

Evaluation of EGFR and HE4 on MagArray High Sensitive Immunoassay Platform

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Abstract (submitted)

Background:

HE4 and EGFR are important clinically relevant biomarkers. We report here that the MagArray platform is particularly suitable for the detection of HE4 and EGFR with ease of use and high sensitivity. Rather than detecting optical labels, the MagArray platform is based on the detection of magnetic labels that are free of optical interference in biological matrices. We report here that MagArray's magnetic biosensors is a sensitive and simple platform for detecting HE4 and EGFR in human sera.

Methods:

Using MagArray biochips, antibodies for EGFR and HE4 (Human epididymis protein4) were spotted on individual sensors. Serum samples were hybridized on the microarrays followed with detection antibody incubations adopting a standard ELISA configuration. Instead of enzymatic labeling and signaling mechanism, magnetic tags were labeled and detected. The accuracy and sensitivity of results on magnetic sensors were compared with standard ELISA assays.

Results:

The detection limits of EGFR is 100 times better than that of ELISA, and HE4 20 times better. The inter assay CV was less than 20% for both biomarkers between 5 runs(→3 runs) in the concentration range studies.

Conclusions: MagArray platform is demonstrated to be a sensitive and easy-to-use platform for immunoassays.

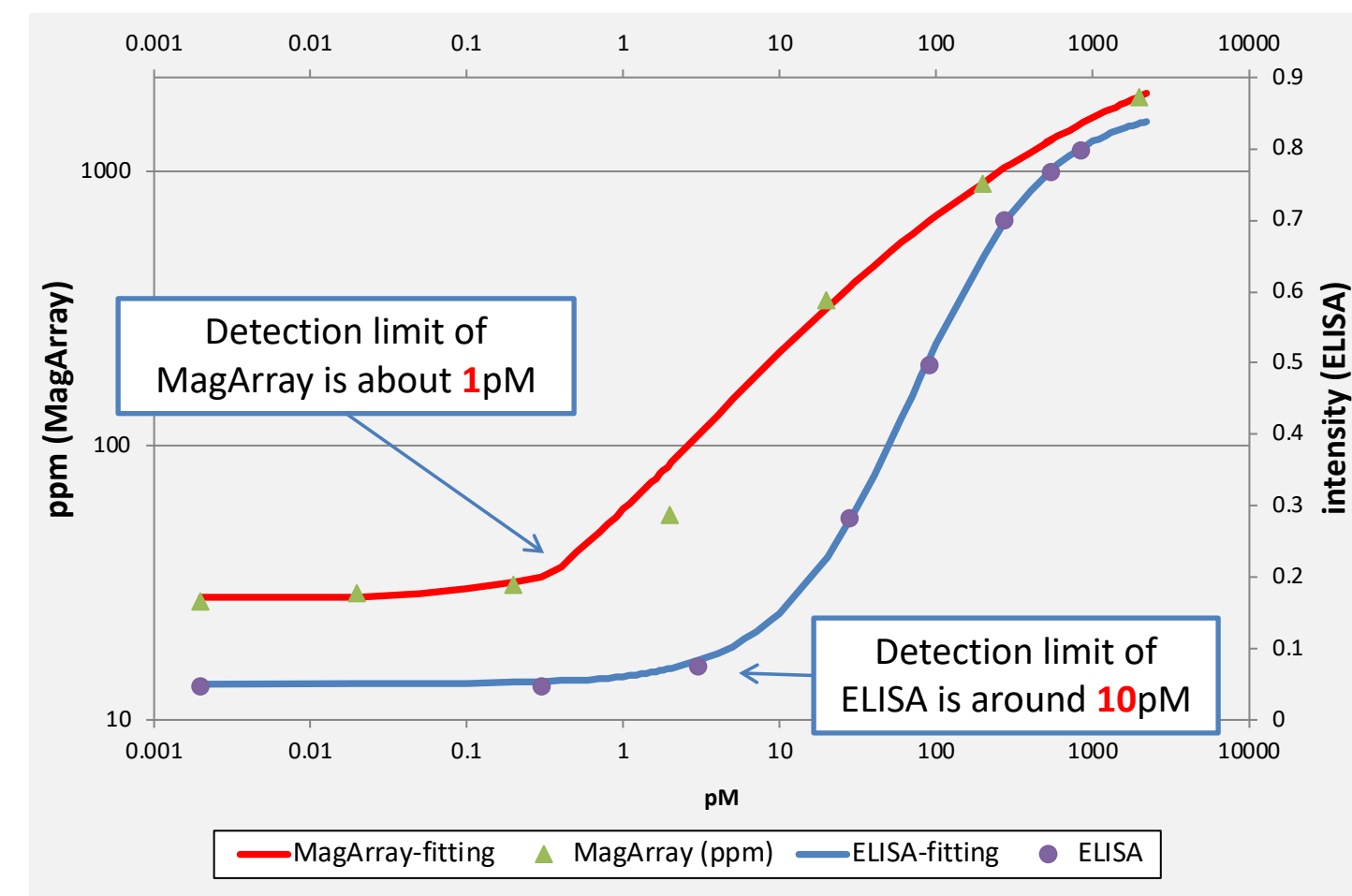
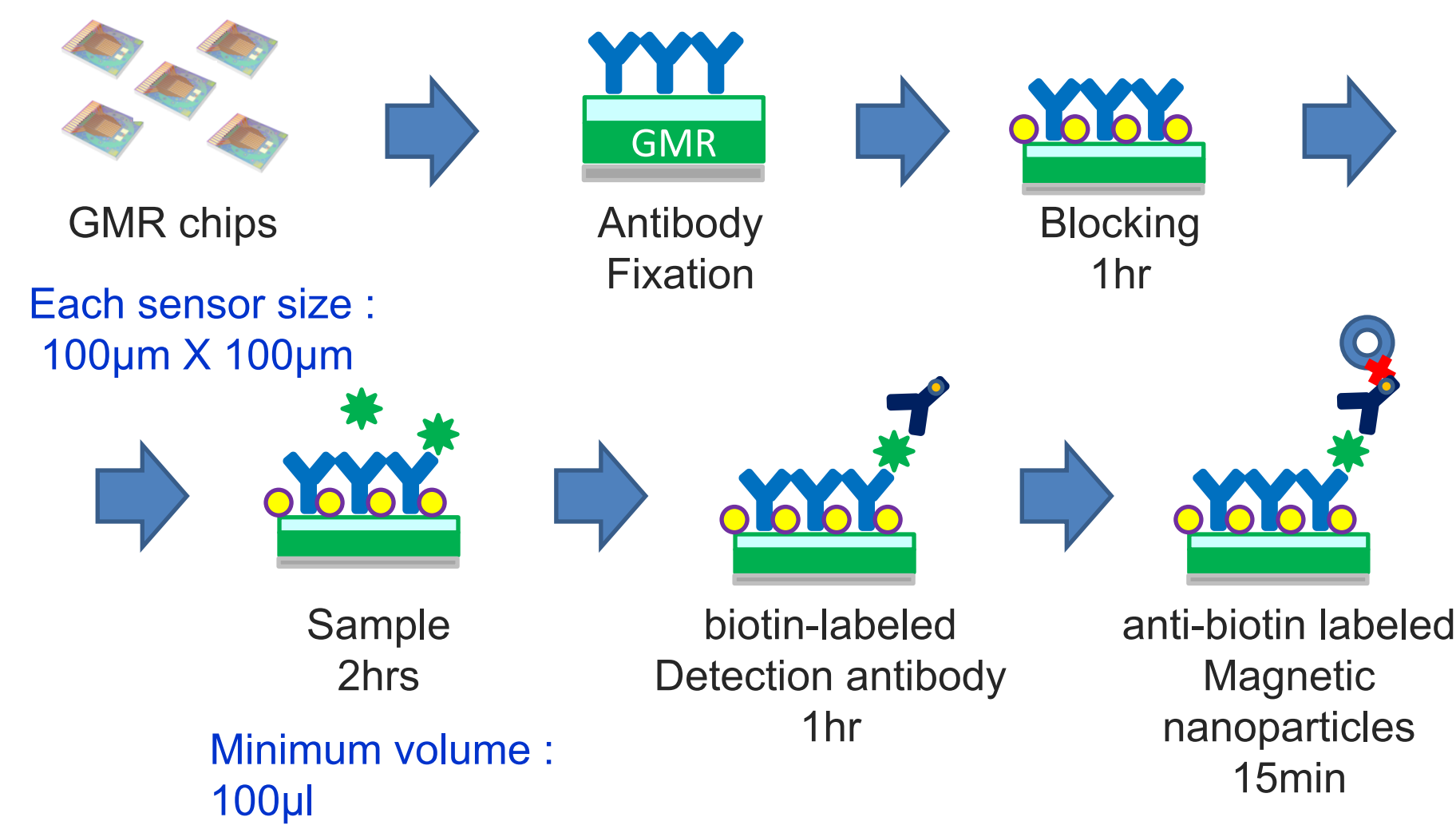


