

# Magnetic sensors as a novel multiplex immunoassay platform with high sensitivity



Heng Yu<sup>1</sup>, Toshiro Saito<sup>2</sup>, Sandy Kamer<sup>1</sup>, Alice Juang<sup>1</sup>, Kalidip Choudhury<sup>1</sup>, Haruki Mizukami<sup>1</sup>, Luis Carbonell<sup>1</sup>



<sup>1</sup>MagArray, Milpitas, CA, <sup>2</sup>Hitachi High-Technologies, Tokyo, Japan

## ABSTRACT

**Background:** MagArray platform is based on the detection of magnetic particles as labels in bioassays. The detection of magnetic and electric signals provides unique advantages over traditional immunoassay platforms based on optical signals. For example, the signals are insensitive to matrix and common interferences, specifically reported here is the high sensitivity of multiplex immunoassays allowed by this platform.

**Objective:** Detection of IL6, IL8, IFN  $\gamma$  and MIP-1 $\alpha$  with a sensitivity of below 0.1 pg/ml is used to demonstrate the potential of magnetic biosensors for multiplex immunoassays with high sensitivity.

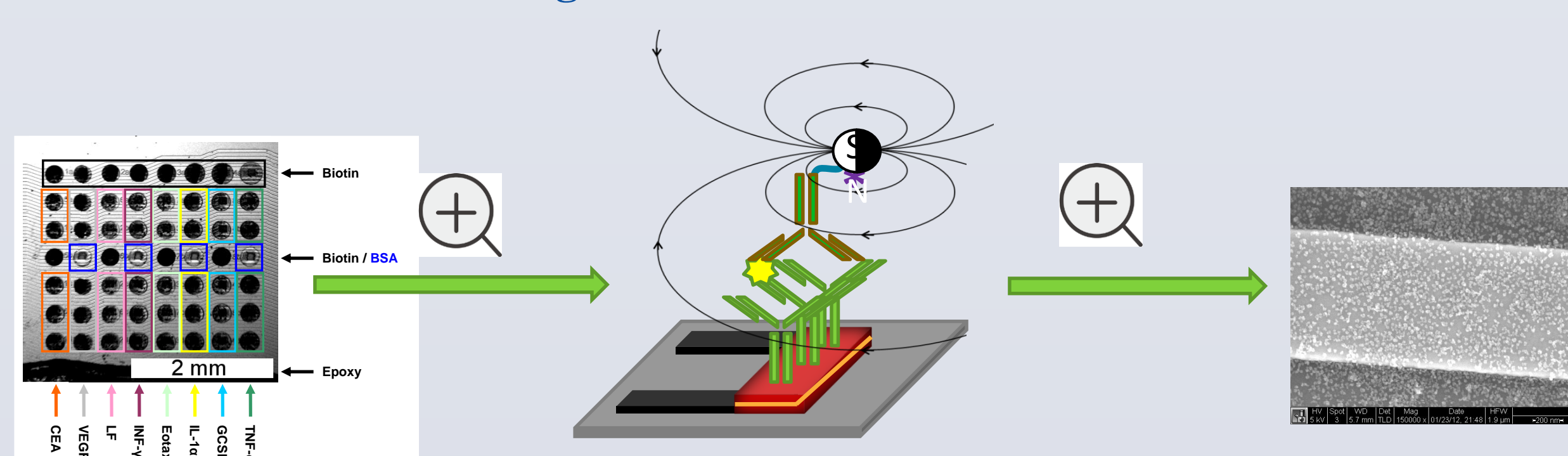
**Methods:** Antibody pairs for the four cytokines were first screened in a parallel mode to quickly identify antibodies with high affinity and minimal cross reactivity. After assay optimization, the sensitivity and dynamic ranges are evaluated according to the CLSI guideline.

