MYRIAD RBM™

Multiplex Development of 35 New Cancer-Related Immunoassays

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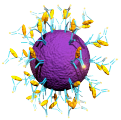
Introduction

Traditional cancer biomarkers such as PSA, CA-125 and CEA have been vital in the diagnosis of cancer, monitoring of therapy, and determination of reoccurrence of disease. While these markers are useful, markers with better sensitivity and specificity are needed. With the support of CPRIT, we are developing a large number of cancer biomarkers and will launch the first 35 cancer markers this year. These assays will be used in conjunction with our Oncology MAP1.0 (100 quantitative assays). Because of the extensive number of analytes and the broad number of pathways that are covered with these two MAPs much valuable information about the detection and treatment of cancer will be discovered.

Methods



Myriad-RBM is a CLIA certified lab offering protein biomarker testing to the clinical and research community. The objectives of the project were to develop new cancer biomarker assays to complement the existing panel of 100. After launching Oncology MAP 1.0 literature searches and discussions with cancer researchers were undertaken to determine potential assays of value to the cancer biomarker community. Antibodies for 35 targets were purchased and conjugated to Luminex beads and biotinylated and screened using the Luminex technology with a recombinant standard and serum and plasma samples from relevant cancer samples and controls. Positive pairs were selected based on sample reactivity and linearity, or in cases where an ELISA was available, correlation to ELISA . The assays were validated for Least Detectable Dose (LDD), lower limit of quantitation (LLOQ), spike-recovery, linearity, precision and sample stability. Assays were scaled up and multiplexed based on dilution and lack of cross-reactivity. The 35 new biomarkers were released as OncologyMAP™ 2.0 and allows for researcher to detect all 35 analytes in less than 100 µl of serum or plasma

Acknowledgements

This project has been funded with funds from the Cancer Prevention and Research Institute of Texas (CPRIT).

Results

1. Assay Development

- Positive assays were selected based on the ability to see differences in cancer serum compared to control serum samples.
- CEACAM-1, IL-18bp and Surfactant protein D assays were selected based on the increased signal seem in lung cancer, as reported in the literature (Fig 1).
- Markers of angiogenesis and metabolic disturbance were shown to increase in cancer serum. (Fig 2)
- Assays where existing ELISAs were available were compared to selected pairs to confirm specificity (Fig 3)

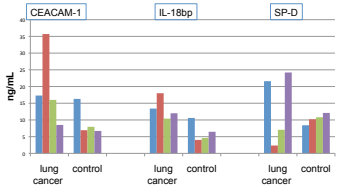


Fig 1. Serum levels of lung cancer biomarkers.
Samples were run as part of the multiplex development, as all 3 markers are reported to change in lung cancer. Patterns differ between patients.

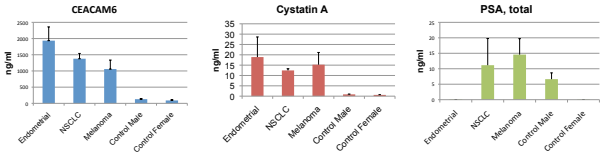


Fig 2. Serum levels in Cancer Serums compared to Control Serum.
Samples were run as part of the multiplex development. The results demonstrate that CECAM6 and Cystatin A increase during the progression of cancer. PSA , as expected, increased in male samples.

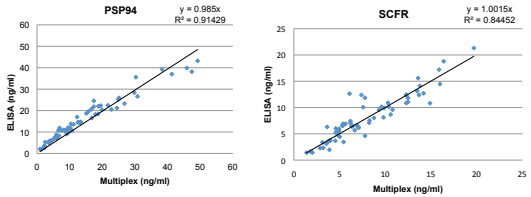


Fig 3. Correlation of serum and plasma samples to ELISA
Developed assays were compared to existing ELISA for PSP94 (Biovendor ELISA;Cat #RD191140200R) and SCF-R (R&D Systems ELISA ;Cat # DSCR00) using serum and plasma samples.

