Development of Biomarker Assays for Obesity, Diabetes, and Metabolic Syndrome

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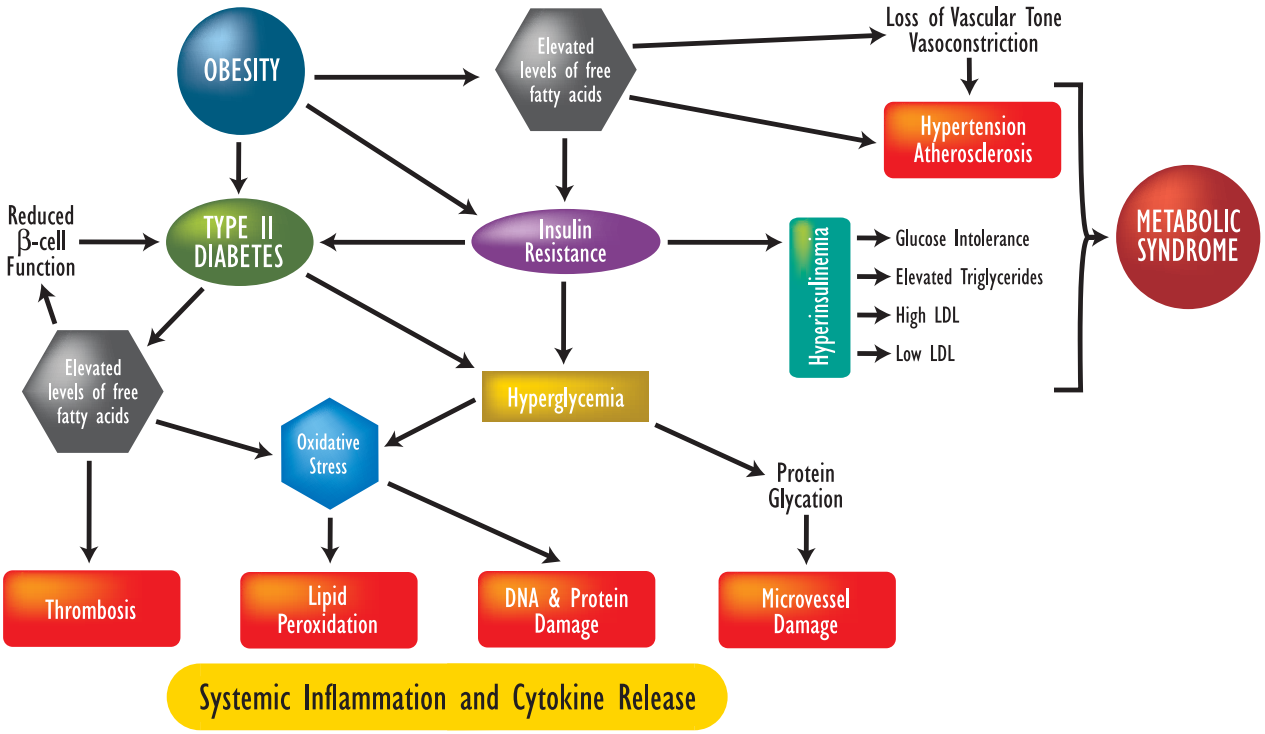
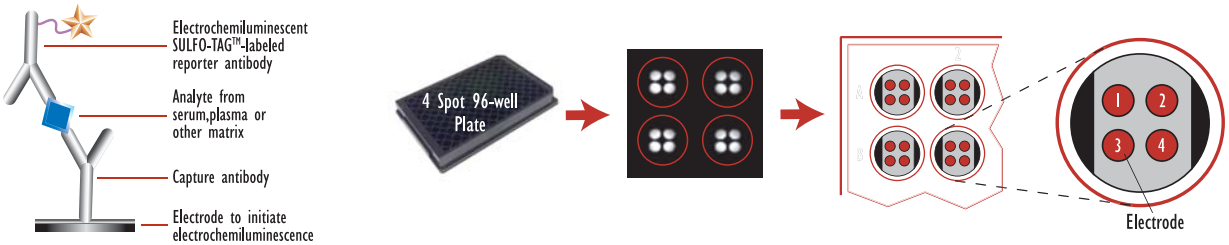
The complex pathology of diabetes, cardiovascular disease and metabolic syndrome has driven an increased demand for quantitative measurement of biomarkers linked to these disease states. Obesity, which has reached epidemic proportions worldwide, is directly related to increased risk for diabetes, hypertension, atherosclerosis and metabolic syndrome. Novel proteomic technologies have helped define key serum biomarkers produced in the gut and adipose tissue and altered in abundance in disease states. Meso Scale Discovery (MSD) has developed quantitative immunoassays that interrogate metabolkine regulators of energy metabolism [Ghrelin (Total)] and glycemic control [Glucagon, GLP-1 (Active & Total)] in serum and plasma samples. These assays, available individually and in multiplex panels, complement an existing selection of MSD metabolic, cytokine and vascular biomarker assays. This broad selection of assays provides for comprehensive and quantitative assessments of biomarkers critical for drug discovery research and monitoring clinical interventions in Obesity and Diabetes.