**Results:** In this multiplex assay of four cytokines, the background or blank signals are caused mainly by the multiplex format using multiple antibodies. The limits of blank were 0.04 pg/ml, 0.05 pg/ml, 0.05 pg/ml, and 0.05 pg/ml for IL6, IL8, IMP-1 $\alpha$ , and IFN- $\gamma$ , respectively. At 0.1pg/ml of each protein in the multiplex assay, the CVs were 7.9%, 6.9%, 10.7%, and 14.5% respectively. We are also applying the multiplex immunoassay described here to test a small scale of lung cancer (NSCLC) samples to evaluate their potential for early diagnosis of lung cancer combining with other biomarkers we have developed.

**Conclusions:** MagArray platform is demonstrated to be an appropriate platform for immunoassays that require both high sensitivity and multiplexity. A sensitivity of <0.1pg/ml is routinely demonstrated in a multiplex configuration. In addition, the detection of magnetic signals, rather than optical signals, offers a unique benefit for protein detection in complex biological matrices.

## INTRODUCTION

Fig. 1. GMR Sensors for MNPs



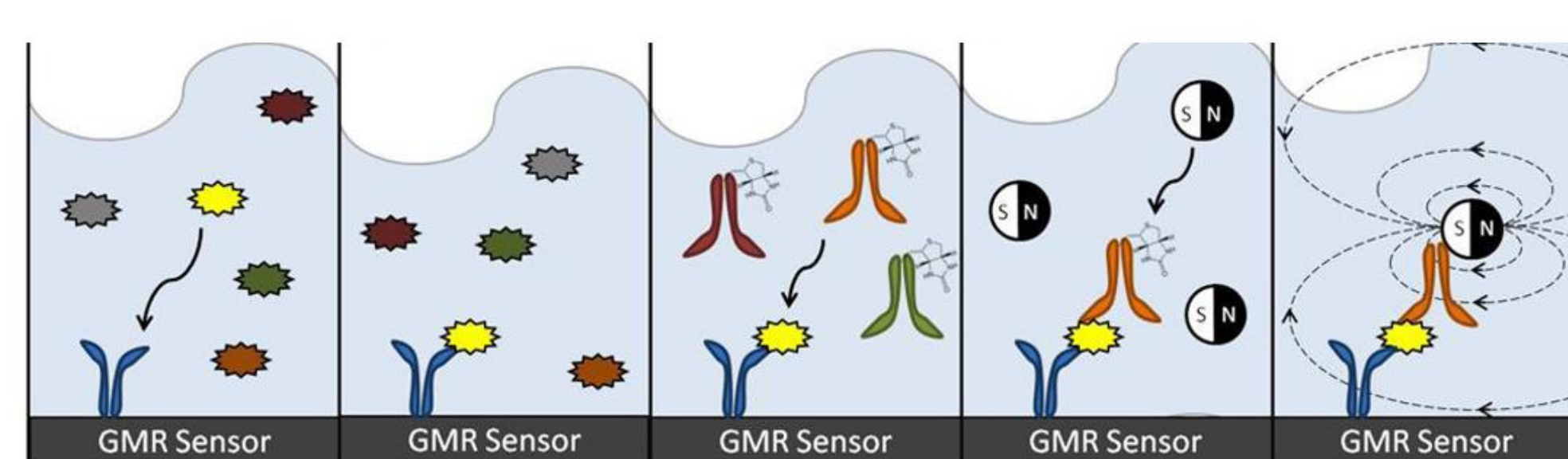
Multiple sensors on the biochip (100x100  $\mu$ m)

GMR sensors are intrinsically proximal in detecting magnetic nanoparticles (MNPs) bound to the sensors surface

MNPs on the sensor surface

## MagArray Assay Principles

Based on standard sandwich ELISA process, with magnetic nanoparticles used as labels



Sample added to chip with pre-functionalized capture antibodies  
Target antigen binds to capture antibody  
Detection antibody binds to target antigen  
Magnetic nanoparticle binds to detection antibody  
Magnetic nanoparticle generates magnetic field that induces electric current in GMR sensor

## METHODS

### Assay development:

1. Antibody pairs are screened and selected for each individual biomarker
2. The cross reactions between all reagents are evaluated
3. Individual biomarker assays are combined to establish the biomarker panel

### Assay performance studies

1. Calibrations including blank baselines are measured to characterize the assay sensitivities of each biomarker in the multiplex panel
2. Values of the limit of blanks and CV at 0.1pg/ml are reported here

## ASSAY and STATISTICAL DATA

Calibration curves for the 4 selected biomarkers are shown here.

