# sciFLEXARRAYER Application Note No. 08021

## Optimization of array - based diagnostic ELISA tests for the sensitive quantification of three inflammation markers

**In clinical chemistry, standard ELISA and Western blotting techniques are often used to determine and quantify inflammation markers. However, these techniques can only measure a single parameter per assay and well. In contrast, considerable cost, time and sample volume savings can be achieved by miniaturizing and multiplexing these immunoassays. Different blocking agents were tested for their effectiveness and analytes. Detection antibodies and signal molecules were titrated against each other in a wide range of buffers to identify the most sensitive assay protocol. The influence of the incubation period and temperature was also investigated.**
**A miniaturized multiplex assay was established for three inflammation markers (h-CRP, h-PCT and h-TnI), with sensitivities comparable to standard ELISA. The optimized multiplex assay makes it possible to detect three inflammatory markers in the lower pg/mL range in only one sample by using less costly antibodies and detection reagents than in the standard ELISA. This leads to a significant reduction of costs and time.**

### Materials and results

For the direct comparison between standard ELISA and microarrays, the same sandwich principle (see Figure 1) was applied in both cases in high binding sciPLEXPLATES Type 1. For this purpose, the identical capture antibodies (cAb), biotinylated detection antibodies (detAb), analytes and PolyHRP conjugated streptavidin were used. All antibodies were purchased from fzmb Bad Langensalza in Germany. For the standard whole well ELISA, a soluble TMB substrate was used and stopped by acid addition. Spot formation in all microarray wells was performed with SCIENION´s highly sensitive substrate sciCOLOR T3. The microarray readout and data evaluation were done with a colorimetric sciREADER CL2 microarray plate reader.

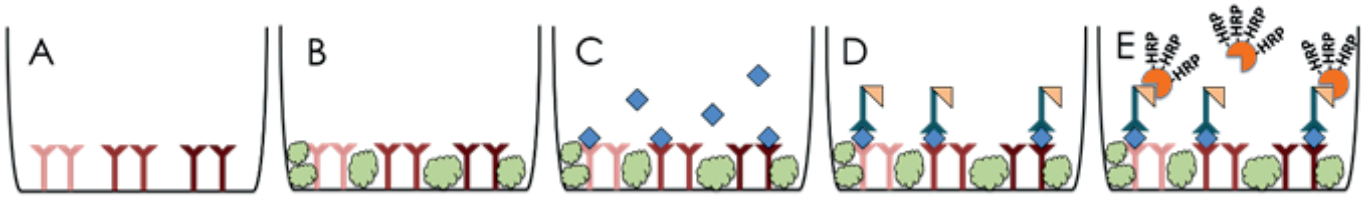


**Fig. 1** Schematic procedure of the stepwise sandwich ELISA. Printing or coating of capture antibodies in sciPLEXPLATES Type1 (A) . (B) SCIENION´s optimized blocking procedure. Investigation for sensitive quantification incubation protocols for (C) analytes and (D) biotinylated detection antibodies. Using Streptavidin-PolyHRP conjugate for increasing the LOD (E). A customized washing procedure with sciWASH Protein D1 between steps (B) to (E) is necessary.

### Results

With SCIENIONs sciFLEXARRAYER SX combined with the sciMULTIPLEXBOX, a quick and easy miniaturization of existing standard ELISA in order to reduce material and personnel costs were shown.
The cross-reactivity of the individual analytes with other capture antibodies was less than 2%. With the optimized multiplex assay, it was possible to detect the analytes with a precision between approx. 80 - 130%, of the expected value. The variation coefficient of the reproduced, optimized multiplex assay was between 10 and 28% for three different repetitions on three different days for the individual analytes.

