report describes basic information of the project together with the employed strategies, applications and technologies is given. Finally, a review of the obtained results is presented in three sections: Fabrication, Biological and Validation results.

Consortium creation, Projects' description and Motivation

The OPTOLABCARD project was successfully submitted to a FP6 call. The project started in September 2005 and was finished in February 2009 with the budget of 2.7 M€. The partners involved were: IKERLAN-IK4 (coordinator), GAIKER-IK4 and Hospital de Donostia (via BIOEF) from Spain; DTU-Nanotech, DTU-Vet from Denmark; ITE from Poland; EVGroup from Autria; Microresist GmbH from Germany, and Silex from Sweden.

Projects' Strategy

The consortium partners have imposed a set of conditions in order to develop LOCs with future exploitation potential. Initially, the most important priority was to integrate the sample preparation using a

low cost Labonachip. The wafer strategy is valid for microelectronics where chips are small in comparison with LOCs, and not disposable. Therefore, a compromise had to be found between performance and cost. This is achieved by providing a set of convergence rules between the life sciences and the micro-nano technology. The applied convergence is described in the following sections.

Applications

The OPTOLABCARD project was focused on the development of a LOC that has two applications: detection of *Salmonella spp*. in human clinical samples and detection of *Campylobacter spp*. in broiler chicken production.

Fabrication and Microfluidic results

The OPTOLABCARD project delivered a methodology to fabricate and package a wide variety of LOCs made of a negative tone photoresist. This fabrication technology allows research laboratories to produce microfluidic channels on top of any biosensors fabricated on wafers within two days, offering an excellent tool for LOC prototyping production. Moreover, the 100% fabrication yield together with its lamination compatibility corroborates that this technique has a potential for future mass-production, guarantying low-cost and high reproducibility. The following sections will summarise some of the achieved results.

Methodology to fabricate LOCs

M. Agirregabiria *et al*. developed a method to integrate 3D microfluidic networks with biosensors to manufacture LOCs[1]. Basically, the method consists of repeating the following steps: (i) photolithography of two negative photoresist layers to pattern the microchannels on a wafer, and the sealing cover on a polyimide film (i.e. Kapton), (ii) bonding of the two photoresist layers together, and (iii) a final releasing step of the polyimide film. The first photoresist process can be carried out on a silicon, pre-processed silicon, polymeric or glass wafer. After these three steps, more SU-8 layers on polyimide films can be prepared, bonded and released, yielding 3D multilayered SU-8 stacks. The estimated manufacturing cost of SU-8 microfluidic circuitry on top of a biosensor with integrated platinium electrodes would be aproximately 2€/cm^2.[2]

This manufacturing process can be modified by substituting the rigid bottom wafer with another SU-8 coated polyimide films. This new process will create an SU-8-only device, since both top and bottom substrates are released at the end of the process from the polyimide films (See Figure 5). Detailed information about this process can be found elsewhere [3]. Using this tecnique, the manufacturing cost decreases dramatically to 0.07€/cm^2 whereas the wafer approach is 2 €/cm^2.[2]

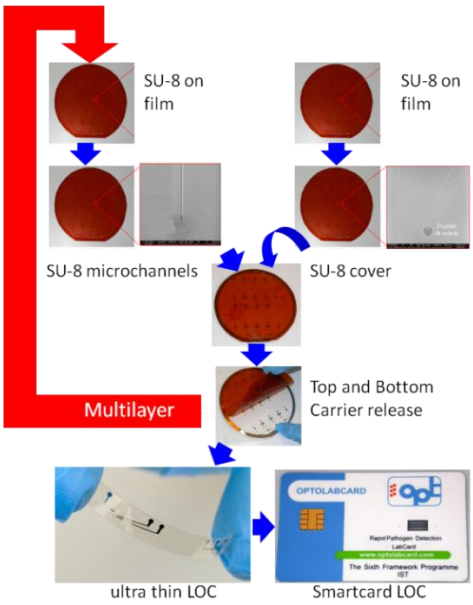

Fig. 3 Scheme of the fabrication process for the manufacture of SU-8 only devices. Both the micro channels and the cover layers are fabricated using a polyimide film as a substrate.