Figure.1 HE4 – MagArray vs. ELISA Standard Curves

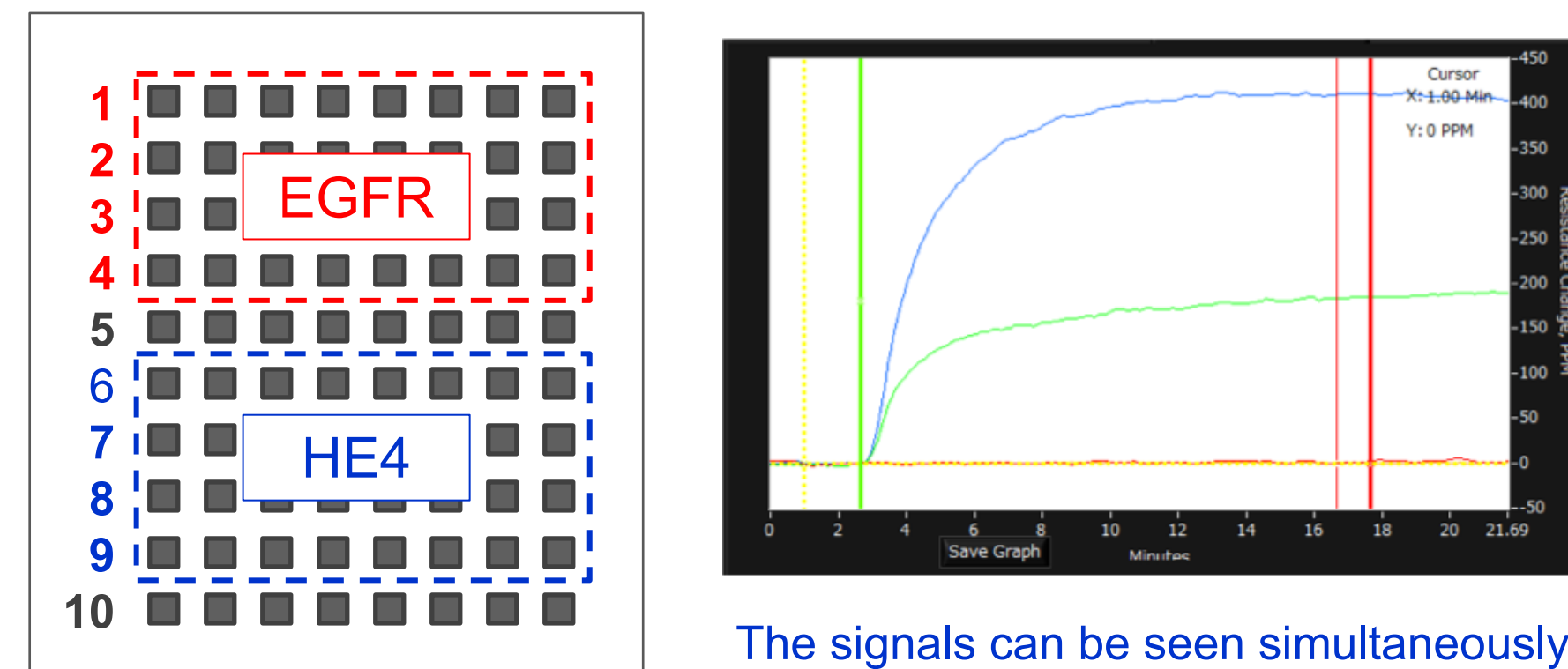
Standard Procedure



Materials and Methods

GMR sensor chip

This platform has 80 sensors per chip (described below) and the top 4 rows were used with EGFR, 6-9 rows were with HE4. Each row has 8 sensors. *It can be free to spot any antibodies.



