sciFLEXARRAYER Application Note No. 08009

Rapid and multi-analyte diagnostic microarrays

Diagnostic methods can be used to detect all kinds of components such as proteins (biomarkers, toxins, food allergens), microorganisms, specific RNA/DNA sequences, toxic chemicals (plasticizers), contaminants (antibiotics/pesticides), and other quality-determining components. Protein-based (e.g. antibody) immuno-diagnostics are crucial tools to address safety and quality issues in fields ranging from plants/crops, environment and food/feed up to veterinary and human applications. An important trend in diagnostics is dedicated multi-analyte detection, i.e. the limited number of relevant components that provides the required answer with acceptable reliability. Often the detection of 5 to 10 different analytes is sufficient to reach this goal. Therefore, microarray platforms are very promising tools for future diagnostics. However, despite the high potential of protein microarrays, they are still not commonly used in the regular diagnostic fields, due to reasons such as the lack of sufficient biological recognition elements (e.g., antibodies), limited sensitivity and specificity, the lack of integrated systems (fluid handling, sample preparation, signal processing), and inacceptable background signals.
A good example of an application that would profit from a microarray approach is bovine mastitis, one of the most costly diseases in dairy cattle. Mastitis can be caused by a wide range of pathogens. Preferably, these analytes are measured with a single diagnostic platform to save time, sample and costs.

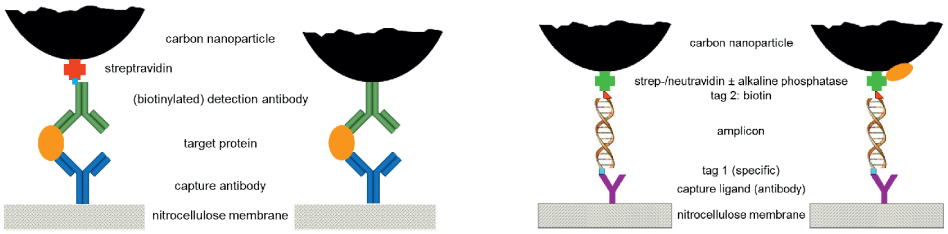


Fig. 1 Several principles to detect proteins (left pictures) or DNA/RNA amplicons (right pictures) with or without streptavidin/neutravidin to amplify the signal by additional staining through substrate conversion by a nanoparticle-immobilized enzyme.

Materials and methods

Various rapid immunoassay platforms are being developed: the Microarray-ELISA (MELISA; generally 64 spots, 8x8 arrays per well; 45 min), the slide-based protein (antibody) microarray assay; generally 100 spots, 10x10 arrays per nitrocellulose pad; 45 min) and the Lateral flow Microarray ImmunoAssay (LMIA; often 25 spots, 5x5 arrays per strip; 10 min).

Example of an assay set up: For the most common mastitis-causing pathogens, a rapid species-specific PCR with tagged-primers was combined with nucleic acid microarray immunoassay platforms; a lateral flow assay and a 96-wells microtiter plate assay, both with a microarray of (antibody) spots per strip or well, respectively. Using biotin as a tag on the reverse primers, unique tags on the forward primers were used to discriminate the individual bacteria. The double-tagged dsDNA molecules were sandwiched between tag-specific antibodies on the nitrocellulose membrane and neutravidin on carbon nanoparticles.

Proteins or compounds were printed by the SCIENION S3 microarrayer (Figure 2 left). This piezo-driven inkjet printer allows the application of droplets down to 200 picoliter on a variety of surfaces, from 2D surfaces such as glass and polystyrene, to 3D surfaces such as nitrocellulose (Figure 2 right).

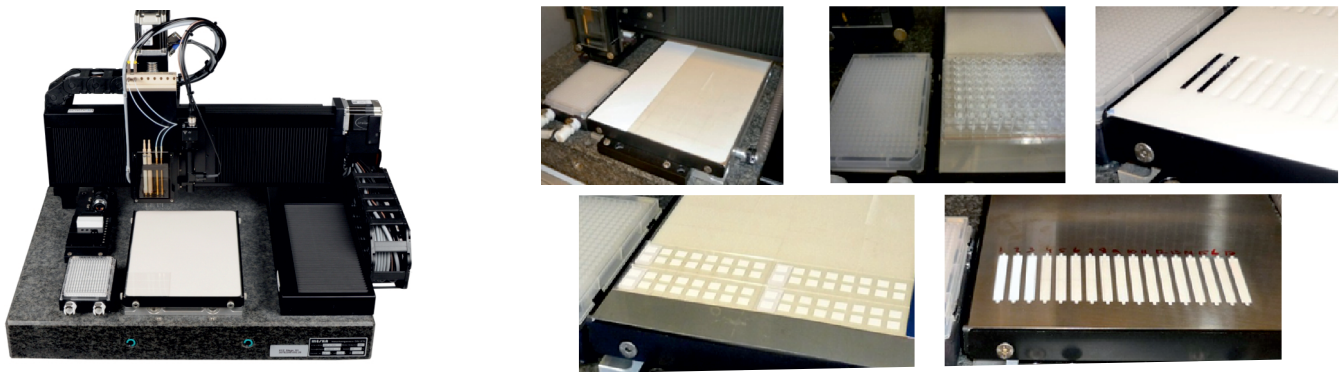
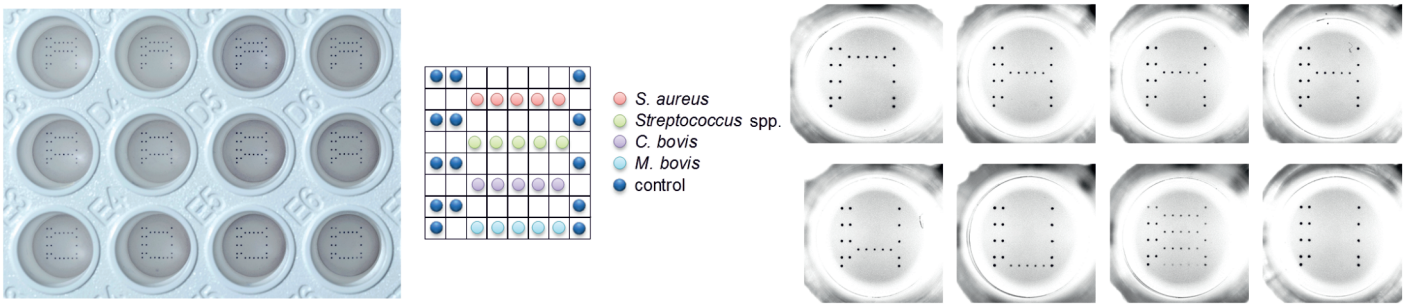


