sciFLEXARRAYER Application Note No. 08022

Development of a new and sensitive multi-analyte lateral flow immunoassay for the verification of three inflammation markers

An effective treatment for a particular disease is preceded by an accurate and early diagnosis. Especially in case of required treatment must usually be decided immediately on a corresponding therapy. For example, in the case of sepsis or stroke, the correct and rapid detection of clinical findings is of great importance. Point of care testing (POCT) is defined as 'testing at or near the site of patient care whenever the medical care is needed' and enables the rapid and reliable determination of the state of health, e.g. initiate on therapies to decisions or further action. These diagnostic tools have the great advantage of a small sample volume, with a short time-to-result duration and are easy to carry out. A POCT can be performed anywhere, e.g. in the ambulance, in a doctor's office, at home or in a nursing home.
Through simultaneous screening of multiple analytes in a single sample with one POCT (multiplexing) a faster and more favourable quantification of the individual markers can be achieved. Therefore, the need for multi-parameter Lateral Flow Assays (LFA) has increased dramatically in the last decade. Since such LFAs quickly meet the requirements of in vitro diagnostics by an ordinary person, this will also accelerate the development of modern home health monitoring systems in the future.
This application note exemplarily shows the development of a highly specific and sensitive multiplex LFA, which is based on capture molecules immobilized in an array format instead of conventional lines. Relying on using the classical sandwich ELISA format, the developed miniaturized POCT allows simultaneous detection of troponin I (TnI), procalcitonin (PCT) and C-reactive protein (CRP) in a single sample of as little as 30 µL. Dose-response curves were obtained for each of the investigated markers in the range of 0.2 – 200 ng/mL.

Materials and results

Patterns (Figure 2) were applied to nitrocellulose membranes using a sciFLEXARRAYER microarray spotter equipped with the piezo-driven sciDROP PICO dispense technology. Replicate strip patterns were printed onto 30 cm x 2.5 cm membranes prior to laminating and cutting into individual strips. Conjugate and absorption pads were made of glass fibre and cellulose, respectively. Multiplex LFAs were realized in the classical sandwich based ELISA microarray format. The detection antibodies were functionalized with SCIENION´s black nanoparticles to reach a high signal-to-noise ratio.

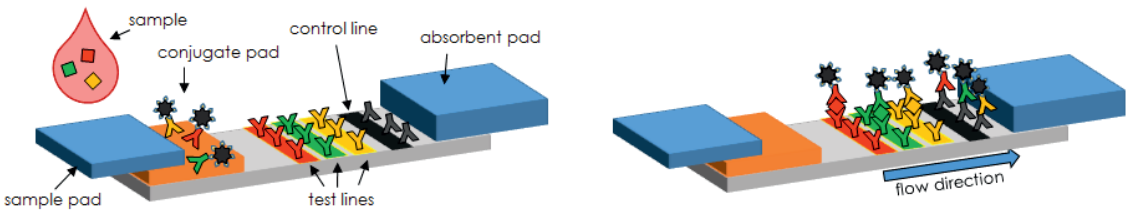

Fig. 1 Conventional multi analyte LFA based on the classical sandwich ELISA format: (A) The nitrocellulose membrane carries test and control lines made up of immobilized capture antibodies (cAb) targeting the analyte and immobilized antibody targeting the detection antibody (detAb), respectively. The conjugate pad is loaded with detAb conjugated to a detection label. (B) Upon sample application, capillary forces transport the sample through the strip. The detAb conjugate mobilizes, binds the analyte and moves further through the strip towards the test line where the complex binds to cAb giving rise to a signal. Vacant detAb is captured at the control line. Tests are only conclusive if a signal emerges at the control line guaranteeing correct handling and sample flow through the whole strip.

Following antibody screening in singleplex assays and design of the sandwich ELISA on the molecular level (data not shown), development of the multiplex LFA was initiated by establishing a compatible array layout (Figure 2). The half-stick LFA setup shown in figure 3 was chosen to speed up assay development allowing efficient variation and optimization of critical assay components. The running buffer composition was optimized and detAb concentrations were titrated against analyte standards maximizing signal-to-noise ratios (data not shown). The results are summarized in figure 4 and figure 5. The influence of membrane flow rates (Figure 4) and spotting-volumes at constant cAb concentrations (Figure 5) are shown.

Results & Summary

The development and production of a user-friendly, multi-analyte diagnostic application requires a lot of investigation and optimization. This application note shows the producibility of a highly sensitive and specific multiplex LFA assay (sandwich ELISA-based). The spatial arrangement of captures as staggered array rather than lines avoids interference of assay components. The use of 3 x 10 nL capture molecule per stripe can save up to 90 % of the applied antibody amount, compared to the common value of 500 nL/cm. The simultaneous detection and quantification of three parameters in a single sample reduces personal cost and time.

