Abstract #16

Sample-Sparing Immunoassays for Early Detection of Cancer

Mingyue Wang[1], Jermaine Brown[1], Daniel Lee[1], Salvia Misaghian[1], Taron Gorham[1], Leonid Dzantiev[1], Anu Mathew[1], Martin Stengelin[1], Ji Qiu[2], Karen Anderson[2], Joshua Labaer[2], George Sigal[1] and Jacob Wohlstadter[1]

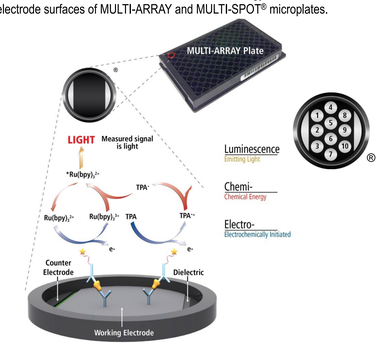
[1]Meso Scale Diagnostics, LLC., Rockville, Maryland, USA; [2]Arizona State University, Tempe, Arizona, USA

1 Abstract

Research Use Only (RUO) sample-sparing MULTI-ARRAY® immunoassays have been developed for several well-established cancer markers. The primary focus of this project is the development of assays to support research of suspicious lung nodules, but many of the selected markers are also relevant to other cancer types. These immunoassays include a serology panel, two biomarker panels, and a single-marker assay. The serology panel includes assays to detect autoantibodies to p53, CTAG-1, and CTAG-2. This panel requires 25 µL of 2,500-fold diluted serum or plasma. A biomarker panel requiring 25 µL of 100-fold diluted serum or plasma detects the following biomarkers: CA15-3, SCFR/Kit, ErbB2, IGFBP-2, MIF, MMP-2, MMP-9 (total), REG4, S100A6, and TNF-RI. A second biomarker panel requiring 25 µL of 10-fold diluted serum or plasma detects the following biomarkers: CA125, Ca19-9, CEACAM-5 (CEA), EGFR, VEGFR-1/Flt-1, FLT3L, HE4, MMP-3 (total), and osteopontin. Finally, an assay for the core lung cancer biomarker cytokeratin-19 was developed. All assays had dynamic ranges that span 3-4 logs and are sufficiently sensitive to measure native levels in commercially sourced samples from apparently healthy individuals. The assays were preliminarily evaluated using more than 100 commercially sourced serum or plasma samples from apparently healthy individuals and more than 100 commercially sourced serum or plasma samples from patients with lung or other cancers. Many of the biomarkers had significantly different concentrations in several cancers compared to samples from apparently healthy individuals. These RUO assays may be useful in identifying biomarkers for a multi-marker panel to study early cancer.

2 Methods

MSD® electrochemiluminescence detection technology uses SULFO-TAG™ labels that emit light upon electrochemical stimulation initiated at the electrode surfaces of MULTI-ARRAY and MULTI-SPOT® microplates.

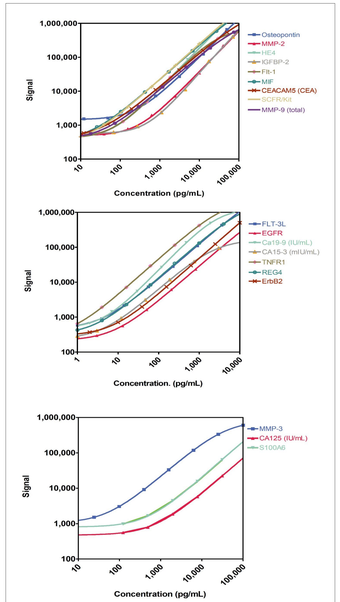


Electrochemiluminescence Technology

- Minimal non-specific background and strong responses to analyte yield high signal-to-background ratios.
- The stimulation mechanism (electricity) is decoupled from the response (light signal), minimizing matrix interference.
- Only labels bound near the electrode surface are excited, enabling non-washed assays.
- Labels are stable, non-radioactive, and directly conjugated to biological molecules.
- Emission at ~620 nm eliminates problems with color quenching.
- Multiple rounds of label excitation and emission enhance light levels and improve sensitivity.
- Carbon electrode surface has 10X greater binding capacity than polystyrene wells.
- Surface coatings can be customized.

3 Multiplex Protein Biomarker Panels

Nineteen assays were combined into two multiplex panels (Table 1) and formulated as kits containing 96-well MULTI-SPOT 10 spot plates, blended calibrators, controls and detection antibody solutions, diluents, and read buffer. The assays were run following the 2-step protocol below using the sample dilutions listed in the table. Lower Limits of Detection (LLOD) are calculated as the concentration that gives a signal 2.5 SD above the blank.