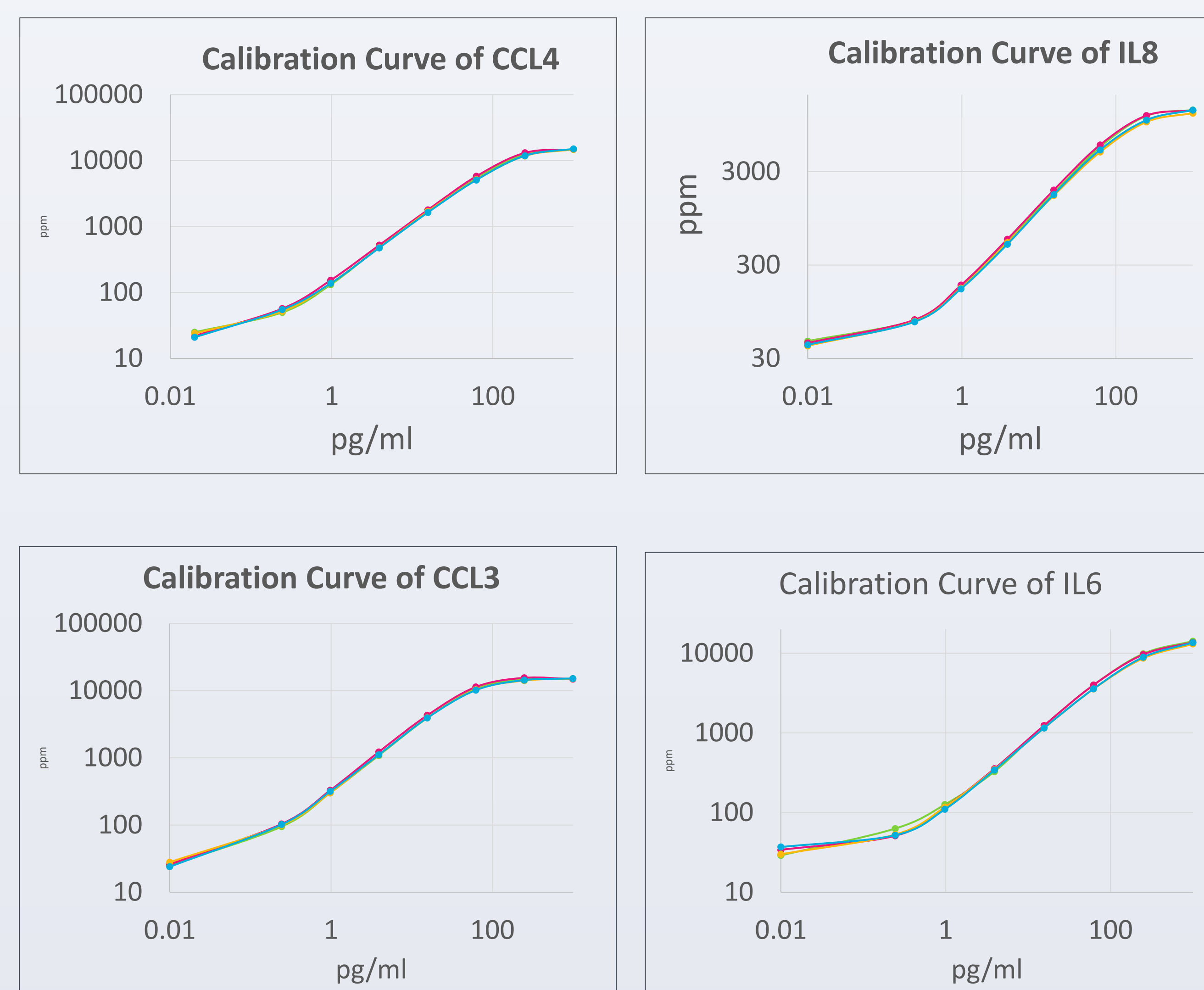


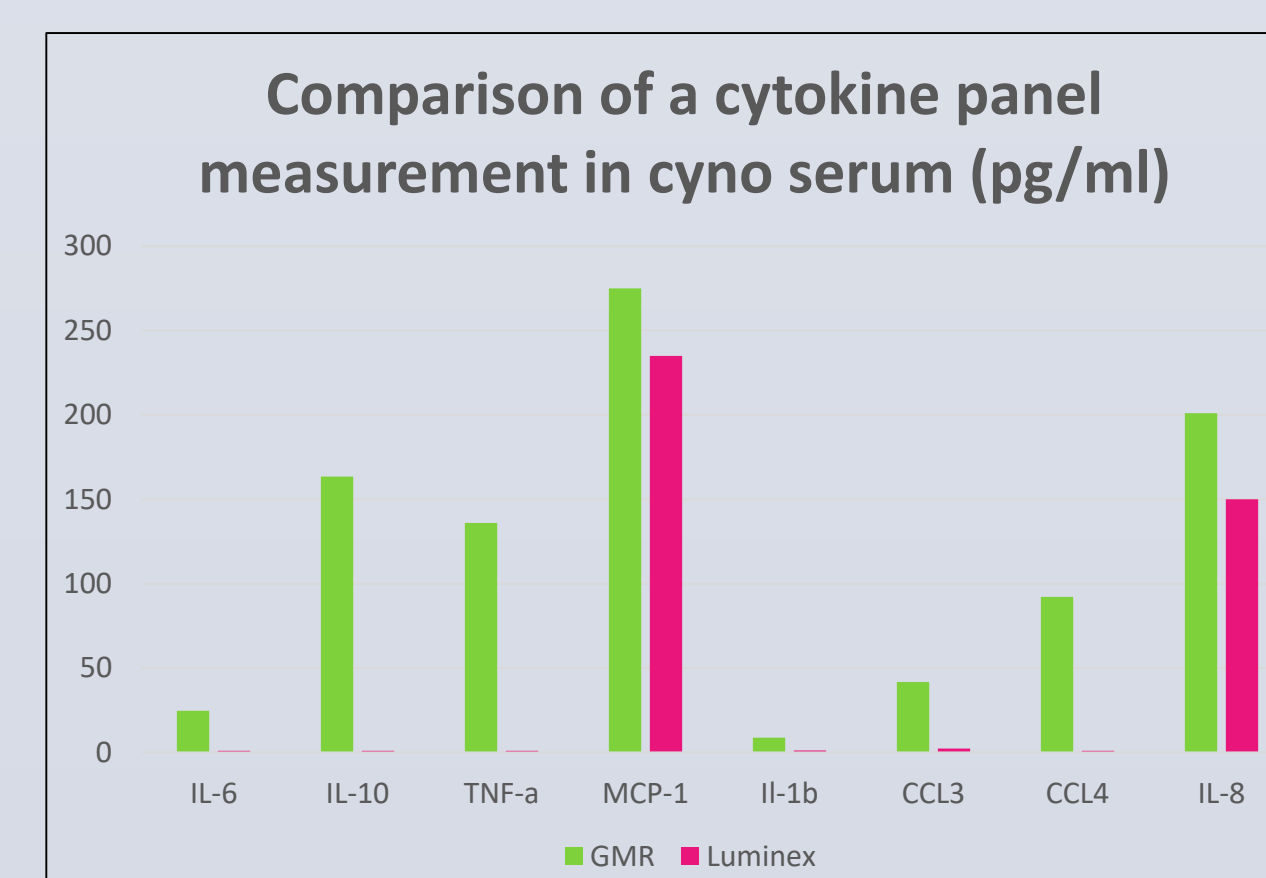
Table 1. Summary of the sensitivities of 4 biomarkers selected.

