sciFLEXARRAYER Application Note
No. 08019

Multiplexed ELISA of cardiovascular disease biomarkers in 96 well plate

The prime motivator for transferring traditional ELISA assays to a miniaturized multiplex ELISA format is the significant saving of time, assay materials and patient samples. The consumption of capture probes for example can be reduced by a factor of 10^5-10^6 yielding equivalent data at the same or even better sensitivity. Using traditional ELISA assays, detection of one analyte is performed per well, with positive and negative controls in separate wells.
With multiplex ELISA tests, multiple analytes can be detected in parallel with all controls included in the same well. Microarrays produced in standard 96 well plates allow assay processing with conventional lab equipment which is also used for traditional ELISA tests.
With some showcase ELISAs, we demonstrate the feasibility of multiplexing these assays in a standard 96 well plate. To this end, a panel of biomarkers correlated to cardiovascular disease was employed.

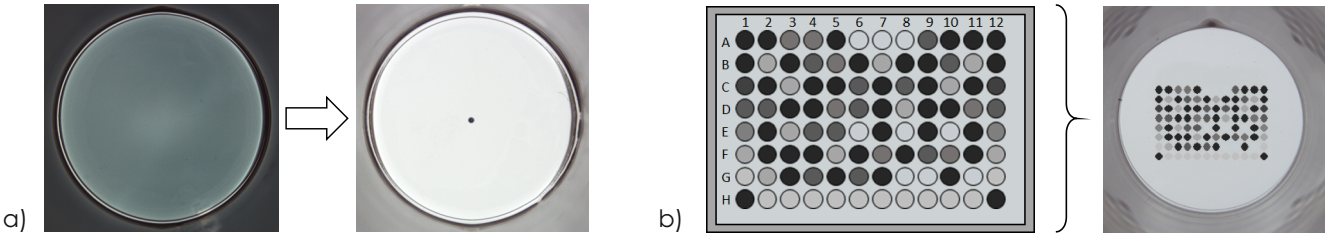

Fig. 1 a: Transferring conventional whole well coating to a single spot means a reduction in material consumption by a factor of 105-106. b: Schematic drawing of a 96 well plate with 96 single ELISA assays (controls + tests). The miniaturized multiplex ELISA with a microarray in a well of such a plate has the potential to include all these tests and controls in one single well.

Materials and Methods

Microarrays were produced with a sciFLEXARRAYER S11 in sciPLEXPLATES Type 1. Capture antibodies were printed in a PBS based buffer with a concentration ranging from 80 to 200 ng/µl. The microarrays were produced with an 8x11 pattern with ~700 pL/spot, resulting in spots with a diameter of ~190 µm. The assays were performed using the antibodies and protein standards from DuoSet® ELISA kits (R&D Systems). Colorimetric staining was performed using HRP conjugated streptavidin (R&D Systems) and sciCOLOR T3 substrate; readout and data evaluation was done with a sciREADER CL2 microarray plate reader.

Results

With SCIENION's sciFLEXARRAYER technology it is easily possible to produce microarrays with individual spot sizes and layout within the wells of a microtiter plate (Figure 2).

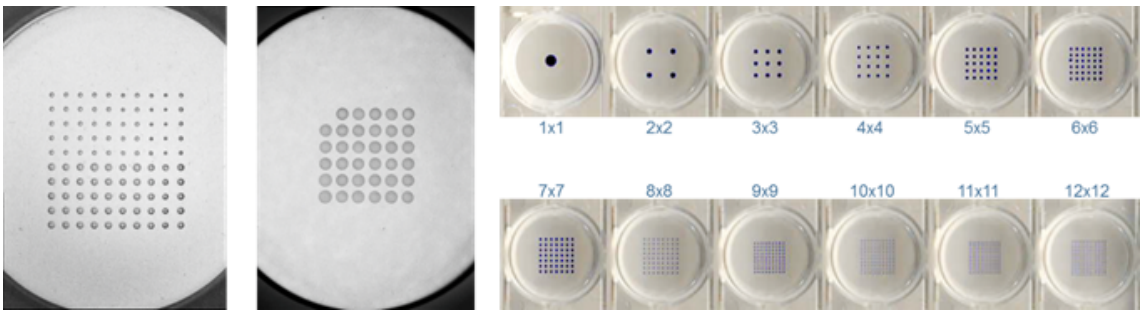

Fig. 2 Example images of microarrays in microtiter plates. Left: Headcam images taken with a sciFLEXARRAYER directly after printing. Right: Examples images of microarrays with systematically varying spot sizes and quantities, produced with a sciFLEXARRAYER.

