GRAVI™- *Chip*: Automation of Microfluics Affinity Assay Using Magnetic Nanoparticles

Joël Rossier*, Sophie Baranek*, Patrick Morier*, François Vulliet*, Christine Vollet* and Frédéric Reymond*

*DiagnoSwiss S.A., Rte de l'Ile-au-Bois 2, CH-1870 Monthey, Switzerland
j. rossier@diagnoswiss.com

ABSTRACT

This paper presents a novel microfluidic analytical platform called GRAVI™ , with particular emphasis on the benchtop robotised version of this system which is dedicated to fast and automated enzyme-linked immunosorbent assays (ELISA) in low volumes. By dramatically reducing time-to-result (assay time of <10 min, including chip regeneration) and significantly decreasing sample/reagent consumption and cost, this microfluidic biosensor system enables to perform multi-menu immunoassays with simplified robotics and laboratory infrastructure.

Keywords: Microfluidics, microsensors, magnetic nanoparticles, ELISA, electroanalytical methods.

1 INTRODUCTION

For more than twenty years, ELISA has been a reference method in bio-analytics, particularly in *in vitro* Diagnostics (IVD) and in Life Science Research (LSR).

In order to meet the increasing demand for accelerated time-to-results and reduced sample and reagent consumption in ELISA, DiagnoSwiss has developed a microfluidics-based biosensor platform, which can be used as a manually operated stand-alone device or which can be coupled to standard robotic stations. The system, currently introduced under the trade name GRAVI™, comprises a compact instrument (GRAVI™-Cell), electrochemical microchip consumables (GRAVI™-Chip) and a computer software (GRAVI™-Soft) for developing assay protocols, running the analyses and processing the results.

Coupled to a sample preparation robot, the GRAVI™ system fully integrates the following functions:

- Sample preparation (dilution, mixing, etc.)
- Mixture of sample and magnetic bead reagents ("Mix")
- Mix incubation (either in a plate or in the GRAVI™-chip)
- Dispensing of the Mix into the GRAVI™-chip
- Bead capture (by a programmable magnet array integrated in the GRAVI™-Cell) and washing in the microchannels
- Addition of the substrate for enzymatic readout and, finally, in-chip detection
- Robotised removal of the magnet array, chip regeneration and washing.

Either 8 or 16 assays are run in parallel, with one loop protocol taking only 8 minutes. As such, this hands-free system is suitable for a throughput of 60 or 120 tests per hour. Combined with standard robotics, the system allows any combination of volume, order and number of reaction steps (including bead menus). The full menu is programmed by means of the GRAVI™-Soft software controlling both the liquid handler and the GRAVI™-Cell instrument.

2 MATERIAL & METHODS

GRAVI™-Chip: GRAVI™-Chip is a cartridge harboring 8 parallel micro-channels produced in a polyimide flex foil with printed board circuitry [1-2] and comprising 30 uL inlet and outlet reservoirs (see Figure 1).

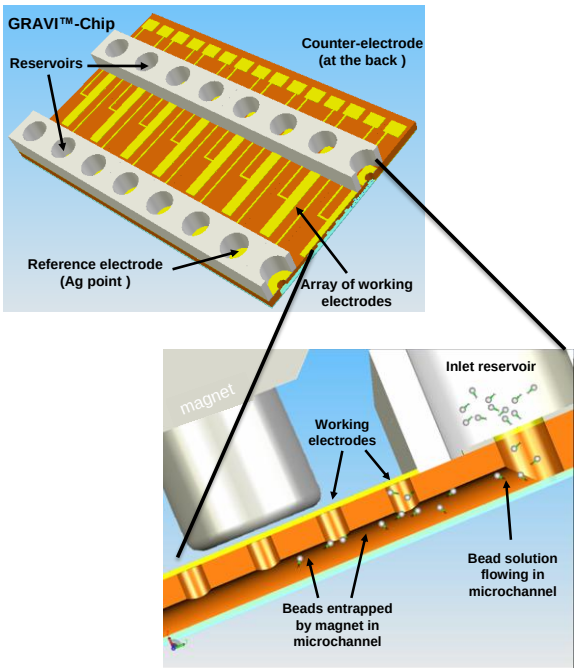

Figure 1: Top: Technical drawing of a GRAVI™-Chip with its network of electrodes and solution reservoirs; Bottom: detailed microchannel cross-section with flowing magnetic beads being captured by a magnet.