Critical Pathways in Metabolic Disease



MSD® MULTI-ARRAY® Technology and MULTI-SPOT® Plates

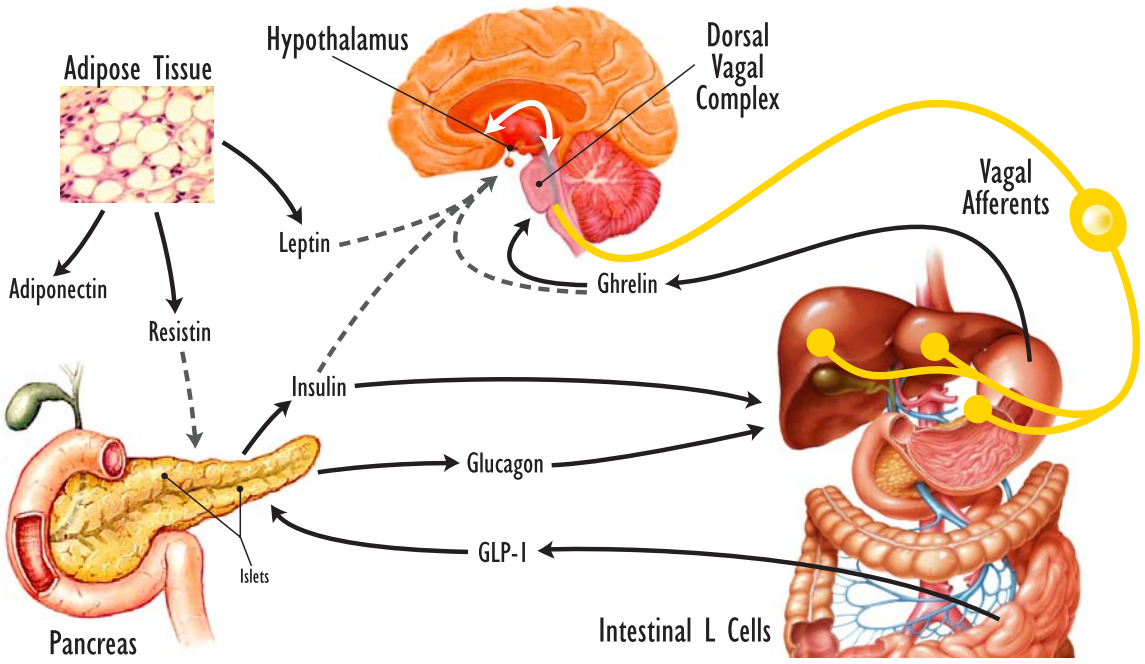
Assay Format



General Protocol

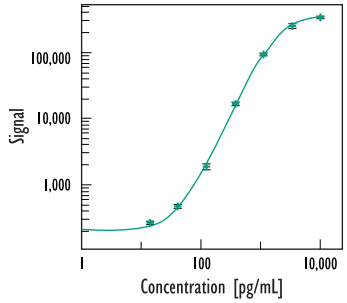
1. MULTI-SPOT 4 Spot 96-Well Plates precoated with capture antibodies. Plates are blocked for 1 hour and washed.
2. Samples or calibrators are incubated for 2 hours with shaking in assay plate with 25 µL assay diluent containing specific protease inhibitors; plates are washed.
3. Antibodies labeled with MSD SULFO-TAG™ are incubated in 25 µL antibody diluent for 1 hour with shaking; plates are washed.
4. MSD Read Buffer T (with surfactant) is added, 150 µL per well and analyzed on MSD SECTOR™ Imager.