| Concentration (pg/ml) | blank | 0.1* | 1     |
|-----------------------|-------|------|-------|
| <b>CCL4</b>           | 23    | 54   | 141   |
| CV                    | 0.08  | 0.06 | 0.07  |
| <b>IL-8</b>           | 43    | 76   | 173   |
| CV                    | 0.05  | 0.02 | 0.04  |
| <b>CCL3</b>           | 26    | 100  | 312   |
| CV                    | 0.04  | 0.10 | 0.05  |
| <b>IL-6</b>           | 32.5  | 54.8 | 117.5 |
| CV                    | 0.11  | 0.10 | 0.06  |

Sensitivities of less than 0.1 pg/ml for detecting these cytokines are demonstrated here. It is noteworthy that all sensitivities are obtained in a 9-plex format that simultaneously measures 9 different cytokines with high sensitivities. A relevant application of this panel is shown in Figure 2, which helps in-depth understanding of the MOA of therapeutic.

Figure 2. Comparison of GMR and Luminex platforms for Cytokine Detection

|       | GMR   | Luminex   |
|-------|-------|-----------|
| IL-6  | 24.6  | No signal |
| IL-10 | 163.4 | No signal |
| TNF-a | 136   | No signal |
| MCP-1 | 275   | 235       |
| IL-1b | 8.7   | No signal |
| CCL-3 | 41.8  | No signal |
| CCL-4 | 92.1  | No signal |
| IL-8  | 201   | 150       |



In order to determine MOA of therapeutic, pharma partner performed a multiplex cytokine analysis on serum samples from cynomolgus monkey showing more sensitive detection by GMR allowed a deeper analysis of MOA.