Packaging of LOCs

Since the LOCs should be disposable, it is essential to use a package which allows an easy replacement of the used LOCs. This package or capsule must include the proper fluidic, electrical and optical connections to the macro world without the need of adhesives or wirebonding. The multilayer approach LOC fabrication simplifies the packaging. All layers are photopatternable, therefore, the inlet and the outlet of the microchannels are kept in contact with the outside world (see Fig. 4). This fact avoids slow drilling or etching steps to the cover, radically simplifying the packaging. Hence, a plastic capsule with external flexible tubes and a low cost O-ring per reservoir are enough to insert liquids into channels, as can be seen in Fig. 4.

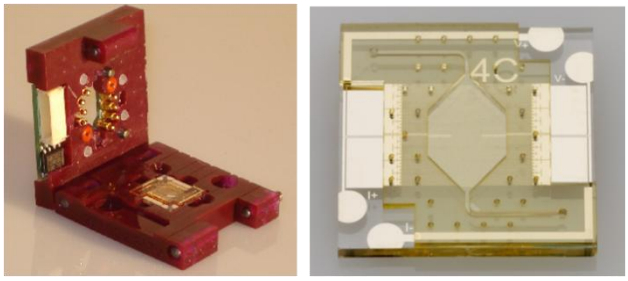

Fig. 4 Pictures of the packaging and the PCR chip where sample concentration and PCR takes place. This package allows an easy replacement of the LOCs since there is not glue or wire bonding. Below, you can find one of the biolical results.

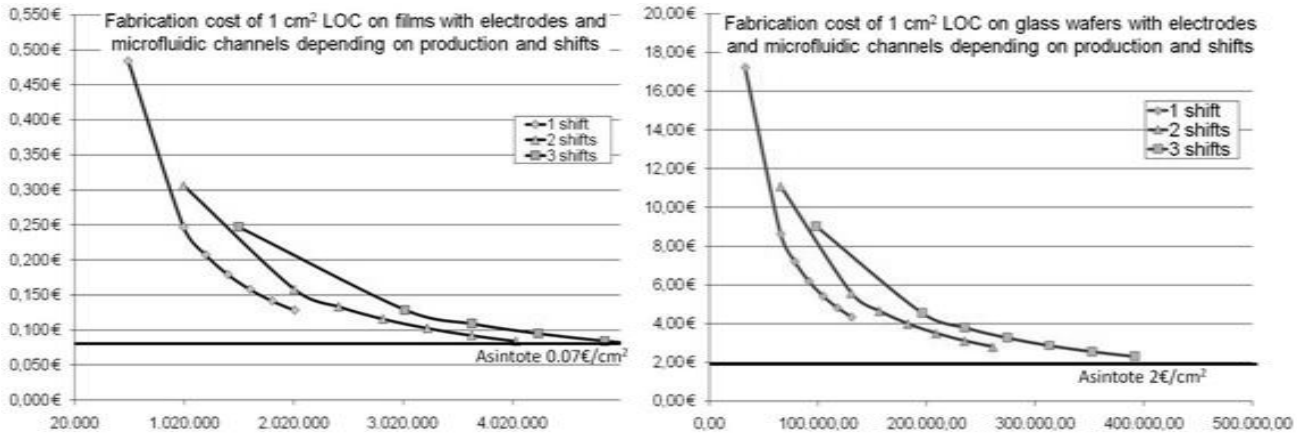

Fig. 5 Manufacturing cost of a LOC of 1 cm^2 versus production and working shifts. The cost is dramatically reduced if the bottom rigid wafer is substituted by another dry SU-8 layer on a flexible substrate.

