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

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

OBESITY
- Reduced $\beta$-cell Function
- Elevated levels of free fatty acids

TYPE II DIABETES
- Insulin Resistance
- Elevated levels of free fatty acids
- Oxidative Stress
- Hyper glycemia

METABOLIC SYNDROME
- Protein Glycation
- Glucose Intolerance
- Elevated Triglycerides
- High LDL
- Low LDL
- Loss of Vascular Tone Vasoconstriction
- Hypertension Atherosclerosis

Systemic Inflammation and Cytokine Release

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

Assay Format
- Electroc chemiluminescent SULFO-TAG™-labeled reporter antibody
- Analyte from serum, plasma or other matrix
- Capture antibody
- Electrode to initiate electroluminescence

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

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

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)

Graph showing the detection of Mouse/Rat GLP-1 (7-37) with calibration data points.

Detection Limit: 4 pg/mL

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.

Spike Recovery:

<table>
<thead>
<tr>
<th>Sample</th>
<th>Average % Recovery</th>
</tr>
</thead>
<tbody>
<tr>
<td>Serum</td>
<td>111</td>
</tr>
<tr>
<td>EDTA Plasma</td>
<td>106</td>
</tr>
<tr>
<td>Hepatic Plasma</td>
<td>119</td>
</tr>
</tbody>
</table>

Detection of Mouse/Rat Total GLP-1

Graph showing the detection of Mouse/Rat Total GLP-1 with calibration data points.

Detection Limit: 22 pg/mL

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.

Spike Recovery:

<table>
<thead>
<tr>
<th>Sample</th>
<th>Average % Recovery</th>
</tr>
</thead>
<tbody>
<tr>
<td>Serum</td>
<td>97</td>
</tr>
<tr>
<td>EDTA Plasma</td>
<td>99</td>
</tr>
<tr>
<td>Hepatic Plasma</td>
<td>110</td>
</tr>
</tbody>
</table>
Detection of Mouse/Rat Total Ghrelin

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)

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.
Mouse/Rat Metabolkinine Panel

![Graph showing metabolkinine panel with different lines representing different metabolites]

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

<table>
<thead>
<tr>
<th>Assay</th>
<th>Detection Limits</th>
</tr>
</thead>
<tbody>
<tr>
<td>IL-6</td>
<td>0.6 3.8</td>
</tr>
<tr>
<td>GM-CSF</td>
<td>1.1 4.6</td>
</tr>
<tr>
<td>IL-1β</td>
<td>125 &lt;100</td>
</tr>
<tr>
<td>MCP-1</td>
<td>1.3 9</td>
</tr>
<tr>
<td>Leptin</td>
<td>23 &lt;100</td>
</tr>
<tr>
<td>Resistin</td>
<td>10 &lt;1</td>
</tr>
<tr>
<td>TNF-α</td>
<td>6.1 3.4</td>
</tr>
</tbody>
</table>

**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.