

A TEN-MINUTE HIGH DENSITY LATERAL FLOW PROTEIN MICROARRAY ASSAY

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ABSTRACT

Protein microarrays are useful tools for highly multiplexed determination of presence or levels of clinically relevant biomarkers in human tissues and biofluids. However, such tools have thus far been restricted to laboratory environments. Here, we present a novel 384-plexed easy to use lateral flow protein microarray device capable of sensitive (<50ng/ml) determination of antigen specific antibodies in less than ten minutes total assay time. Results were developed with gold nanobeads and could be recorded by a cell-phone camera or table top scanner. Excellent accuracy (AUC=99.4%) was achieved in comparison with an established glass microarray assay for 26 antigen-specific antibodies.

KEYWORDS: Protein microarray, Lateral flow, Point of care,

INTRODUCTION

Clinical multiplexed affinity-based proteomics is a rapidly expanding field which holds the potential to provide early and accurate diagnosis or prediction of for instance various cancers, cardiovascular diseases, autoimmune diseases and dementias where single biomarkers cannot provide an adequate specificity [1].

Through protein microarrays, researchers and clinicians may look for fingerprints of proteomic manifestations of diseases and adverse conditions. In the case of many cancers, recognizing the subtle proteomic pattern of a pre-malignant state may mean the difference between life and death for the patient. In some cases, the relevant biomarker panel can be predicted, for instance in the case of immunogenic surface proteins on pathogens resulting in specific antibodies that can be detected [3]. In other cases typical patterns in the levels of biomarkers can be found and used to predict or classify disease although the underlying mechanisms are not yet known. For this reason, diagnostic multiplexed tools for proteomic investigation need to be flexible enough to allow for low-plex as well as high-plex investigation.

Current protein microarray technologies (fluorescence-detection on glass-substrate microarrays and suspension bead arrays), while providing excellent platforms for laboratory-based analysis, cannot easily be transferred into a point of care context, and thus simple, rapid and portable solutions are urgently needed for multiplexed proteomic diagnostics to reach its potential [4]. Here, we present a paper-based lateral flow protein microarray with 384 peptide affinity binders, which can sensitively and reliably determine levels of specific antibodies within less than ten minutes.

EXPERIMENTAL

A microarray printer (ArrayJet Marathon) was used to pattern arrays of 24*16 microspots with 384 different protein epitope-specific tags (PrESTs) [5] of 50-100 amino acids long per array into nitrocellulose membranes (HiFlow 90, Millipore) mounted on glass slides (Figure 1). A grease line was applied 3 mm from one end of the strip to create a sample application window, and a tissue-pad was applied at the strip-end to provide a sink for the capillary flow.

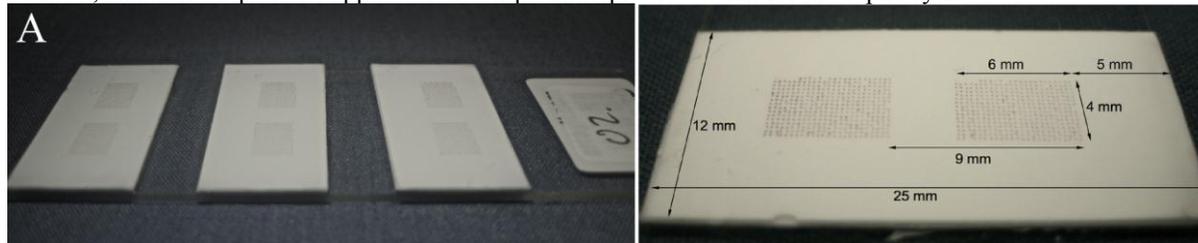


Figure 1 A) Three nitrocellulose strips glued to a glass array slide B) Two microarrays per strip. Here, an anti-HisABP antibody that binds a molecular tag on each printed peptide was used to reveal the printing pattern.

26 rabbit IgG antibodies previously raised using the PrESTs as immunogens were diluted to 0.1-0.5 μ g/ml in assay buffer (PBS+0.5% Tween, 1% Sucrose, 3% BSA) and analyzed using the lateral flow microarray as well as an established glass microarray assay [6]. A biotinylated anti-rabbit-IgG was then applied, followed by gold nanobeads coated with an anti-biotin antibody (British Biocell International). Due to the intense red color resulting from the gold nanobeads accumulating on the microspots, the array could be recorded by means of a tabletop scanner or a cell phone camera. After image acquisition, images were analyzed using the software Gene Pix 5.1 (Axon Laboratories).

RESULTS AND DISCUSSION

The binding patterns were compared to those of a conventional glass microarray assay with fluorescence-based detection (Fig 2A). An excellent concordance with AUC=99.4 % was achieved in the comparison with the common glass microarray assay with fluorescence readout for the same antibodies (Fig 2B). All the 26 antibodies gave rise to strong correct signals for both methods. For five of the antibody samples, we saw secondary PrEST-spot displaying an above-threshold signal for both methods. Further, 7 antibody samples gave rise to secondary signals with the glass-array not found with the lateral flow array, and 3 samples gave secondary signals by the lateral flow array but not the glass array. While the thresholds may be varied according to the need for balance between specificity and sensitivity of the assays, it appears that the lateral flow array performs similarly to the established assay in detecting the expected antibodies, and allows a similar gap between the positive signals and the sub-threshold ones as the glass assay. Further, it is conceivable that while both assays seem adequate for detecting the expected antibody analytes, the substantial differences in assay conditions (ELISA-like/immunochromatographic) underlying the binding and unbinding could explain why some secondary binders appear on the lateral flow array, and some on the glass array.