Integrated SU8 Labonachip examples

This fabrication concept created several fabrication versions to be applied in different applications. Many of them made use of this fabrication to monolithically integrate SU-8 microfluidic circuitry on top of biosensors or, in other cases, to create different SU-8 microfluidic circuits on top of wafers. For example, an SU-8 microchannel was integrated on top of a Mach Zendher interferometer fabricated in silicon[4]. A SDS-CGE chip was succesfully tested to separate proteins on a glass wafer[5] and on polymethylmethacrylate (PMMA) wafer[6]. A flow sensor has been designed and fabricated[7]. SU-8 free-standing structures embedded in microchannels for valves and pumps have been designed[8] and fabricated[9,10]. A SU-8 microfluidic circuitry was monolithically integrated on top of a magnetoresistive immunosensor[11] to detect *Escherichia coli O157:H7*. A SU-8 LOC performed the purification and concentration of 1mL of raw sample plus to a final volume of 2.6µL for Real Time PCR[12] (see Fig. 5.). The concentration method was characterised obtaining a 90% bead capturing efficiency at a realistic flowrate (1mL/min)[13,14,2215]. PCR reagents were also freeze-dried within this PCR chip[16]. Other devices were developed using the mentioned alternative method, where the rigid substrate is substituted by another flexible film [3]. This technique was used to fabricate SU-8 microfluidic chips[2] and microneedles for drug delivery in *ex vivo* neural tissue[17] and for ischemia monitoring in a rat kidney[18].

Biological results

In order to get specific and rapid pathogen detection, it is crucial to reduce the processing time and the number of steps of sample pre-treatment. An important application of microfluidic systems is the ability to efficiently process crude biological samples and subsequently perform the required analytical assays "on-chip", as practical applications are demanding. The integration of sample pretreatment such as target bacteria concentration and specific detection by PCR into microfluidic devices need to be solved in LOC devices to enable a fully automated operation.

A SU-8 LOC has confirmed to be very appropriate for the fully integrated analysis system. A complete sample preparation plus qPCR-based assay for specific pathogen detection in crude biological samples, able to elude/avoid the effect of inhibitory compounds, has been proved. A model system for presence of *Salmonella*[xxii] and *Campylobacter* in stool and broiler chicken samples, respectively, has been used (Figure 4). Procedure for sample collection consists in target concentration using magnetic beads and real-time PCR for specific bacteria detection. All experimental steps, from sample preparation to qPCR detection, are carried out on a SU-8 microdevice based on a single 2.6 µl chamber.

Methodology for sample preparation on chip

The technique for clinical application is described in the following figure. Sample (a swab from stool samples or virus transport medium from nasopharyngeal samples) was transferred to lysis buffer and left to lyse the bacteria or virus. Next, resuspended magnetic beads were added to capture extracted nucleic acids. Sample containing beads was inserted in the packaged SU-8 microdevice using a syringe. The magnetic field, created by magnets placed under and above the device, captured the complex beads-(nucleic acid) while the liquid flowed away along the chamber. Subsequently, qPCR or RT-qPCR mastermix was inserted, the inlet/outlet were sealed, magnets were carried off and amplification was performed. The whole protocol takes about 30 and 75 minutes for stool and nasopharyngeal samples, respectively, including sample purification and amplification. [19].

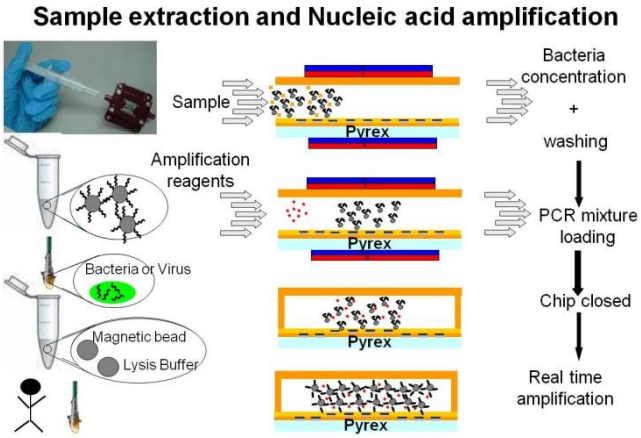

Fig. 6: Schematic representation of the sample extraction and nucleic acid amplification. [20]

Validation results

To characterize in terms of specificity, sensitivity and accuracy, the performance of the whole system for the diagnosis of Salmonella spp. and Campylobacter spp. detection the results were evaluated under EN ISO 16140:2003 standard against the "reference" methods. According to this standard some parameters were defined: (i) Relative accuracy, (ii) Relative sensitivity and (iii) Relative specificity.