Metabolic Regulators of Glycemic and Appetite Control



MSD MULTI-ARRAY assays are now available for high-throughput, quantitative measurements of metabolic serum and plasma Biomarkers

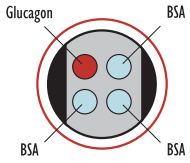
Detection of Mouse/Rat Glucagon



Calibrator (pg/mL)	Mouse/Rat Glucagon		
	Average	StdDev	%CV
0	185	23	13
14	265	8	3
41	477	13	3
123	1,864	186	10
370	16,483	397	2
1,111	94,445	1,433	2
3,333	249,107	19,315	8
10,000	339,538	16,461	5

Glucagon Detection Limit:	19 pg/mL

MSD MULTI-SPOT
96-Well 4-Spot Plate



Spike Recovery

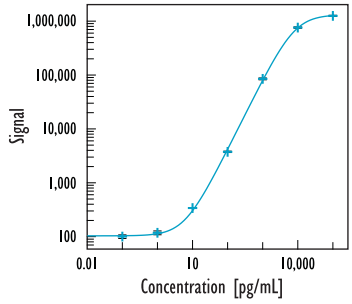
Sample	Average % Recovery
Serum*	68
EDTA Plasma	88
Heparin Plasma	86

* MSD recommends Plasma for best assay performance

Recombinant Glucagon was diluted in serum-based diluent with specific protease inhibitors to limit degradation. Calibrators, serum and plasma samples were assayed on MSD MULTI-SPOT 4-spot plates pre-coated with anti-Glucagon antibody. Glucagon was detected with MSD SULFO-TAG-labeled anti-Glucagon antibody. Mean signals are the average of triplicate wells from a representative experiment to generate a standard curve.

Recovery values presented are averages of 4-6 pooled mouse serum and plasma samples including high, mid and low calibrator spikes.

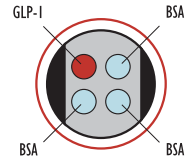
Detection of Mouse/Rat GLP-1 (7-36)amide



Calibrator (pg/mL)	Mouse/Rat GLP-1 (7-36)amide		
	Average	StdDev	%CV
0	101	7	7
0.1	100	8	8
1	118	10	8
10	340	5	1
100	3,753	112	3
1,000	84,894	3,303	4
10,000	760,502	21,437	3
100,000	1,253,062	31,134	2

GLP-1 (7-36)amide Detection Limit:	3 pg/mL

MSD MULTI-SPOT
96-Well 4-Spot Plate



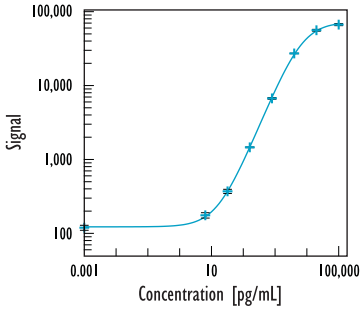
Spike Recovery

Sample	Average % Recovery
Serum	100
EDTA Plasma	108
Heparin Plasma	113

Synthetic GLP-1 (7-36)amide was diluted in serum-based diluent with specific protease inhibitors to limit degradation. Calibrators, serum and plasma samples were assayed on MSD MULTI-SPOT 4-spot plate, pre-coated with anti-GLP-1 (Total) antibody. GLP-1 (7-36)amide was detected with MSD SULFO-TAG labeled anti-GLP-1 (7-36)amide antibody. Mean signals are the average of triplicate wells from a representative experiment to generate a standard curve.

Recovery values presented are averages of 4-6 pooled mouse serum and plasma samples including high, mid and low calibrator spikes.