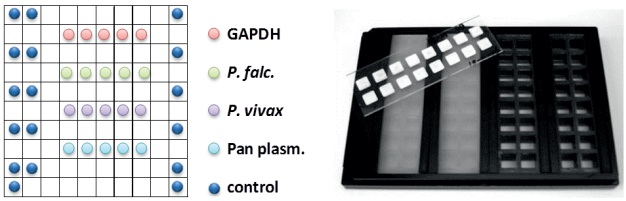
Fig. 2 sciFLEXARRAYER S3; Examples of surfaces onto which binding proteins are being printed (from left to right and top to bottom): Nitrocellulose membrane sheets, ELISA plates with transparant or non-transparant bottom, biosensor chips in a Delrin template, slides with nitrocellulose pads and nitrocellulose lateral flow membranes for microarray applications in a metal template.

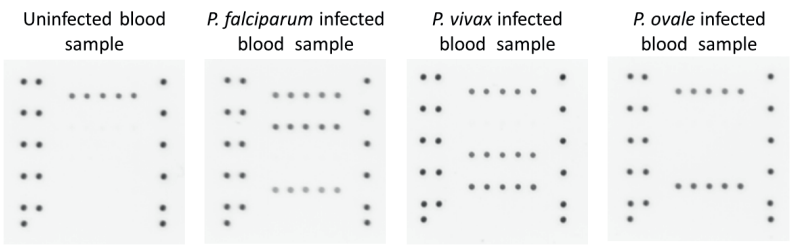
Results

Microarray-ELISA for the detection of specific amplicons from microorganisms that cause mastitis in cows. Detection following a 30 min incubation with carbon nanoparticles conjugated to neutravidin - alkaline phosphatase (AP) and for 10 min with AP substrate.



Slide-based protein microarray assay for identification of malaria species. Following PCR, amplicons were incubated for 30 min with carbon nanoparticles conjugated to neutravidin - alkaline phosphatase (AP) and for 10 min with AP substrate to enhance the signal.





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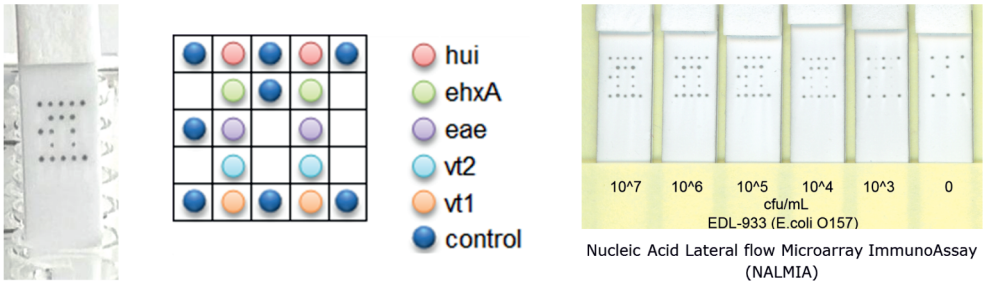
Lateral flow microarray immunoassay to detect genes that code for virulence factors from Escherichia coli O157. Amplicons from a rapid multiplex PCR (30 min) were run through lateral flow membranes and sandwiched between specific antibodies immobilized on the membrane and neutravidin bound to carbon nanoparticles (10 min).



Nucleic Acid Lateral flow Microarray ImmunoAssay (NALMIA)

Conclusion

SCIENION technology can be used to produce user-friendly, multi-analyte diagnostic applications. Different platforms are being developed e.g. Microarray-ELISAs, Slide-based Protein Microarray Assays and Lateral flow (Microarray) Immunoassays. In addition to proteins and other compounds the platforms are suited to detect specific DNA/RNA amplicons. A combination of a rapid amplification step with a fast detection protocol results in a total assay time of less than 60 min and 75 min, respectively, and a large reduction in time-to-answer for treatment compared to traditional methods.

Courtesy of Dr. Aart van Amerongen
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