Multiplex Autoantibody Detection Using MagArray GMR Biosensors

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MagArray

"Enabling Better Outcomes through Better Diagnostics"

Abstract

Background: We are developing multiplex autoantibody assays based on magnetic sensors of the MagArray platform. The serum autoantibodies were selected for their demonstrated potential in the diagnosis and prediagnosis of lung cancer. The MagArray platform utilizes magnetic nanotags for signal generation and detection. By monitoring multiplex sensors on a single chip, multiplexed detection of autoantibodies is made possible. Also, since magnetic signals rather than optical signals are generated and detected form the tags, interferences with optical outputs by biological matrices in a conventional optical-based detection system have no effect in current platform.[1]

Objective: Feasibility study of multiplex detection of autoantibodies in serum samples that are related to lung cancer, which are anti-14-3-3 theta, anti-LAMR1, and anti-ANXA1 autoantibodies.

Methodology: We have screened and selected recombinant human proteins of 14-3-**3** theta, ANXA1, and LAMR1 as targets of interested autoantibodies. The antigens were spotted onto magnetic sensors in replicates on individual MagArray chips. Individual sera collected from 5 patients were hybridized to individual microarrays. The electronic readout of the magnetic signals from the assays were recorded and compared with corresponding fluorescence-based microarray data.

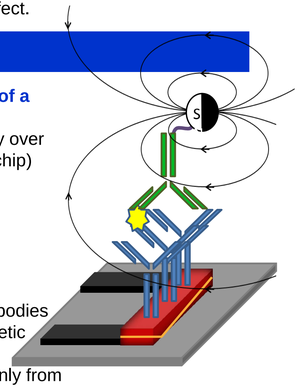
Results: Detection of autoantibodies on diluted serum samples is demonstrated and the results compared with regular florescence microarray data. Most of the data points obtained from MagArray chips qualitatively agreed with fluorescent results with the exception of 2 out of 20 data points that don't agree. Intra assay CVs are below 10% for the range of autoantibody concentrations studies, and inter-assay CVs are below 15% as defined as chip to chip signal variation when measuring the same sample.

Conclusions: MagArray platform shows excellent performance in the multiplex detection of autoantibodies related to the diagnosis of lung cancer. The instrument is novel, multiplex, easy to use, and free of matrix effect.

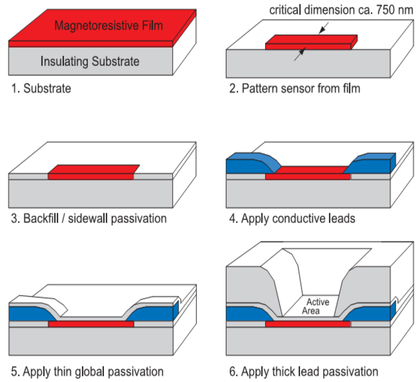
Introduction

1. Principle of Operation: Case of a Sandwich Immunoassay

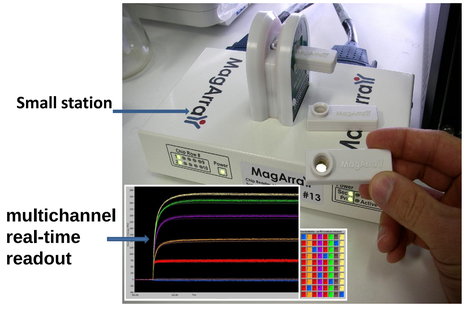
1) Spot a unique capture antibody over each sensor (80 sensors per chip)
2) Incubate with Fluid of Interest
   - Blood
   - Plasma
   - Serum
   - Urine
   - Saliva
3) Add biotinylated detection antibodies
4) Add streptavidin labeled magnetic nanoparticle tags
5) Detect magnetic signal - but only from surface-bound nanoparticles!