## DISCUSSIONS

### High-sensitive detection of cytokines is achieved in the following conditions:

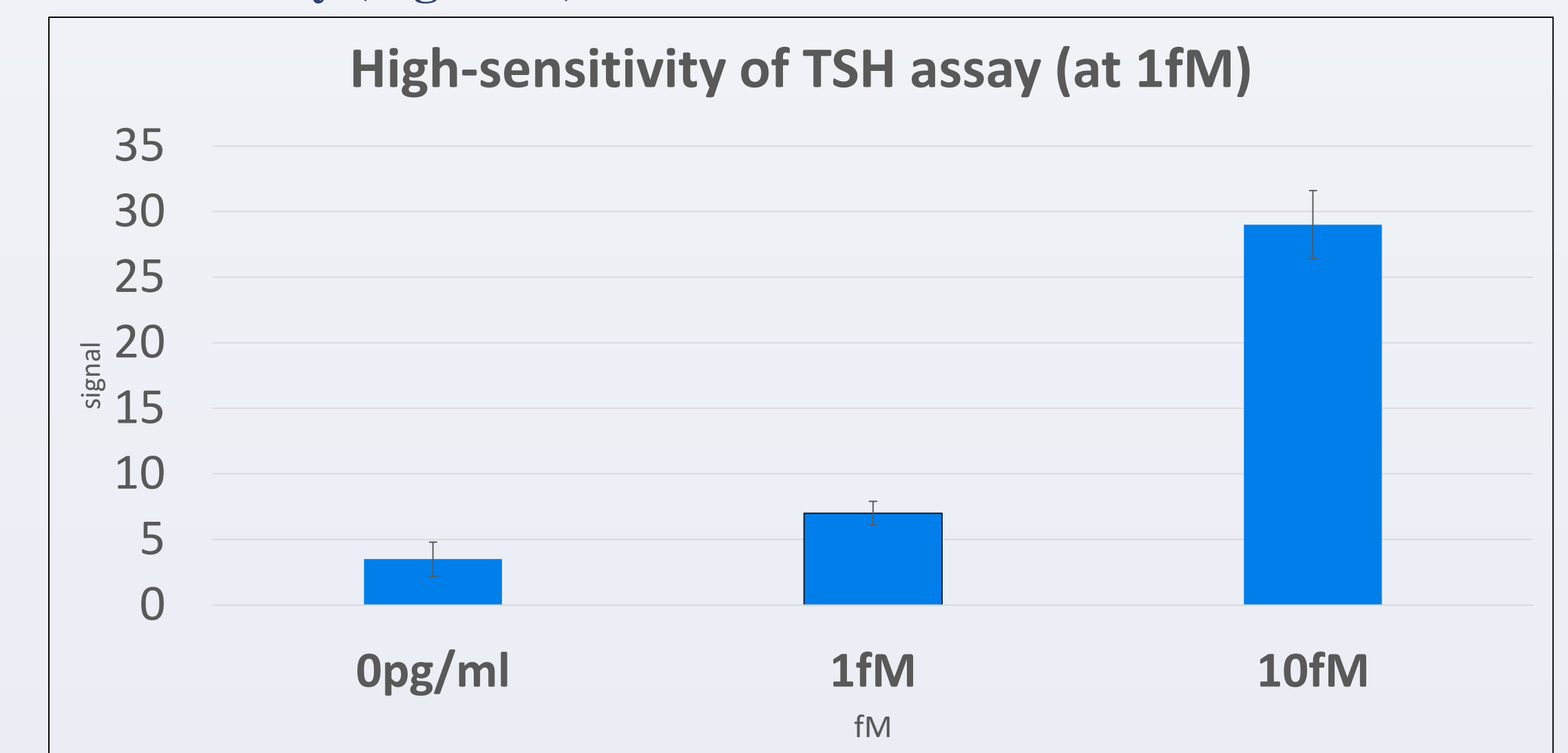
- Multiplex format that detects 9 cytokines simultaneously
- Plasma or serum samples with little effect from the sample matrix
- Relatively short assay time (< 4 hrs of total assays)
- Possibly even higher sensitivity with assay time

### High sensitivity from magnetic biosensor is attributed to:

- The optimal design of the sensors
- A **matrix-insensitive** and **proximity** mechanism of detection
- Uniquely developed proprietary MNPs

## EXAMPLES OF OTHER APPLICATIONS

High sensitivity of TSH assay: a benchmark of platform test at 1fM of sensitivity (Figure 4.)



Sub-pg/ml sensitivity of Cytokine panel for IL-4, IL-12, IL-13 and IFN- $\gamma$  (Figure 3.)