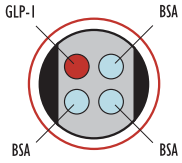
Detection of Mouse/Rat GLP-1 (7-37)



Calibrator (pg/mL)	Mouse/Rat GLP-1 (7-37)		
	Average	StdDev	%CV
6	174	3	2
6.4	227	25	11
32	458	22	5
160	1,618	86	5
800	7,960	96	1
4,000	34,011	1,038	3
20,000	65,633	3,919	6
100,000	79,449	3,894	5

GLP-1 (7-37) Detection Limit:	4 pg/mL

MSD MULTI-SPOT 96-Well 4-Spot Plate



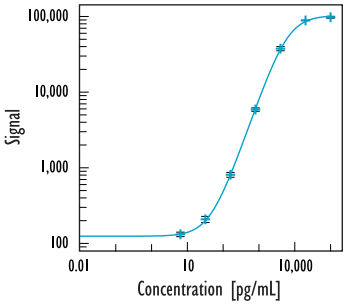
Spike Recovery

Sample	Average % Recovery
Serum	101
EDTA Plasma	106
Heparin Plasma	119

Synthetic GLP-1 (7-37) was diluted in serum-based diluent with specific protease inhibitors to limit degradation. Calibrators, serum and plasma samples were assayed on MSD MULTI-SPOT 4-spot plates pre-coated with anti-GLP-1 (Total) antibody. GLP-1 (7-37) was detected with MSD SULFO-TAG labeled anti-GLP-1 (7-37) antibody. Mean signals are the average of triplicate wells from a representative experiment to generate a standard curve.

Recovery values presented are averages of 4-6 pooled mouse serum and plasma samples including high, mid and low calibrator spikes.

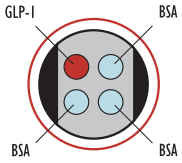
Detection of Mouse/Rat Total GLP-1



Calibrator (pg/mL)	Mouse/Rat GLP-1 (Total)		
	Average	StdDev	%CV
6	193	20	11
6.4	155	1	1
32	218	16	7
160	713	23	3
800	5,376	172	3
4,000	35,243	1,377	4
20,000	101,219	3,076	3
100,000	127,265	4,409	3

GLP-1 (Total) Detection Limit:	22 pg/mL

MSD MULTI-SPOT 96-Well 4-Spot Plate



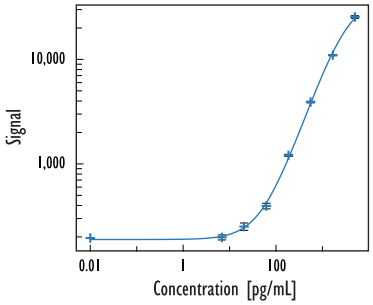
Spike Recovery

Sample	Average % Recovery
Serum	97
EDTA Plasma	99
Heparin Plasma	110

Synthetic GLP-1 (7-36)amide was diluted in serum-based diluent with specific protease inhibitors to limit degradation. Calibrators, serum and plasma samples were assayed on MSD MULTI-SPOT 4-spot plates, pre-coated with anti-GLP-1 (Total) antibody. GLP-1 (Total) was detected with MSD SULFO-TAG labeled anti-GLP-1 (Total) antibody. Mean signals are the average of triplicate wells from a representative experiment to generate a standard curve.

Recovery values presented are averages of 4-6 pooled mouse serum and plasma samples including high, mid and low calibrator spikes.

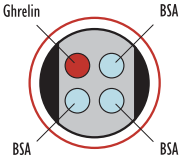
Detection of Mouse/Rat Total Ghrelin



Calibrator (pg/mL)	Mouse/Rat Ghrelin (Total)		
	Average	StdDev	%CV
0	195	2	1
6.8	197	15	8
20.5	251	21	8
61.7	396	25	6
185	1,209	27	2
555	3,910	70	2
1,667	10,989	146	1
5,000	25,471	706	3

Ghrelin (Total) Detection Limit:	11 pg/mL

MSD MULTI-SPOT 96-Well 4-Spot Plate



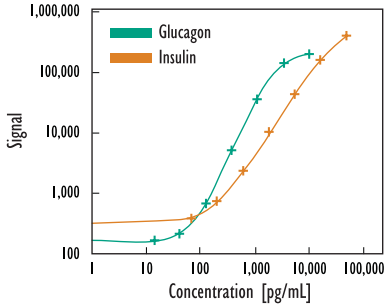
Spike Recovery

Sample	Average % Recovery
Serum	87
EDTA Plasma	106
Heparin Plasma	92

Recombinant rat Des-Ghrelin was diluted in serum-based diluent with specific protease inhibitors to limit degradation. Calibrators, serum and plasma samples were assayed on MSD MULTI-SPOT 4-spot plates, pre-coated with Ghrelin antibody. Ghrelin was detected with MSD SULFO-TAG labeled anti-Ghrelin (Total) antibody. Mean signals are the average of triplicate wells from a representative experiment to generate a standard curve.