Analyte	Fold Sample Dilution	LLOD	TOC	Units
CA125	10	122	500,000	mIU/mL
CEACAM5 (CEA)		5.40	50,000	pg/mL
HE4		14.9	20,000	pg/mL
Osteopontin		21.3	50,000	pg/mL
Ca19-9		0.91	1,900	IU/mL
EGFR		2.84	13,100	pg/mL
Flt-1		16.7	30,000	pg/mL
FLT-3L		0.71	3,600	pg/mL
MMP-3		3.86	100,000	pg/mL
CA15-3	100	2.38	3,000	mIU/mL
ErbB2		1.74	10,000	pg/mL
IGFBP-2		167	70,000	pg/mL
MIF		4.50	27,000	pg/mL
MMP-2		65.5	40,000	pg/mL
MMP-9 (total)		10.3	75,000	pg/mL
REG4		0.63	4,000	pg/mL
S100A6		79.7	500,000	pg/mL
SCFR/Kit		4.48	40,000	pg/mL
TNFR1		0.21	1,000	pg/mL

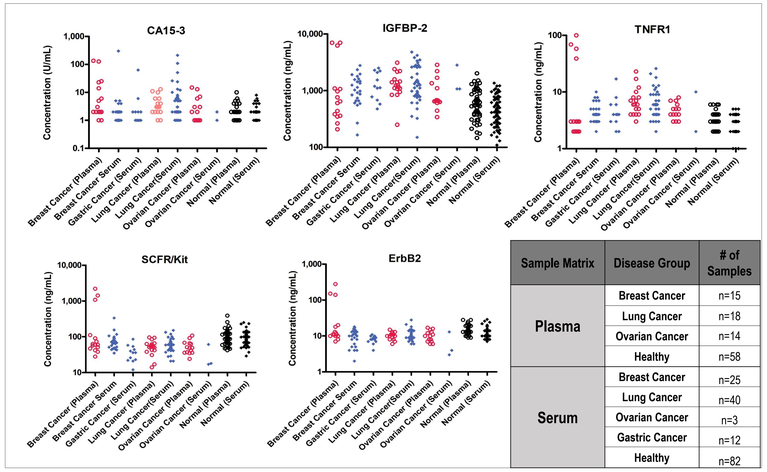
Protocol

1. Add calibrator, control, or sample to assay plate (25 µL/per well). Incubate 1 hour at room temperature (RT).
2. Wash plate and add detection antibody solution (25 µL per well). Incubate 1 hour at RT.
3. Wash plate and add read buffer (150 µL per well). Analyze with MSD instrument.

Figure 1 (A) Typical calibration curves for the assays in two multiplex panels. **(B)** Table of the Lower Limits of Detection (LLOD) and Top of Curve (TOC) concentrations for each analyte in the multiplex panels. Values in this table are not corrected for sample dilution.

4 Individual Serum and Plasma Sample Testing

The biomarker panel of 10 assays requiring 100-fold diluted serum or plasma samples was evaluated using the following commercially sourced samples: 140 serum or plasma samples from apparently healthy individuals and 120 serum or plasma samples from individuals with lung, breast, gastric or ovarian cancer. Five of the biomarkers in this panel displayed significantly different concentrations in various cancer samples compared to samples from apparently healthy individuals.



Sample Matrix	Disease Group	# of Samples
Plasma	Breast Cancer	n=15
	Lung Cancer	n=18
	Ovarian Cancer	n=14
	Healthy	n=58
Serum	Breast Cancer	n=25
	Lung Cancer	n=40
	Ovarian Cancer	n=3
	Gastric Cancer	n=12
	Healthy	n=82

Figure 2 Sample concentrations (corrected for sample dilution) for five of the markers in the 100-fold diluted panel. Samples with concentrations above TOC concentrations are assigned those respective values.

The biomarker panel of nine assays that require 10-fold diluted samples was evaluated using 16 serum samples from apparently healthy individuals and 16 serum samples from individuals with lung cancer. Six of the biomarkers (data shown below) had significantly different concentrations in samples from lung cancer individuals compared to apparently healthy individuals.

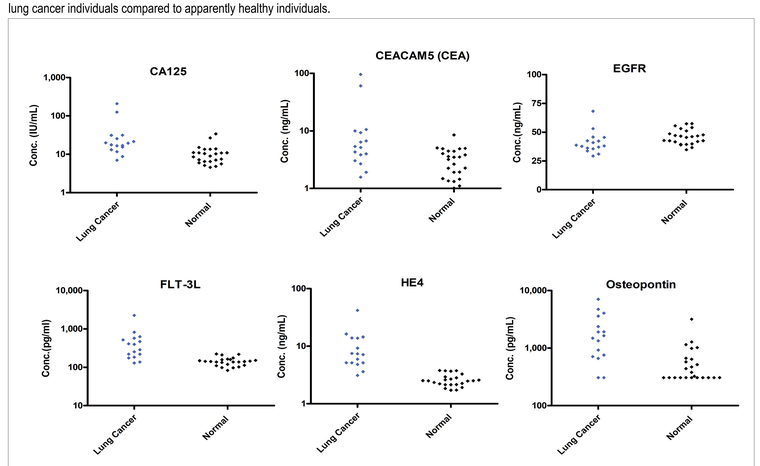


Figure 3 Sample concentrations (corrected for sample dilution) for six markers in the 10-fold diluted panel. Samples with concentrations below LLOD or above TOC concentrations are assigned those respective values.

