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Development and Characterization of Human, Mouse, and Rat Metabolic Assays on the U-PLEX® Platform

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PURPOSE

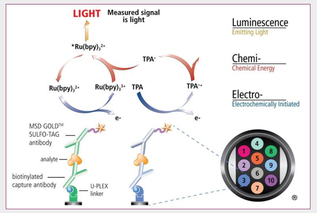
Metabolic-related diseases, including diabetes, obesity, and metabolic syndrome, have driven an increased demand for the sensitive measurement of biomarkers linked to these disease states. The expression levels of biomarkers, produced in the gut, adipose tissue, and the brain, are frequently altered during disease and many can be detected in samples such as plasma and serum. Here we report the development of 34 assays targeting both traditional and emergent metabolic biomarkers for use in human, mouse, and rat samples. After extensive screening, antibodies with high selectivity and affinity have been developed for key markers such as GLP-1 and glucagon.

METHODS

MSD's 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. The U-PLEX assay platform uses 10 unique linkers that specifically bind to individual spots, enabling simple and flexible creation of multiplex immunoassays.

Electrochemiluminescence Technology

- Minimal background combined with strong response to analyte yields high signal-to-noise 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.



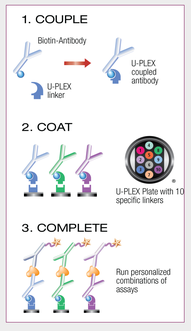
Metabolic U-PLEX Protocol

Couple and Coat the U-PLEX Plate:

- Add 200 µL of the biotinylated capture antibody to 300 µL of the assigned linker. Vortex. Incubate for 30 minutes.
- Add 200 µL of Stop Solution and vortex. Incubate for 30 minutes.
- Combine each U-PLEX-coupled antibody solution into a single tube and vortex. Add 50 µL of multiplex coating solution to each well.
- Incubate with shaking for 1 hour then wash the plate.

Complete the Assay:

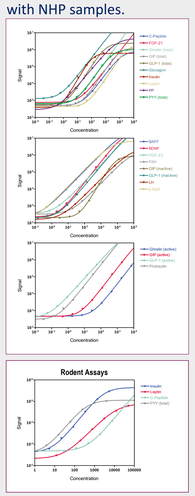
- Add 50 µL of sample, calibrator, or control to each well.
- Incubate the plate for 2 hours, then wash the plate.
- Add 50 µL of detection antibody solution to each well.
- Incubate the plate for 1 hour, then wash the plate.
- Add 150 µL of MSD® Read Buffer to each well and read the plate.



RESULTS

Assay Characteristics

Calibrator curves, lower limits of detection (LLODs), and species cross-reactivity for 22 human assays and 4 assays specific for mouse and rat are shown below. LLODs were calculated from 3 runs each with >20 blank wells. Control samples for each assay showed expected precision and accuracy, with intra-run CVs less than 10%, inter-run CVs less than 25%, and recoveries largely within 70-130% of target concentrations (data not shown). Key metabolic assays such as glucagon and GLP-1 (total) featured exceptional LLODs of 0.13 pM and 0.59 pM, respectively. Note that 8 of the human assays (marked with an asterisk) are cross-reactive with NHP samples. All but two assays are cross-reactive with NHP samples.



Human Assay	Units	LLOD	NHP	Mouse	Rat	Canine
BAFF	pg/mL	0.05	+	-	-	+
BDNF*	pg/mL	0.72	+	+	+	-
C-Peptide	pg/mL	13.7	+	-	-	-
FGF-21*	pg/mL	2.75	+	+	+	+
FGF-23	pg/mL	0.75	+	-	-	-
FSH	µIU/mL	9.02	+	-	-	-
Ghrelin (active)*	pg/mL	13.1	+	+	+	+
Ghrelin (total)*	pg/mL	1.74	+	+	+	+
GIP (active)	pg/mL	1.25	+	-	+	+
GIP (inactive)	pg/mL	25.8	+	-	+	-
GIP (total)	pg/mL	3.69	+	-	+	-
GLP-1 (active)*	pM	0.01	+	+	+	+
GLP-1 (inactive)*	pM	0.86	+	+	+	+
GLP-1 (total)*	pM	0.59	+	+	+	+
Glucagon*	pM	0.13	+	+	+	+
Insulin	µIU/mL	0.32	+	-	-	-
Leptin	pg/mL	14.3	+	-	-	-
LH	µIU/mL	1.63	+	-	-	-
PP	pg/mL	0.19	+	-	-	-
Proinsulin	pM	0.05	+	-	-	-
PYY (total)	pg/mL	2.7	-	-	-	-
β-NGF	pg/mL	0.05	-	-	-	-

Rodent Assay	Units	LLOD	Mouse	Rat
C-Peptide	pg/mL	112	+	+
Insulin	pg/mL	50.1	+	+
Leptin	pg/mL	11.6	+	+
PYY (total)	pg/mL	1.10	+	+

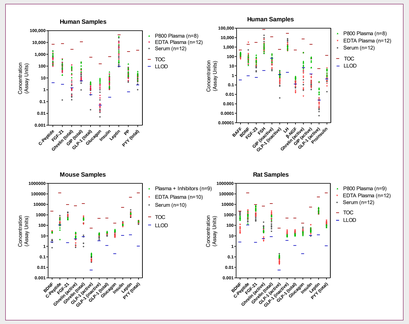
U-PLEX Biomarker Compatibility

Other assays in the U-PLEX product line were tested for compatibility with the new metabolic assays using performance criteria such as dynamic range, sensitivity, sample detection, and non-specific binding between assays of <2%. 65 existing human U-PLEX assays were found to be compatible, creating a human U-PLEX Metabolic Group with 87 biomarkers (see the table below) that can be run together in multiplexed panels. Similarly, 46 existing mouse U-PLEX assays were found to be compatible with the new mouse metabolic assays, making a total of 58 mouse metabolic assays (assay list not shown).