The signals can be seen simultaneously.

Standard curve using purified samples, 1/10 dilution series

EGFR : 10fg/ml ~ 100ng/ml

HE4 : 50aM ~ 500pM

Diluted buffer ; PBS

Blocking : BSA

Detection antibody : 1µg/ml , 0.05% Tween20 in PBS

Multiplex assay study

MagArray system has 80 sensors per chip. Calculation each row was evaluated for multi-biomarkers.

Recovery check

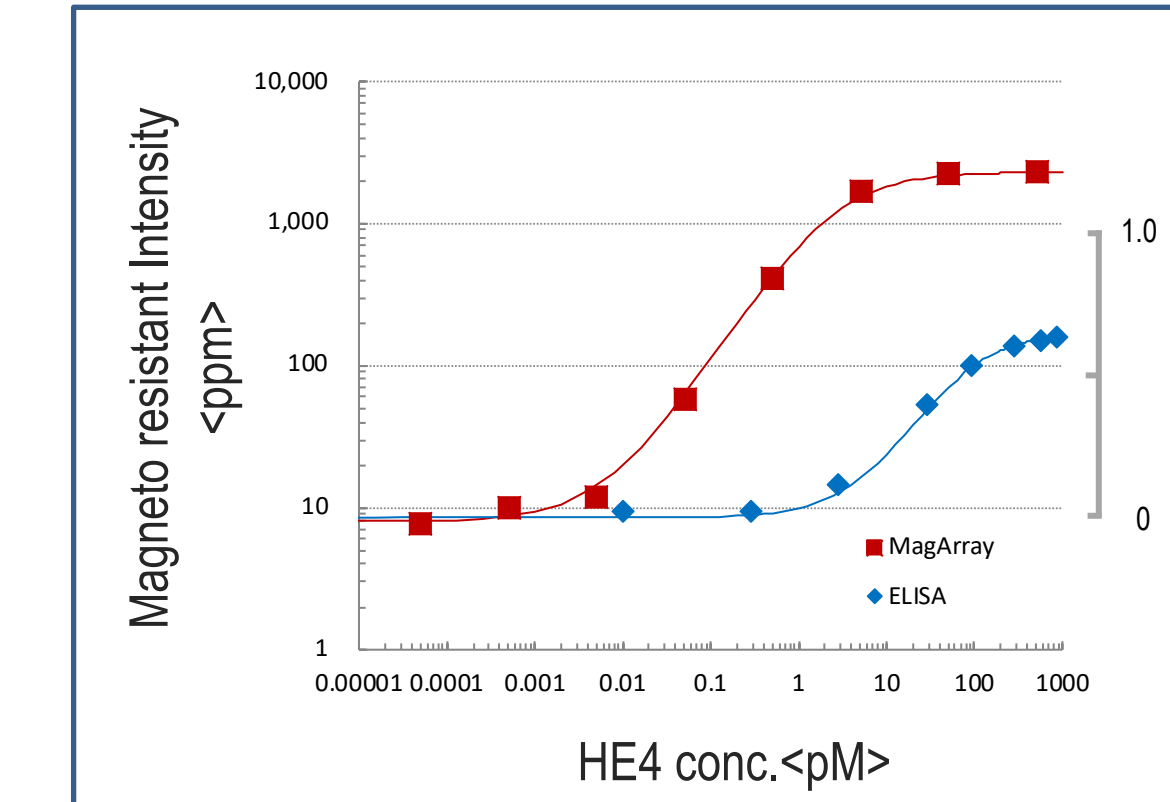
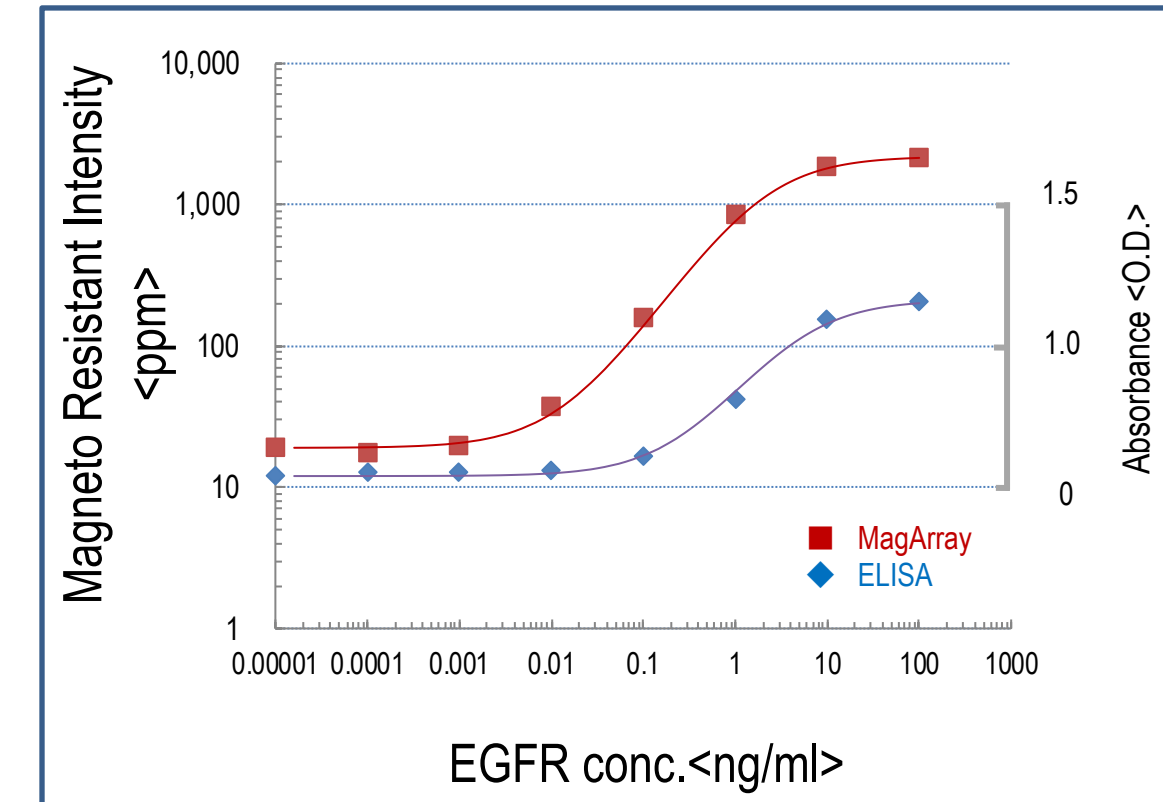
Samples were spiked with known standards into serum and were compared with purified samples.