With a panel of protein biomarkers related to cardiovascular disease, sandwich immunoassays were conducted on top of microarrays containing the whole set of capture antibodies. Calibration curves of each parameter were created with a dilution series of protein standards (Figure 3). Sensitivity and dynamic range are comparable to standard whole well ELISAs (30 – 2000 pg/ml or 100 – 2000 pg/ml, respectively).

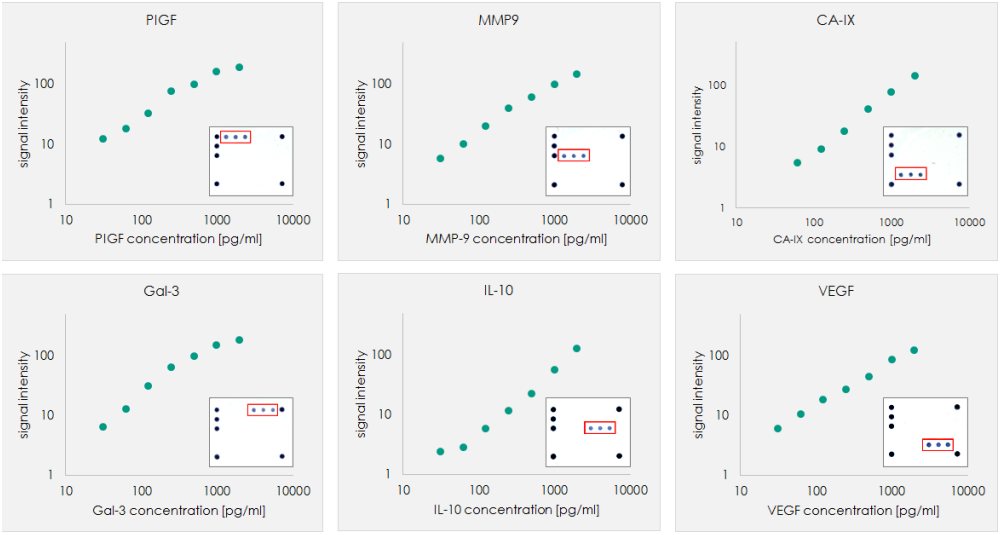

Fig. 3 Calibration curves of six protein biomarkers, created with dilution series of protein standard and a sandwich immunoassay on capture antibody microarrays. One example microarray image is shown in each case as insert.

Two different detection formats were successfully tested on microarray ELISA. The biotinylated detection antibodies were stained with streptavidin conjugated either with HRP for colorimetric readout or with Cy3 for fluorescent readout (Figure 4).

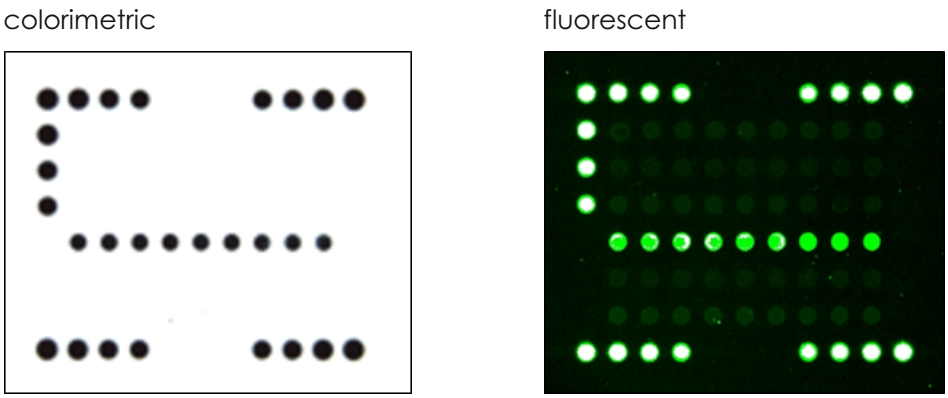

Fig. 4 Example images of microarrays after conducting a Gal-3 assay. Left: Colorimetric staining with HRP and sciCOLOR T3 and readout with the sciREADER CL2. Right: Fluorescent staining with Cy3 and readout with a Tecan LS reloaded scanner.

Summary

We demonstrated the performance of a microarray ELISA based on a panel of cardiovascular biomarkers. By this example the sciMULTIPLEX platform is visualized: from producing the microarrays, successful development of microarray based immunoassays and readout complete with data evaluation.

September 2016

SCIENION AG / Volmerstr. 7b / 12489 Berlin / Germany
SCIENION US, Inc. / 11 Deerpark Drive, Suite 100 / Monmouth Junction NJ 08852 / USA
Fon +49 30 6392 1700 or +1 888 988 3842 / Fax +49 30 6392 1701
support@scienion.com or USsupport@scienion.com / www.scienion.com

scienion
ENABLING LIFE SCIENCE