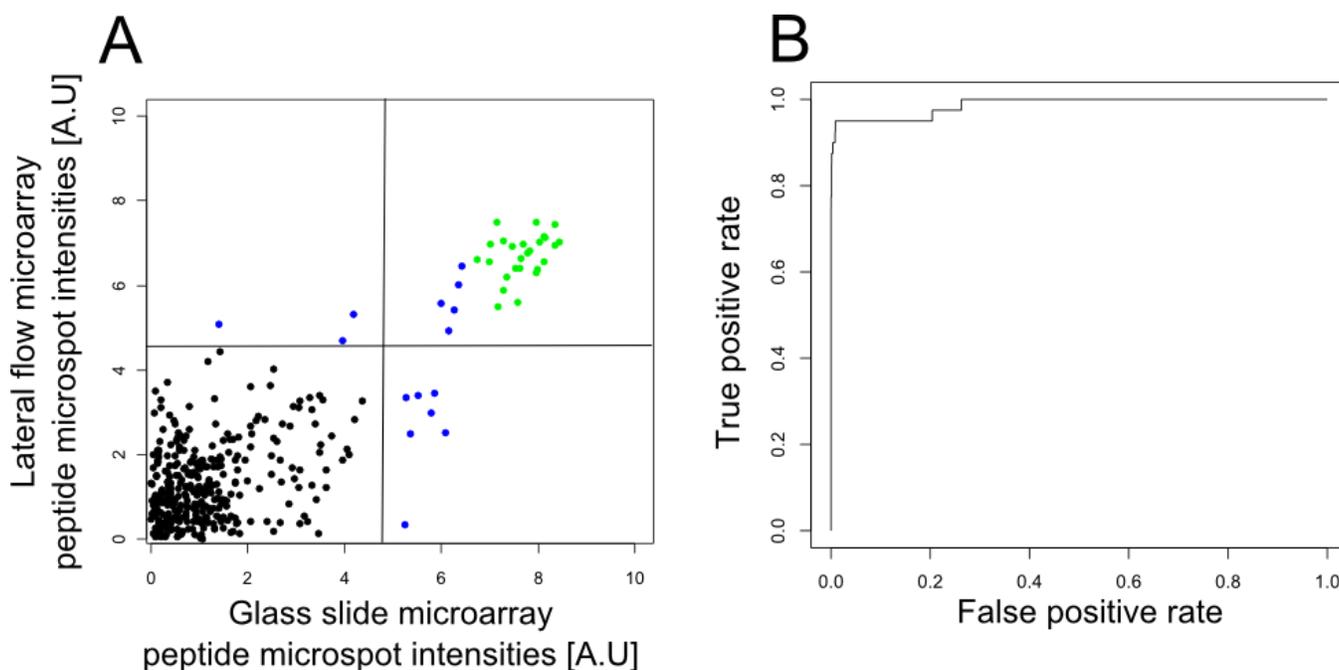


Figure 2 A) Scatterplot comparing the lateral flow microarray and corresponding glass microarray results for 26 antibody samples. Each dot indicates one microarray spot result (only spots that were equal to or higher than the array mean are shown). Black dots indicate spots yielding sub-threshold results on both methods. Green dots are the expected positive signals, in accordance with the immunization pattern, and the blue dots are spots that presented above-threshold signals on either method, but were not among the expected B) Employing the vertical threshold indicated in (A), the receiver operator characteristics curve could be calculated and the area under curve (AUC) was estimated to 99.4%.

All assays were run in triplicates, and thus an average inter-assay variability could be estimated to 13.42 %. Two antibodies were selected and diluted 1:100, 1:1000, 1:10.000 and 1:100.000 for a sensitivity analysis. For both antibodies, the response in the interval was roughly linear to the log of the dilution rate, and both antibodies gave significant signals at 1:10.000 but not 100.000 dilutions, suggesting a sensitivity of better than 34 ng/ml and 28 ng/ml, respectively (Figure 3).

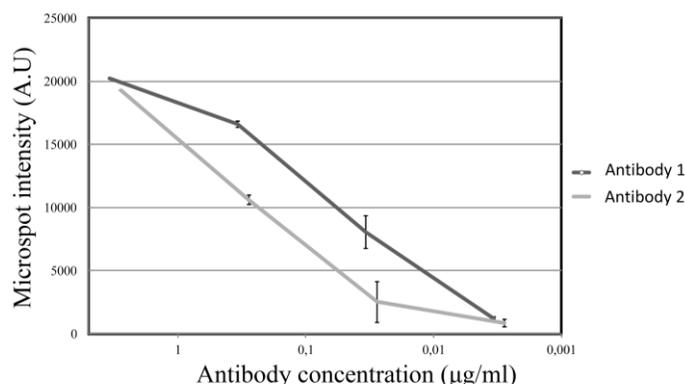


Figure 3 Dilution series of two antibodies, spanning 1:100, 1:1000, 1:10.000 and 1:100.000 from the original concentration. A signal above noise could be detected at dilution 1:10.000 (34 ng/ml and 28 ng/ml).

CONCLUSION

Analogously to common lateral flow-based rapid tests, the presented protein microarray assay should be amenable for end-user handling, and may therefore carry the potential to provide better diagnostics to patients and relieve medical institutions at the same time. The short assay time, the simple procedure and the inexpensive components used further benefits the potential applicability of the assay in low-resource contexts. Due to the excellent concordance with an established protein microarray assay, we suggest that the high density lateral flow microarray could become a useful tool in rapid and point of care diagnosis of for instance cancers, autoimmune diseases, infections, cardiovascular diseases and neurodegenerative diseases. Future work will focus on implementing plasma-separation and dilution modules and on-chip buffer handling, as well as benchmarking the platform towards a clinically relevant sample cohort.

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