Evaluation of the correlation with patient samples

The evaluation of correlation was done by ROC method.

Results and Discussions

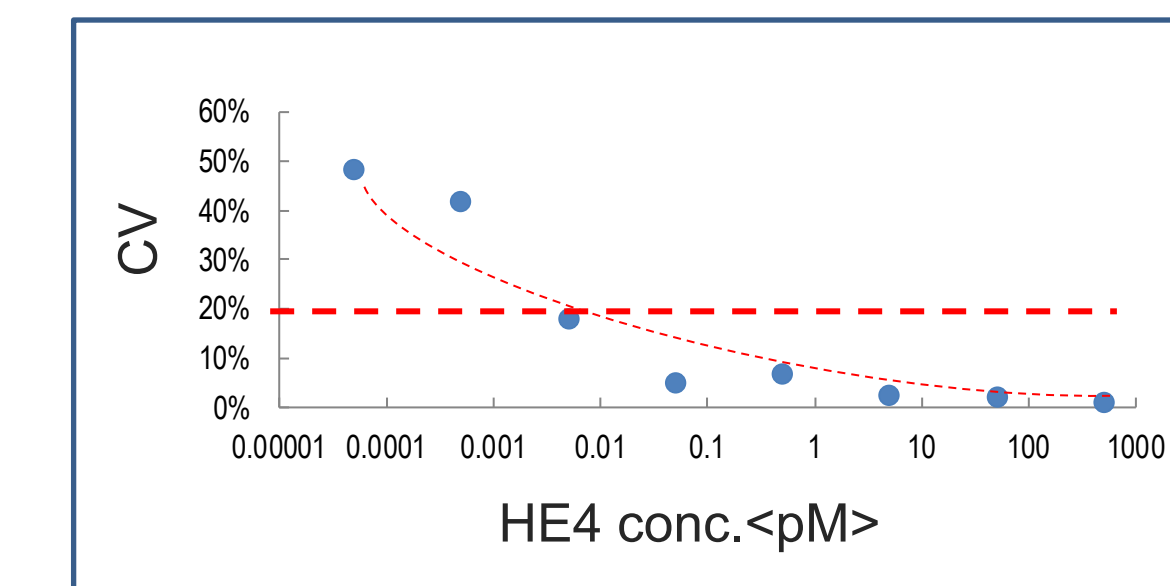
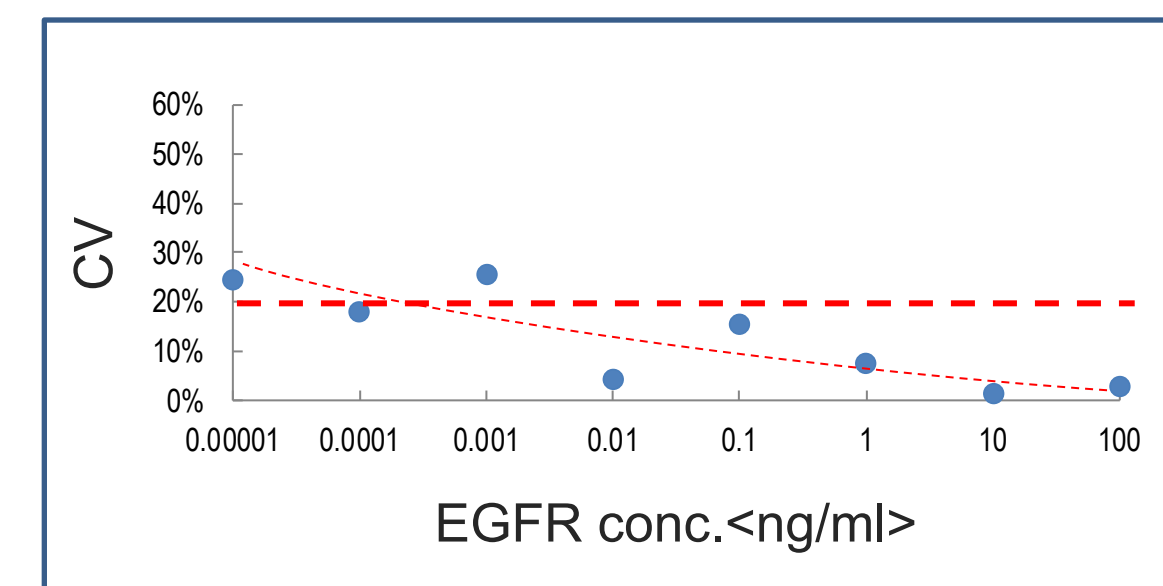
Standard curves of EGFR and HE4



The background noise depend on cross-reaction was improved and it could be high-sensitive.

Accuracy Profile

3 runs at each concentration was done and CV was calculated. In general, it is said to be good that CV could be less than 20%.



After all, the detection limit was following:

EGFR : MagArray 10pg/ml vs ELISA 1ng/ml , HE4 : MagArray 50fM vs ELISA 10pM
This shows that MagArray system is about 100 times higher than ELISA , and has wide range.