Prevalidation for Salmonella *spp.* sample preparation and PCR

27 clinical samples were analysed using the microdevice under procedure described previously, with the aim of identifying *Salmonella spp* [7]. The real samples had been previously diagnosed by Hospital Donostia, reference method ,to establish Salmonella spp. load and to compare the results with the microdevice ones.

End point PCR product from reference and micro device methods	*Salmonella* spp
Relative accuracy	95.6 %
Relative sensitivity	95.6 %
Relative specificity	100 %

Fig.7 Result comparison between reference method and the microdevice for *Salmonella spp*. detection out of 27 clinical samples.

Prevalidation for Campylobacter *spp.* sample preparation and PCR

Of 48 farm sample tested, 36 were cloacal swabs samples collected from chickens at slaughter; 4 were socks samples and 8 were packed chicken from supermarkets. For more details see [8]. Next table presents the results of microdevice PCR detection compared to on tube conventional PCR. All the 4 sock samples were positive in both methods, while all 8 packed chickens are negative in both methods. In this case, samples were analysed using the detection system mentioned in figure 3.

End point PCR product from reference and micro device methods	Campylobacter spp.
Relative accuracy	95.8 %
Relative sensitivity	94.7 %
Relative specificity	100 %

Fig.8 Result comparison between reference method and the microdevice for *Campylobacter spp*. detection out of 48 chicken samples.

Spin off company

As a result of sound achievements of this project, a company namely microLIQUID[21] was created. This new company provides a set of standard LOCs together with customised fluidic solutions, based on the fabrication principles obtained through the OPTOLABCARD work.

Conclusions

The OPTOLABCARD device was validated and compare with a reference method for the diagnosis of *Salmonella spp*. in 27 clinical samples (human samples supplied by Hospital de Donostia) and *Campylobacter spp.* in 48 real samples (chicken cloacal swabs, socks and packed chicken) supplied by farmers and supermarkets. These analyses have studied the relative sensitivity, specificity and accuracy of the developed system and they have performed on the basis of EN ISO 16140.

All these data tohether with its portabibility confirm the good performance of the device developed when real samples are assayed, making it very suitable and appropriated for further pre-industrialization projects and to be used in other applications with real samples.

Patents

Apart from the 45 conferences, 8 fairs, 21 scientific articles and 20 new, an extensive work has been carried out in order to obtain a patent portfolio. The following table compiles the obtained patents and ideas.

OWNER	*ID*	*PATENT OR INTELECTUAL PROPERTY*
IKERLAN	1	Procedure for the fab. of µfluidic polimeric structures
	2	Flexible Microfluidic MicroNanoDevices
	3	Micro-Nanofluidic control devices
	4	New active valves based on flexible membranes and SU8
GAIKER-IKERLAN	5	Method and device to detect genetic material by amplification
EVG	6	Megasonic developer bath
Mrt	7	Waveguiding material
VET	8	Freeze dried reagents
ITE	9	Optics external integration

Future Work

Confidential exploitation actions are being launched and carried out to exploit the diagnostic results. On the

other hand, a new EU project has been granted to the consortium and new partners called LABONFOIL (www.labonfoil.eu). This new project has a wider scope. The project aims at developing four LOCs with four different applications and one fabrication equipment (See Fig. 9). The project will validate the developed LOCs in four fields:

- Labcard for marine algae analysis by NASBA.
- Labcard for pathogen typing in broiler production.
- Labcard for monitoring biomarkers of Colorectal Cancer by immuno-Q-PCR of a blood droplet.
- Skin patch to detect cocaine consumption by professional drivers.