2. Assay Validation

- The assays were validated for Least Detectable Dose (LDD), lower limit of quantitation (LLOQ), cross-reactivity, spike-recovery, linearity, precision and sample stability.
- Spike Recovery, Freeze-thaw (up to 3X) and bench top stability all met acceptance criteria of 70-130% (data not shown)
- Assays within multiplex showed no cross-reactivity for samples and detection.
- Precision (n=2) was all well within acceptance criteria.
- Linearity in serum met acceptance criteria with the exception of IL-18bp, E-Cadherin and CECAM-1; however linearity of plasma met acceptance (data not shown). We are in the process of investigating the cause of non-linear serum results.

Table 1. Precision and Linearity of Serum-Based Controls

		LOW QC			MED QC			HIGH QC			
	Unit	Obs	Intra CV	Inter CV	Obs	Intra CV	Inter CV	Obs	Intra CV	Inter CV	Serum Linearity
C1qR1	ug/ml	0.24	12%	12%	1.9	4%	6%	9.4	7%	10%	96%
ALP	ng/mL	0.59	13%	18%	14	2%	10%	29	2%	7%	97%
ANG-1	ug/mL	5.7	5%	12%	43	8%	10%	259	2%	4%	117%
CA-9	ng/mL	0.52	10%	21%	3.1	3%	7%	17	3%	9%	127%
Cad-13	ng/mL	3.1	11%	17%	26	4%	9%	65	2%	6%	160%
CEACAM1	ng/mL	8.9	5%	11%	111	2%	9%	235	2%	7%	134%
CEACAM6	ng/mL	95	5%	6%	370	1%	2%	1968	3%	7%	77%
COMP	ng/ml	103	8%	11%	712	5%	5%	2809	5%	9%	104%
CTSB	ng/mL	3.6	6%	15%	32	6%	8%	162	5%	7%	81%
Cystatin A	ng/mL	0.68	7%	27%	3.0	2%	5%	30	2%	3%	73%
Cystatin-B	ng/mL	0.74	14%	13%	13	4%	9%	31	6%	10%	90%
Decorin	ng/mL	0.22	8%	9%	2.4	2%	12%	12	3%	4%	117%
DPP4	ng/ml	91	14%	14%	448	3%	6%	1699	6%	11%	86%
E-Cad	ng/mL	11.8	8%	21%	934	3%	13%	2585	3%	13%	74%
IL-18bp	ng/mL	0.27	10%	18%	2.2	7%	8%	16	2%	4%	62%
Lactoferrin	ng/mL	1.1	25%	24%	607	5%	11%	1279	4%	10%	88%
Lipocalin-1	ng/mL	0.29	2%	49%	1.3	1%	15%	10	3%	4%	78%
Midkine	ng/mL	6.1	5%	14%	50	2%	15%	469	8%	21%	85%
PECAM1	ng/mL	21	8%	19%	117	3%	10%	628	2%	8%	123%
PEPD	ug/ml	1.5	3%	10%	7.2	3%	8%	30	5%	10%	106%
PSA, total	ng/mL	0.039	9%	14%	0.18	5%	7%	1.8	5%	3%	77%
PSP94	ng/mL	1.2	4%	13%	13	2%	4%	26	2%	4%	75%
SCFR	ng/mL	1.9	5%	16%	20	4%	12%	41	6%	13%	94%
SP-D	ng/mL	0.76	10%	14%	16	4%	9%	60	2%	6%	102%
TATI	ng/mL	0.36	11%	16%	6.3	3%	13%	16	5%	10%	77%
Tie-1	ng/mL	1.8	9%	8%	27	4%	3%	73	2%	4%	74%
TIMP-2	ng/mL	7.7	6%	11%	73	3%	7%	162	4%	9%	90%
TNXB	ng/mL	36	7%	9%	201	1%	4%	470	3%	7%	79%
TSP4	ug/ml	1.6	7%	11%	7.5	5%	9%	31	4%	8%	105%

Conclusions

- This enlargement of the Myriad RBM's Oncology panel will increase the scope and diversity of biomarker analysis for clinical cancer research.
- Validation data shows that the new panels will give accurate results.

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