Multiplex assay study

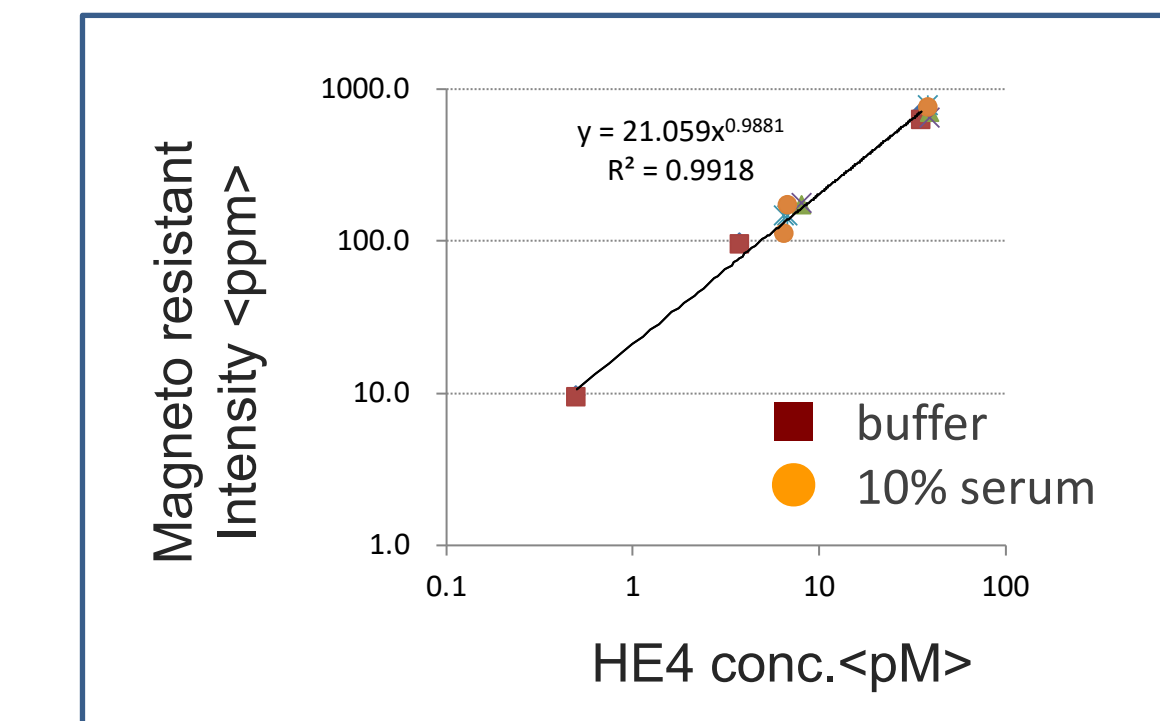
The re-calculation about 100pg/ml EGFR (because these were the largest CV) were done with each row digested. This shows that the average and CV of each row was as good as of all and 10 kinds of biomarkers could be measured simultaneously.

	n1	n2	n3	Ave	SD	inter CV
row 1(8 sensors)	132.9	161.1	178.4	157.5	23.0	14.6%
row 2(8 sensors)	133.9	165.8	177.9	159.2	22.7	14.3%
row 3(8 sensors)	139.4	159.1	179.0	159.2	19.8	12.5%
row 4(8 sensors)	134.8	169.6	185.1	163.1	25.8	15.8%
32 sensors	134.1	166.1	182.4	160.9	24.6	15.3%

Definitely, it was confirmed that the cross-reaction never happen and non-specific bind.