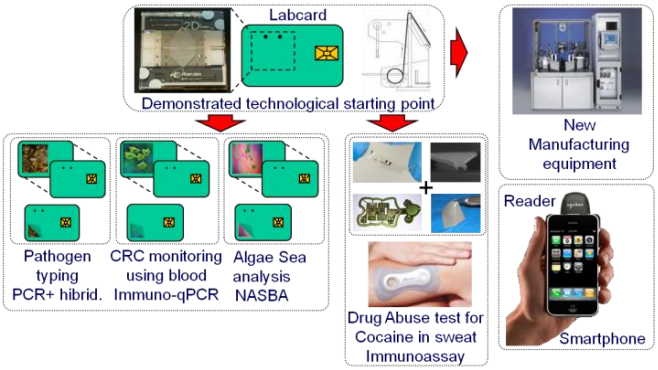

Fig. 9 Description of the equipment and applications. Three Labcards, a Skinpatch, a reader-smartphone and Dry film Lamination equipment.

Contacts

Coordinator: Dr. Jesus M. Ruano-Lopez, Ikerlan-IK4, Pº. Arizmedarrieta N.2, 20500 Arrasate, Spain, Fax:+34 943 712 400, Tel:+34 943 716 944; email:jmruano@ikerlan.es

Dr. Emilio Pérez-Trallero, Microbiology Department, Hospital Donostia, Paseo Dr Beguiristain s/n, 20014 San Sebastián, Spain, Tel:+34 943007046; e-mail:mikrobiol@terra.es

Dr. Anders Wolff, DTU Nanotech, Technical University of Denmark, Building 345 East, DTU, Kgs. Lyngby, DK-2800, Denmark, Tel: +45 45256305; email: Anders.Wolff@nanotech.dtu.dk

Dr. Anja Voigt, micro resist technology GmbH, Koepenicker Str. 325, Haus 211, D-12555 Berlin, Germany, Tel. +49-30-65762192; email:a.voigt@microresist.de

Dr. Garbiñe Olabarria, Gaiker-IK4, Centro Tecnológico, Ed. 202, 48170 Zamudio, Spain, Tel: +34 946002323, e-mail: olabarria@gaiker.es

Dr. Rafał Walczak, Politechnika Wrocławska-WEMiF, Janiszewski str. 11/17, 50-372 Wrocław, Poland, email:rafal.walczak@pwr.wroc.pl

Dr. Markus Norén, Silex Microsystems AB,Box 595, Bruttovägen 1, SE-175 26 Järfälla, Sweden, Tel: +46 8 580 249 02, email:markus.noren@silexmicrosystems.com

Dr. Markus Wimplinger, EV Group E.Thallner GmbH, Rechtsform: GmbH, Sitz: St. Florian am Inn, FN 215218y. Tel: +43 7712 5311 0, email:M.Wimplinger@EVGroup.com,

Dr. Dang D. Bang, Laboratory of Applied Micro-Nanotechnolog (LAMINATE), National Veterinary Institut, Tech nical University of Denmark, Hangøvej2, DK-8200 Aarhus N Denmark, Tel: + 45 7234 6892, email: ddba@vet.dtu.dk

References

[1] M. Agirregabiria, F. J. Blanco, J. Berganzo, M.T. Arroyo, A. Fullaondo, K. Mayora, J.M. Ruano-Lopez, 2005, *Lab on a Chip* **5**, 545-552.

[2] J.M. Ruano-López, M. Agirregabiria, V. Calvo, R. Vilares, I. Ugarte, A. Altuna, J. Berganzo, L.J. Fernández, J. Elizalde, M. Tijero, A. Ezkerra, I. Aranburu, K. Mayora, Commercialization of Micro and Nano Systems Conference, August 31st-Sept. 4th Puerto Vallarta, Mexico, 2008.

[3] I. Zugasti, J.M. Ruano-López, J.M. Goenaga, K. Mayora, Commercialization of Micro and Nano Systems Conference, August 31st-Sept. 4th Puerto Vallarta, Mexico, 2008.