5 Cytokeratin-19 Assay

An assay for the core lung cancer biomarker cytokeratin-19 was developed using a one-step protocol where the detection antibody and samples were added simultaneously. The assay performance was evaluated using commercially sourced samples, including 81 plasma or serum samples from apparently healthy individuals and 76 plasma or serum samples from breast, lung, and ovarian cancer patients. This assay demonstrated sufficient sensitivity in measuring native cytokeratin-19 levels in apparently healthy individuals. Samples can be further 2-fold diluted when sample volume is limited.

Analyte	LLOD	TOC	Units
Cytokeratin-19	33.5	100,000	pg/mL

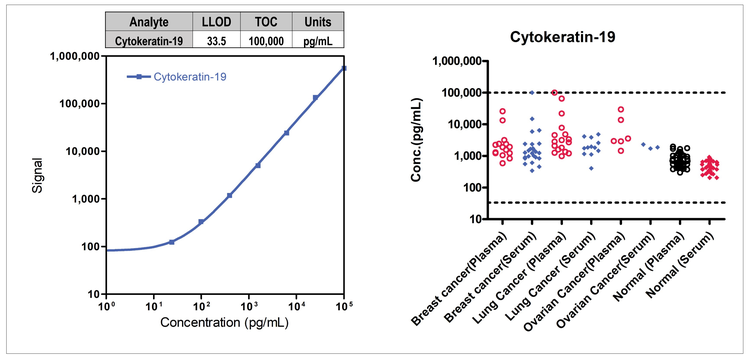


Figure 4 (A) Typical calibration curve for the cytokeratin-19 assay. **(B)** Cytokeratin-19 concentrations in commercially sourced samples. Dashed black lines show the assay's lower limit of detection (LLOD) and top of curve (TOC) concentrations. Concentrations below LLOD or above TOC are assigned those respective values

6 Serology Assay

A multiplexed serology panel was developed to detect autoantibodies against p53, CTAG-1, and CTAG-2. Plates are provided with antigens on spots in the wells of a 96-well plate. Antibodies in the sample bind to the antigens on the spots and anti-human IgG antibody conjugated with MSD SULFO-TAG is used for detection. Commercially sourced plasma or serum samples were tested at 2,500-fold dilution on the serology panel.

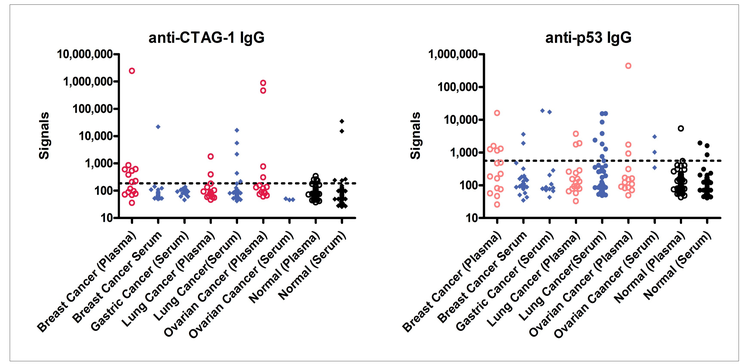


Figure 5 Absolute ECL signals for assays detecting autoantibodies against CTAG1 and p53 in commercially sourced samples. Dashed black lines show the 95th percentile of signals from 98 serum and plasma samples from apparently healthy individuals.

7 Conclusions

- We successfully developed two multiplex panels, one singleplex immunoassay, and a serology panel to measure biomarkers that are relevant to lung or other cancer types.
- The assays performed well in the assessment of samples (including cancer and normal samples) from commercial sources.
- The assays are sensitive enough to detect all markers in most samples.
- These RUO assays, including CA15-3, CA125, CEACAM5 (CEA), IGFBP-2, TNFR1, SCFR/Kit, ERbB2, EGFR, FLT-3L, HE4, osteopontin, and cytokeratin-19, as well as autoantibodies against p53, CTAG-1, and CTAG-2, may be useful in identifying biomarkers for a multi-marker panel to study early cancer.

8 Acknowledgement

Research reported in this poster was supported by the National Cancer Institute of the National Institutes of Health under Award Number U2CCA271903. The content is solely the responsibility of the authors and does not necessarily represent the official views of the National Institutes of Health.

DOWNLOAD POSTER

Meso Scale Discovery®
A division of Meso Scale Diagnostics, LLC.
www.mesoscale.com®

For Research Use Only. Not for use in diagnostic procedures.