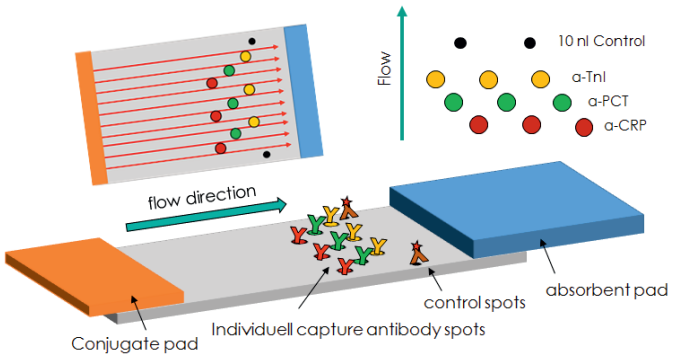

Fig. 2 Schematic representation of a perfectly staggered multiplex microarray pattern printed onto LFA strips. Triplicates are arranged perpendicular to the flow. The 3x3 array is shifted such way that laterally flowing assay components are only passing a single capture zone avoiding unintendingly influencing assay results caused by depletion and interference. Schematic presentation of printed pattern is shown.

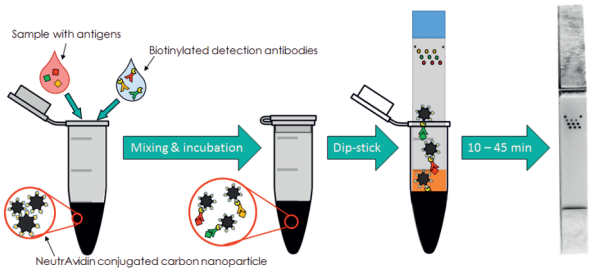

Fig. 3 Overview of the half-stick assay format chosen for assay development. After mixing and incubation of the sample containing target analytes with biotinylated detAb and NeutrAvidin-functionalized black nanoparticles in optimized running buffer, the half stick is incubated in 100 µL thereof. The half stick lacks a sample pad and only carries a non-loaded conjugate pad for submersing into the test mix.

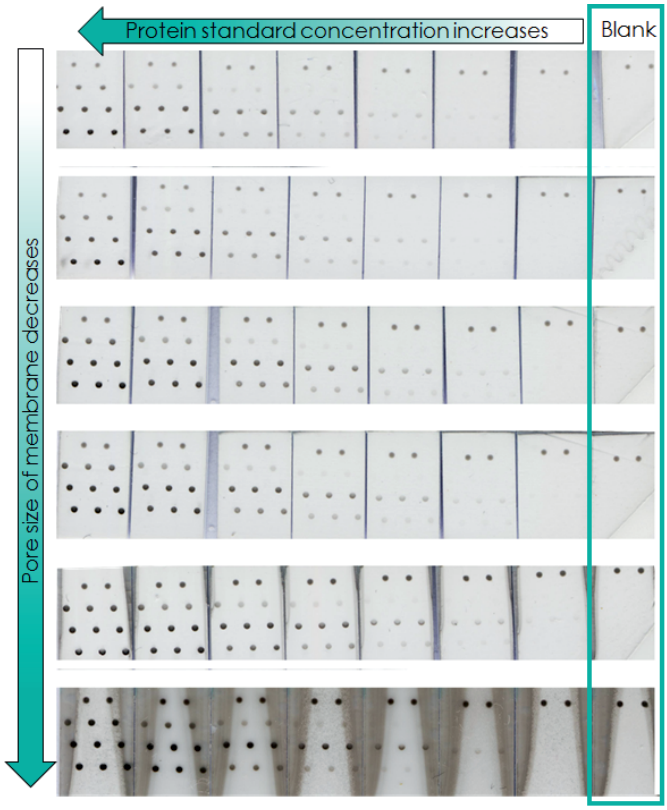

Fig. 4 Assay performance in dependence of the nitrocellulose flow rate. 10 nL cAb were deposited per spot on membranes varying in flow rate (max and min of the range).

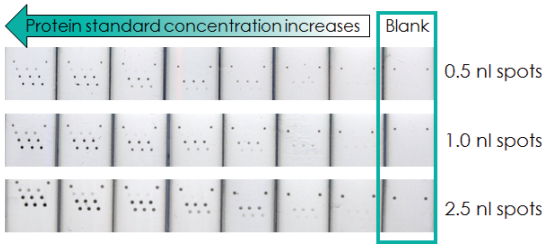

Fig. 5 Optimized multiplex assay on a sensitive membrane with different ultra-low spotted volume per cAb.

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