[4] F.J.Blanco, M.Agirregabiria, J.Berganzo, K.Mayora, J.Elizalde, A.Calle, C.Dominguez and L M. Lechuga, Microfluidic-optical integrated CMOS compatible devices for label-free biochemical sensing. Journal of Micromechanics and Microengineering, Vol. 16, No.5, 1006-1016.

[5] M. Agirregabiria, F. Javier Blanco, J. Berganzo, A. Fullaondo, A. M. Zubiaga, K. Mayora and J. M. Ruano-López, Electrophoresis, 2006, Vol. 27, No. 18, 3627-3634 .

[6] M. T. Arroyo, L. J. Fernández, M. Agirregabiria, N. Ibañez, J. Aurrekoetxea and F. J. Blanco, Journal of Micromechanics and Microengineering, 2007, Vol 17, No. 7,1289-1298.

[7] R. Vilares, I. Ugarte, I. Aranburu, J. Berganzo, L. J. Fernandez, 2008, Eurosensors XII, Dresden, Germany, 338.

[8] A. Ezkerra, P.A. Wilson, K. Mayora, J.M. Ruano-López, 2008, J. Micromech. Microeng. 18, 095011, 12pp.

[9] Ezkerra A, Fernández L J, Mayora K, Ruano-López J M, 2007, J. Micromech. Microeng. 17, 2264-2271.

[10] A. Ezkerra, L.J. Fernández, A. Wolff, K. Mayora, J.M. Ruano-López, 2008, Proceedings of the 12th International Conference on Miniaturized Systems for Chemistry and Life Sciences µTAS'08, San Diego (California), 182-184.

[11] M.Mujika, S.Arana, E.Castaño, M.Tijero, R.Vilares, J.M.Ruano-López, A.Cruz, L.Sainz and J.Berganza , 2009, Biosensors and Bioelectronics, Vol. 24, N. 5, 1253-1258.

[12] M. Agirregabiria, D. Verdoy, J. Berganzo, L. J. Fernandez, G. Olabarria, J. Berganza, M. Pascual de Zulueta, P. Aldamiz-Echevarria, K. Mayora and J. M. Ruano-López, 2007, 11th International Conference on Miniaturized Systems for Chemistry and Life Sciences µTAS'07, Paris, France, Vol. 1 pp.584-586.

[13] M. Bu, T. B. Christensen, K. Smistrup, A. Wolff, M.l F. Hansen, Sensors and Actuators A 145–146 (2008) 430-436

[14] K. Smistrup, M. Bu, A. Wolff ,H. Bruus, M. F. Hansen, 2008, Microfluid Nanofluid, 4:565-573

[15] D. Verdoy, M. Agirregabiria, J. Berganza, J. Berganzo, J.M. Ruano-López and G. Olabarria. 2008. Rapid Methods in Food and Feed Quality Conference, Amsterdam, 21-23 January 2008

[16] Monica Brivio, D. D. Bang, Yiping Li , Bastian Gaardsvig Kjeldsen, Jakob Leffland Reimers, Minqiang Bu, Anders Wolff. 2007. 11th International Conference on Miniaturized Systems for Chemistry and Life Sciences µTAS'07, Paris, France,59-61.

[17] L. J. Fernández, A. Altuna, M. Tijero, G. Gabriel, R. Villa, M. J. Rodríguez, M. Batlle, R. Vilares, J. Berganzo and F. J. Blanco, Journal of Micromechanics and Microengineering, 2009, Vol. 19, No 2, 025007 (8pp).

[18] M. Tijero, G. Gabriel, J. Caro, A. Altuna, R. Hernández, R. Villa, J. Berganzo, F.J. Blanco, R. Salido and L.J. Fernández, Biosensors and Bioelectronics, 2009, In press, Accepted manuscript, Available online 24 December 2008.

19 Jesus M. Ruano-López et al., Perspective article, LabonaChip Journal, 9, 1495 - 1499, 2009

20 WO Pat. 2008116941, 2008.

[21] www.microLIQUID.com