An array of microelectrodes positioned along the 1.5 cm micro-channel of 90x250 um^2 section serves for the readout of the enzymatic signal. Multiplexing is feasible by using different enzyme labels, e.g. alkaline phosphatase (ALP) or β-galactosidase.

The GRAVI™-Chip is simply inserted into the USB-powered reader (GRAVI™-Cell) which ensures proper electrical contact and current readout during the enzymatic detection (see Ref. [3] for further details about the detection principles). The GRAVI™-Cell apparatus further comprises a magnet array that can be opened and closed automatically for capture and release of magnetic beads within the microchannels. As shown in Figure 2, the chip is positioned on a tilted plane so that microfluidics is solely driven by capillary force and gravity, thus avoiding the need for any serviceable motors, pumps and tubing.

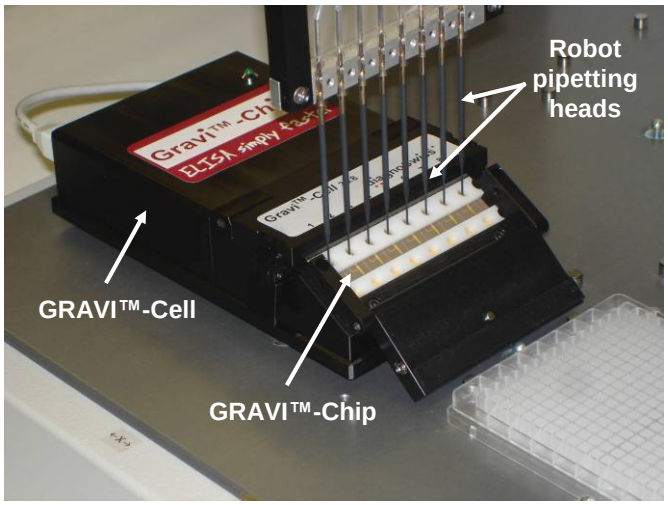

Figure 2: Picture showing a GRAVI™-Cell instrument coupled to a sample preparation robot (Carvo MSP, Tecan, CH)

Correctly placed in the GRAVI™-Cell, the inlet reservoirs of the GRAVI™-Chip is positioned 1 cm above the microchannel outlets, thereby inducing a pressure difference which is sufficient to generate a flow rate of up to 1 $uLmin^{-1}$ [4]. Thus, with an internal channel-volume of 300 nL, this gravity-induced flow allows to replace the total microchannel volume 3 times per minute.

In order to stop the flow (which is notably required for proper signal detection during enzyme-substrate incubation) excess liquid is simply removed from the upper reservoir. As the capillary action is strong at the small dimensions of the microchannels, the capillary forces are larger that the gravity force, thereby preventing emptying of the microchannel and providing efficient valving of the system (which, for instance, ensures stagnation of the solution within the microchannels during the detection).

In order to run bead-based immunoassays, superparamagnetic beads of 1 micron or less are used to carry the affinity capture probes.

Robotic handling: The GRAVI™-Cell is snapped on a plate holder of a sample preparation robot (Cavro MSP9250, Tecan, CH), which is fully controlled by the GRAVI™-Soft computer software. Each fluidic measurement and dispensing step is pre-programmed in a flexible and user-friendly protocol, which can work for hours without need for manual operation.

GRAVI™-Chip for the detection of recombinant human antibody: Aliquots of a mammalian cell culture provided by Selexis (Geneva, CH) containing a theoretical IgG concentration of 400 ug/mL were diluted 100-fold. Calibration samples were then prepared by repeated 2-fold dilutions of the above aliquots so as to obtain IgG standards of 4, 2, 1, 0.5, 0.25, 0.125, 0.063 and 0.031 ug/mL.

Reagents composed of protein A beads (New England Biolabs, Ipswich, MA, USA) at a concentration of 0.015 mg/mL, goat antihuman FAB-ALP conjugate (Sigma, Buchs, CH) at a concentration ratio of 1/1000, washing buffer (Tris pH 9, Tween 0.1%) and p-aminophenyl-phosphate (DiagnoSwiss, CH) as enzyme substrate at a concentration of 10 mM in MAE buffer pH 9 were dispensed in a deep plate. The test consisted in: a) mixing beads, sample and conjugate (5 uL each) in the GRAVI™-Chip inlet; b) permitting flow through the microchannels during 1 min (with the magnet array in place to capture the beads); c) 3 washing of the microchannels three times with 20 uL of washing buffer disposed in the inlet reservoirs; d) adding 20 uL of enzyme substrate solution in the inlet and removing the excess solution after 20 seconds in order to stop the flow. Successive amperometric measurements were then performed in the 8 microchannels simultaneously using the multiplexed potentiostat of the GRAVI™-Cell apparatus, thereby enabling to follow the kinetics of the enzymatic reaction during 30 seconds. Using a fitting procedure integrated in the GRAVI™-Soft, the analyte concentrations were then deduced from the slopes at origin of the obtained current versus time curves.