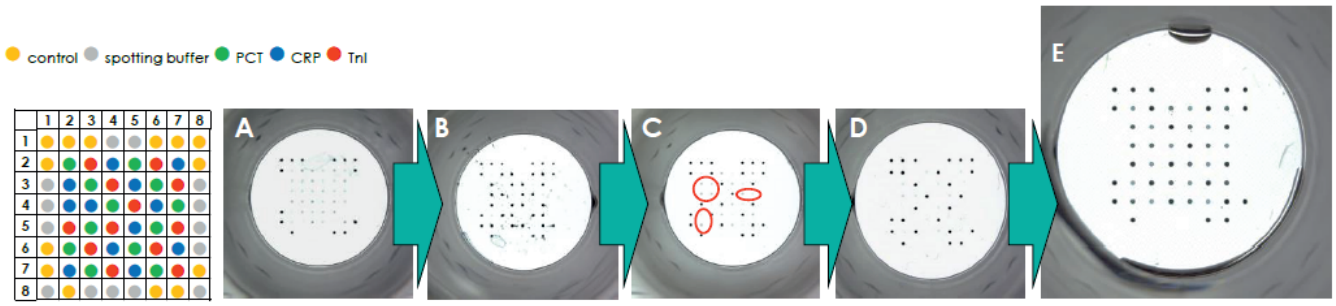


**Fig. 2** Development of the array-based ELISA. (A) weak signals and high background make a sensitive assay nearly impossible. (B) comet formation or bleeding out of cAb make the automatic spot recognition by the sciREADER CL2 software more difficult. (C) false positive signals due to cross-reactivity (red circle) require the design of an individualized blocking-, washing- and incubation-protocol. (D & E) final microarray with high specificity and excellent contrast, similarly sensitive to the standard ELISA with cross-reactivity below 2%.

Capture antibodies were printed in different buffers with a concentration ranging from 100 to 250 µg/mL, resulting in spots with a diameter of ~120 µm. For the whole well standard ELISA, 1 µg/L capture antibodies were coated over night at 4°C in a typical ELISA coating buffer. SCIENIONs high binding and ultra-planar 96x well sciPLEXPLATES Type1 used in this miniaturization shows significantly higher signals in combination with sciSPOT Protein D4 and sciSTAB S3 as print buffer, compared to the competitors ones. With for proteins adjusted spotting buffer formula in all sciCONSUMABLES it is possible to increase the signal intensity enormously. The comparison of different incubation buffers for the sensitive and specific quantification of the three analytes shows that sciBIND Protein D1 increases the assay sensitivity hugely and decreases the unspecific (false positive) signals (data not shown).

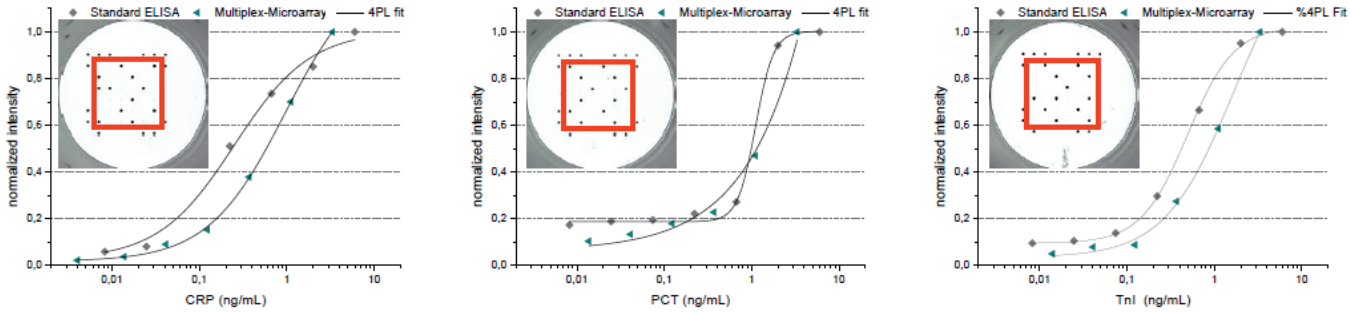


**Fig. 3** Comparison of the calibration curves between standard ELISA and multiplexed microarray for the three protein inflammation markers, created with dilution series of protein standard. One example microarray image is shown in each case as insert. The corresponding array-design is shown in figure 2, left side.

## Summary

SCIENION´s non-contact printing technology in combination with the optimized formulas in all sciCONSUMABLES offers all necessary tools for your own immunoassay miniaturization and multiplexing. Using the example of the three biomarkers CRP, PCT and TnI, a successful miniaturization and multiplexing of the individual standard ELISA was demonstrated in a sensitive and robust assay. It was also shown that the dynamic range in all multiplex assays is greater compared to the corresponding standard ELISA. The sensitivity is similar to the standard ELISA. The simultaneous quantification of CRP, PCT and TnI with only 30 µL of sample in one well is feasible with the performed multiplex assay. Thus, up to 99.5% of the amount of capture antibodies used in the standard ELISA can be saved using this assay. For each well, up to 64 data points can be analysed with the shown 8 x 8 pattern. The enormous cost savings are obvious. SCIENIONs growing sciMULTIPLEX expertise gives you a strong provider platform for your own customized multiplexing application.

Nov 2017

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