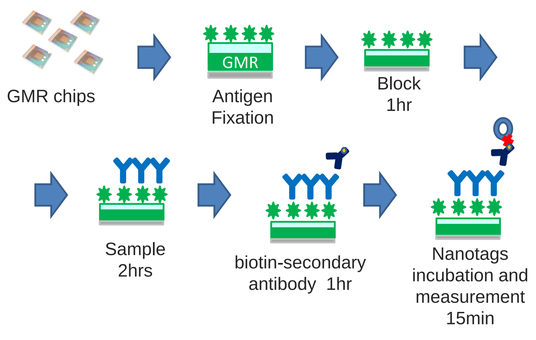
2. Fabrication of Chips – Simplified



3. A Miniaturized Test Station



Assay Format



Methods

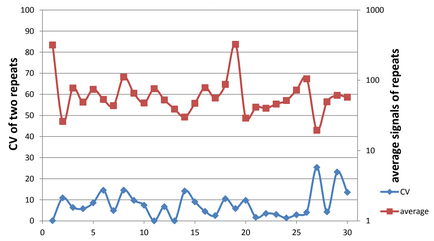
Sample measurements: For each assay, 1 µL of patient serum was diluted into 100 µL assay buffer and incubated with antigen-spotted chips. Each patient sample was run in duplicate with 100 µL diluted sample for each chip. Incubation time for the samples was 2 hrs, followed with 1hr secondary antibody incubation and 15 min of magnetic particle incubation and measurement.

Reproducibility of autoantibody assays: CVs of the duplicate measurement of 30 samples were compared and listed here. Using one selected sample, we also studied the variation of measurement results on five different days and have compared them.

Clinical studies: the clinical utility of selected autoantibody panel to the EGFR/HE4 duel marker lung cancer panel developed at FHCRC was evaluated by ROC analysis.

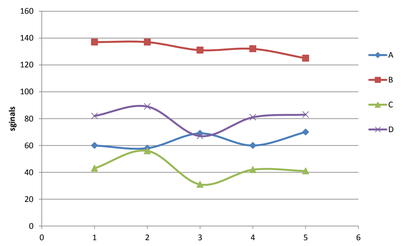
Results

Performance of assay: CV values of 30 samples



CV distribution of 14-3-3θ of 30 samples measured in duplicates. In general, CV is larger for lower signals, and better with larger signals.

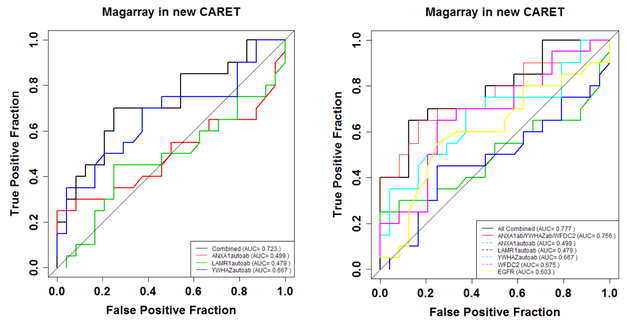
Signals of the same sample on different days



Again, the results demonstrated that measurements of antibodies with lower signals have higher CVs.

Utility of autoantibody panel for lung cancer: ROC analysis

Cases from the new CARET include 20 positive and 20 negative diagnosis. Four autoantibodies were analyzed using linear combination method. The combination of three autoantibodies gave an AUC of 0.723.



Combination of protein biomarkers and autoantibody panels:

FHCRC has developed both protein biomarkers and autoantibody panel for lung cancer. MagArray platform has also been used to measure the biomarkers of EGFR and HE4, and the combination of all the markers had improved the AUC for EGFR/HE4 from 0.723 to 0.777 as shown above.

Comparison to conventional protein microarray: ROC analysis was also analyzed based on conventional fluorescence protein microarrays and compared with the results based on data from MagArray platform. The corresponding AUC values for the conventional microarray are 0.685, and 0.718, respectively for autoantibodies alone and all biomarkers combined.

Conclusions

An autoantibody panel for lung cancer has been developed on MagArray platform:

Sample Volume:	the volume of sample is 1µL with 100 µL working volume after dilution
Precision:	for most concentrations, CV is smaller than 10%, with slightly larger values for low signals
Convenience:	small test station permits the possibility of a POC instrument

Reference:

1. J. Qiu, G. Choi, L. Li, H. Wang, S.J. Pitteri, S.R. Pereira-Faca, A.L. Krasnoselsky, T.W. Randolph, G.S. Randolph, G.S. Omenn, M.J. Barnett, M.D. Thornquist, G.E. Goodman, D.E. Brenner, Z. Feng, S.M. Hanash. *J Clin Oncol* 2008 Nov 1;26(31):5060-6.