3 RESULTS AND DISCUSSION

The IgG concentration of mammalian cell culture samples at 8 different concentrations (from 4 ug/mL down to 0.031 ug/mL) have been determined by bead-based immunoassay using the robotised GRAVI™-Cell system.

Eight assays were performed simultaneously within less than 10 minutes (including chip regeneration), and Figure 3 shows the calibration curves obtained for 5 series of 8 assays performed successively in the same chip after regeneration between each series.

As can be seen in Figure 3, the obtained dynamic range covers more than 3 orders of magnitude and the CV is roughly 5 % in the higher concentrations.

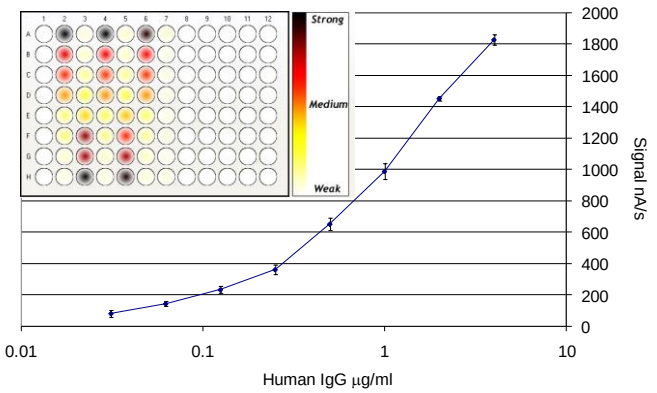

Figure 3: Example of calibration curve for the detection of IgG in samples of mamalian cell cultures using protein A, goat antihuman FAB-ALP conjugate, and p-aminophenyl-phosphate substrate. (Insert: color plate representation for the repeated assays shown in the graph and as dispensed in the microtiterplate).

The GRAVI™-soft also enables to display the results as intensity color plate (see inlet in Figure 3). Each vertical line corresponds to the signals obtained for a row of samples present in the micro-titerplate after the dilution steps processed by the robot as described in section 2. In the present example, a calibration was measured by distributing the samples from high concentration in well A to low concentration in row H of the microtiterplate. The samples are then automatically mixed with the magnetic beads and injected in the microfluidic system for rapid readout. In the second raw, the same calibration is injected, but with the highest concentration in row H and the lowest in row A in order to show that carry over can be reduced to the minimum.

These experiments show that the use of the GRAVI™ platform allows to perform 16 immunoassays in less than 10 minutes. In the 8-channel version, the GRAVI™ system is thus able to perform ~50 tests/h, while the 16-channel option can process about one microtiter-plate per hour.

The concept is amenable to any SBS liquid handler, and different robotic systems can be equipped with the GRAVI™-Cell platform.

4 CONCLUSION

Requiring no maintenance, the pumpless GRAVI™-Chip concept provides an innovative link between rapid and economic microfluidic systems and standard laboratory robotics. Compatible with the conventional 96-well plate format, the GRAVI™ platform can run on standard liquid handling robots used in R&D laboratories. It is a scalable technology which can be declined from portable and bench-top devices to large automated workstations (all with identical assay performances), and it represents a fast, flexible and cost-efficient immunoassay platform.

ACKNOWLEDGEMENT

The authors thank the European Commission for financial support of part of this work (NeuroTAS project of the 6th EU Research program, grant number NMP4-CT-2003-505311).

REFERENCES

[1] J.S. Rossier, F. Reymond and P.E. Michel, Electrophoresis, *23*, 858-867, 2002.

[2] J.S. Rossier, C. Vollet, A. Carnal, G. Lagger, V. Gobry, P. Michel, F. Reymond, and H.H. Girault, Lab Chip, *2*, 145-150, 2002.

[3] P. Morier, C. Vollet, P. Michel, F. Reymond and J.S. Rossier, Electrophoresis, 25, 3761-3768, 2004.

[4] D. Hoegger, P. Morier, C. Vollet, D. Heini, F. Reymond and J.S. Rossier, Anal. Bioanal. Chem., 387 (1), 267-275, 2007.