Recovery check

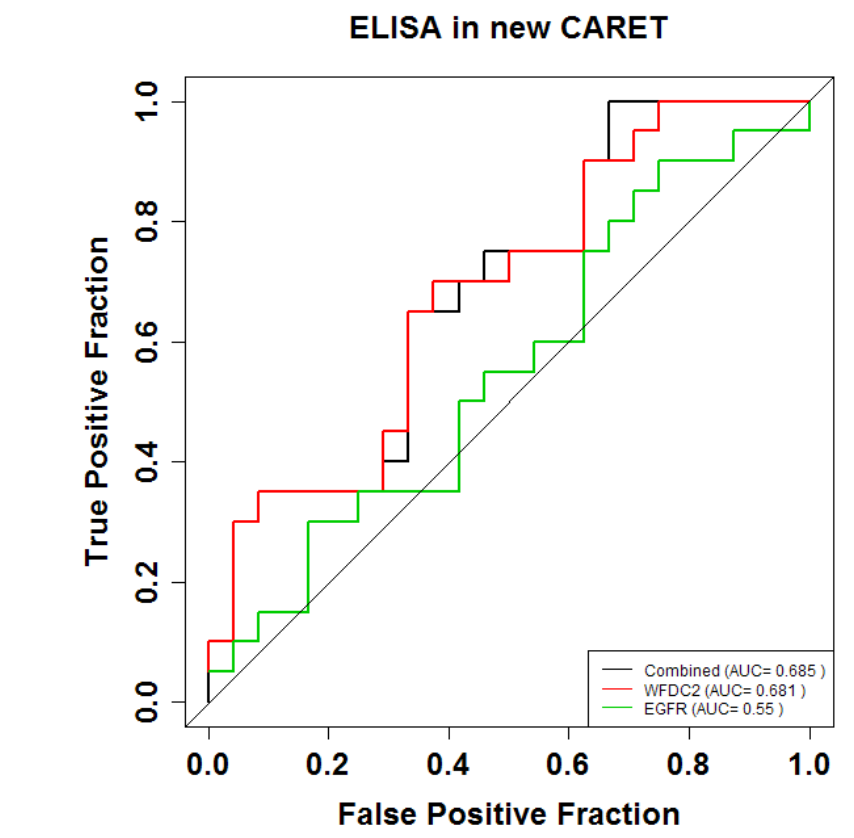
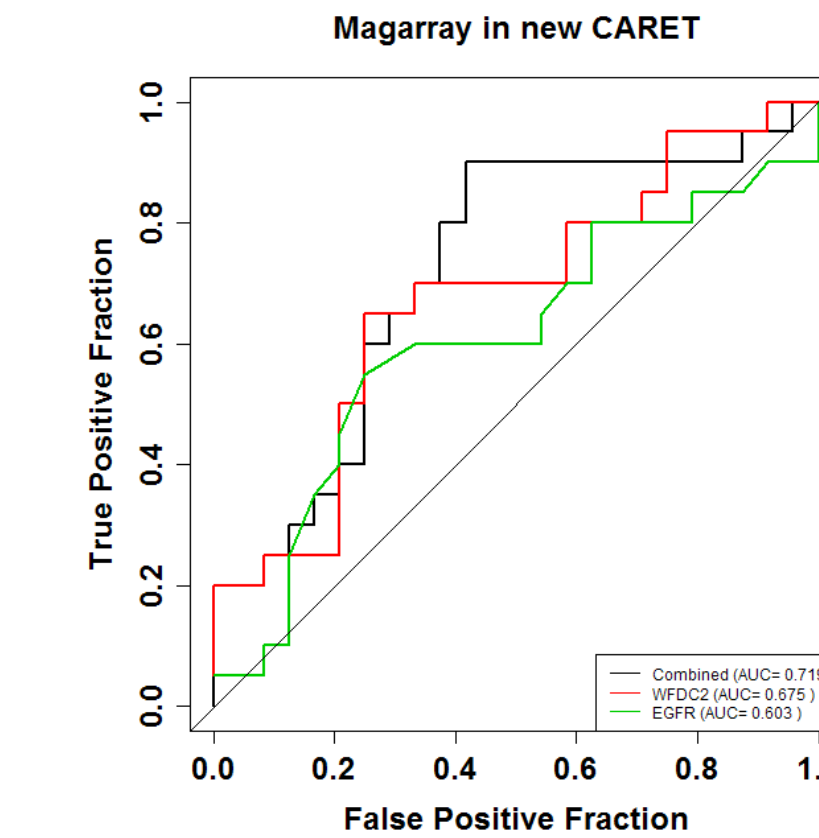
The real measurement might be 10% serum at most and it shows the comparison with buffer at 10% serum.



EGFR was same.

Correlation with patient samples

The patient were made diagnoses as lung cancer. The serum sample were diluted 1:100 to PBS and were detected.



High-sensitivity caused to improve 'AUC' value (0.719 ← 0.685 of ELISA) though these were preliminary data.

Conclusions

It is good to say the following:

1. This novel platform is a higher sensitive than usual ELISA method. The detection limit could be lower proximately 1/100 times.
 2. High-sensitivity could improve to select or develop better antibodies.
 3. Many sensors /chip could detect multi-biomarkers.
- We hope this platform could be very useful for the clinical in the future.