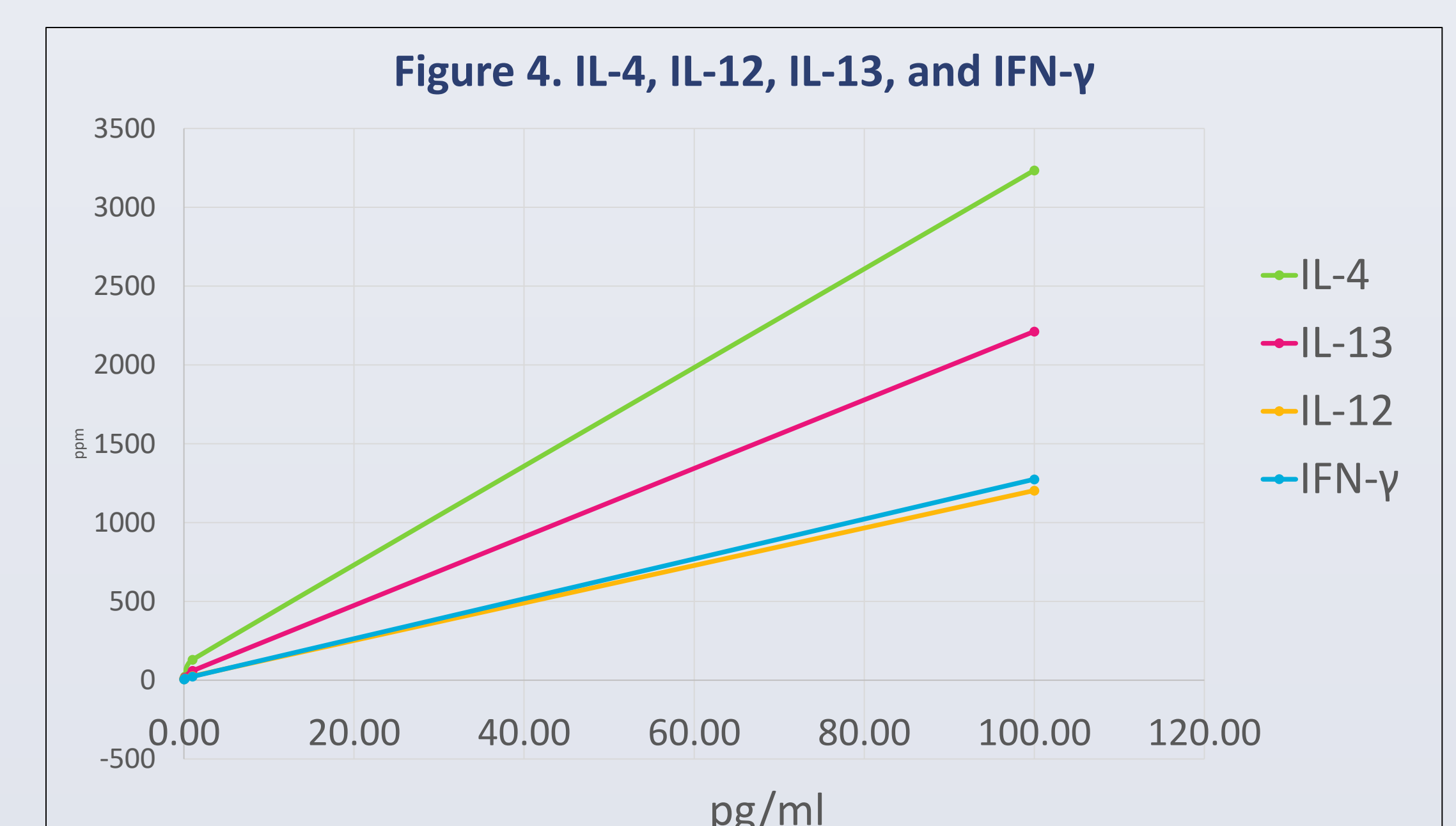


Table 2. Signals of four cytokines from the multiplex assays at different concentrations

| pg/ml                          | 0  | 0.10 | 1.00 | 100.00 |
|--------------------------------|----|------|------|--------|
| <b>IL-4</b>                    | 10 | 23   | 130  | 3235   |
| <b>IL-13</b>                   | 7  | 16   | 60   | 2212   |
| <b>IL-12</b>                   | 6  | 12   | 26   | 1203   |
| <b>IFN-<math>\gamma</math></b> | 5  | 10   | 23   | 1274   |

## CONCLUSIONS

- Magnetic biosensors shown here demonstrate high-sensitivity of cytokine measurement in a multiplex assay
- The sensitivities of cytokine detection are routinely in the sub-pg/ml range, specifically, below 0.1pg/ml
- The application of this novel immunoassay platform is expanding rapidly for sensitive detection of clinically-relevant biomarkers
- The matrix-insensitivity and real-time reading of signals also allow the study of ligand binding such as protein-protein interactions