Recovery values presented are averages of 4-6 pooled mouse serum and plasma samples including high, mid and low calibrator spikes.

Mouse Metabolic Panel II: (Glucagon/Insulin)



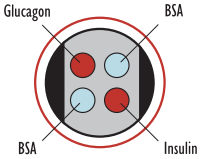
Calibrator (pg/mL)	Mouse Glucagon		
	Average	StdDev	%CV
0	168	12	7
14	164	1	0
41	216	12	6
123	684	64	9
370	5035	299	6
1,111	35,932	2182	6
3,333	141,444	5788	4
10,000	204,486	11382	6

Glucagon Detection Limit:	29 pg/mL

Calibrator (pg/mL)	Mouse Insulin		
	Average	StdDev	%CV
0	274	21	8
69	387	7	2
206	721	26	4
617	2,320	87	4
1,825	10,214	390	4
5,556	43,799	3,078	7
16,667	169,039	11,038	7
50,000	400,496	10,699	3

Insulin Detection Limit:	45 pg/mL

MSD MULTI-SPOT 96-Well 4-Spot Plate



MSD has developed a multiplex assay combining Glucagon and Insulin assays on MULTI-SPOT 4-spot plates. Coating and detection antibodies show no cross-reactivity and assay detection limit and sensitivity for both analytes are equivalent to their respective stand-alone assays.

Recovery values presented are averages of 4-6 pooled mouse serum and plasma samples including high, mid and low calibrator spikes.

Spike Recovery

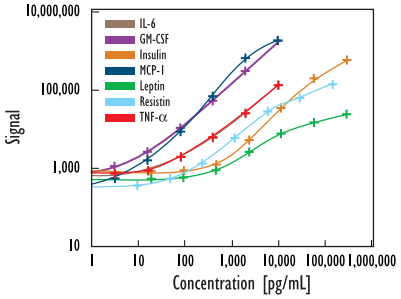
Sample	Average % Recovery	
	Glucagon	Insulin
Serum*	77	73
EDTA Plasma	96	99
Heparin Plasma	95	96

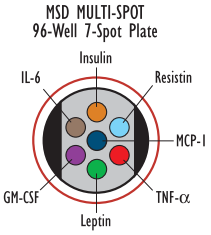
* MSD recommends Plasma for best assay performance

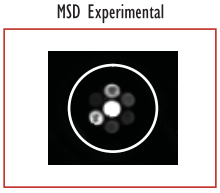
MSD Experimental

10,000 pg/mL Glucagon
50,000 pg/mL Insulin

Mouse/Rat Metabolkine Panel







MSD MULTI-ARRAY technology allows for simultaneous measurement of Metabolic, Cytokine and Vascular biomarkers in multiplex with excellent performance compared to individual assays.

	Detection Limits	
Assay	Multiplex pg/mL	Historical pg/mL
IL-6	4.3	7.7
GM-CSF	1.1	4.4
Insulin	125	~100
MCP-1	1.3	9
Leptin	83	~100
Resistin	10	<1
TNF-α	6.1	3.4

Assay	Native Levels		% Recovery					
			High Spike		Mid Spike		Low Spike	
	Serum pg/mL	Plasma pg/mL	Serum	EDTA Plasma	Serum	EDTA Plasma	Serum	EDTA Plasma
IL-6	17	30	125	95	103	93	106	66
GM-CSF	0	0	102	102	102	90	97	80
Insulin	1,772	2,816	85	122	80	109	95	128
MCP-1	65	44	102	95	100	92	nd	nd
Leptin	3,806	1,224	118	95	92	70	94	121
Resistin	42,760	17,800	114	112	121	131	129	136
TNF-α	3	4	102	125	107	134	102	107

Conclusions

- We present highly specific individual and multiplex assays for the detection of plasma and serum biomarkers critical to Diabetes, Obesity and Metabolic Syndrome.
- We show the ability to multiplex these assays with other cytokine, vascular and serum biomarkers related to inflammatory states. Thus, multiple analytes can be assayed simultaneously in a single well.
- MULTI-ARRAY technology-based assays are powerful replacements for established methods because the assays save time, labor and precious sample volume..
- MSD MULTI-SPOT technology provides highly quantitative and sensitive immunoassays with broad dynamic range that are superior to existing techniques.