Human assays included in U-PLEX Metabolic Group					
BAFF	G-CSF	IFN-g	IL-12p70	IL-29/IFN-?1	PP
BDNF	Ghrelin (active)	IL-1a	IL-13	IL-31	Proinsulin
ß-NGF	Ghrelin (total)	IL-1ß	IL-15	IL-33	PYY (total)
C-Peptide	GIP (active)	IL-1RA	IL-16	Insulin	SDF-1α (CXCL12)
CTACK (CCL27)	GIP (inactive)	IL-2	IL-17A	IP-10 (CXCL10)	TARC (CCL17)
ENA-78 (CXCL5)	GIP (total)	IL-2Ra	IL-17A/F	Leptin	TNF-a
Eotaxin (CCL11)	GLP-1 (active)	IL-3	IL-17C	LH	TNF-ß
Eotaxin-2 (CCL24)	GLP-1 (inactive)	IL-4	IL-17D	MCP-1 (CCL2)	TPO
Eotaxin-3 (CCL26)	GLP-1 (total)	IL-5	IL-17E/IL-25	MCP-2 (CCL8)	TRAIL
EPO	Glucagon	IL-6	IL-17F	MCP-4 (CCL13)	TSLP
FGF-21	GM-CSF	IL-7	IL-18	M-CSF	VEGF-A
FGF-23	GRO-a (CXCL1)	IL-8 (CXCL8)	IL-21	MDC (CCL22)	YKL-40
FLT3L	I-309 (CCL1)	IL-9	IL-22	MIF	
Fractalkine (CX3CL1)	IFN-a2a	IL-10	IL-23	MIP-1a (CCL3)	
FSH	IFN-ß	IL-12/IL-23p40	IL-27	MIP-5	

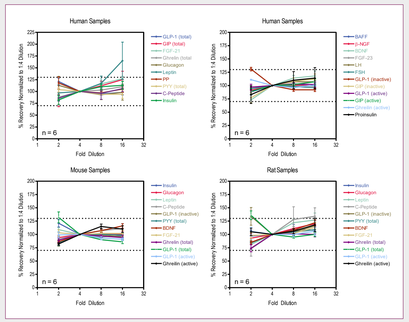
Native Sample Testing

Metabolic assays were evaluated for the ability to detect their respective analytes in P800 plasma, EDTA plasma, and serum. Samples were diluted 4-fold and tested in the presence of aprotinin and diprotin A protease inhibitors. All analytes were detected in P800 plasma samples. Differences in concentrations measured in P800 plasma and EDTA plasma for several analytes such as Glucagon, Ghrelin (active), GIP (active), and GLP-1 (active), demonstrates the necessity for sample collection in P800 tubes.



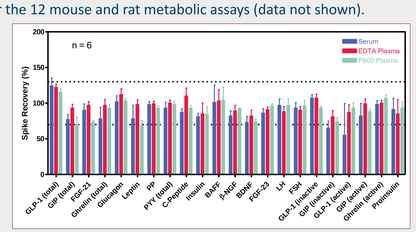
Dilution Linearity

Plasma samples were spiked with calibrator and diluted 2-, 4-, 8-, and 16-fold before testing. Most analytes recover within 70-130%. Data were normalized to the 4-fold sample dilution. Similar results were obtained with serum samples (data not shown).



Spike Recovery

Normal human serum, EDTA plasma, and P800 plasma samples were spiked with calibrators at 3 levels (high, mid, and low). The recovery values in the chart below represent averages from the 3 spike levels. Most analytes recover within 70-130%. Similar spike recovery results were observed for the 12 mouse and rat metabolic assays (data not shown).



Assay Specificity

Human metabolic assays were evaluated for cross-reactivity and interference against homologous and/or related analytes. Data are shown for selected human assays. Interference and cross-reactivity were measured by spiking a blank or mid-level calibrator (Cal 3) with a physiological level of the potential interferent (other analytes). In both cases, signals were obtained and backfitted to the calibrator curve to generate a concentration. No unexpected cross-reactivity or interference was observed.

Interference = Measured concentration (Cal-3+test analyte)/Cal-3 concentration X 100

Cross-reactivity = Measured test analyte concentration/expected concentration of the tested analyte X 100

Assay	Potential Interferent	% Interference	% Cross-reactivity
GLP-1 (active)	GLP-1 (9-36)	n.d.	n.d.
	Glucagon	n.d.	n.d.
	Exendin-4	n.d.	n.d.
	Glicentin	n.d.	n.d.
	GLP-1 (1-36) NH_2	n.d.	n.d.
	GLP-1 (9-36)	n.d.	n.d.
	GLP-2	n.d.	n.d.
	MPGF	n.d.	n.d.
	Oxytomodulin	n.d.	n.d.
GLP-1 (total)	GLP-1 (7-36)	310%	111%
	GLP-1 (9-36)	308%	113%
	Glucagon	n.d.	n.d.
	Glicentin	n.d.	n.d.
	GLP-1 (1-36) NH_2	n.d.	46.7%
	GLP-1 (7-37)	n.d.	6.4%
	GLP-2	n.d.	n.d.
	MPGF	n.d.	n.d.
	Oxytomodulin	n.d.	n.d.
Glucagon	Glicentin	n.d.	n.d.
	GLP-1 (7-36)	n.d.	n.d.
	GLP-1 (9-36)	n.d.	n.d.
	GLP-2	n.d.	n.d.
	Oxytomodulin	n.d.	n.d.
	GLP-1 (1-36)NH2	n.d.	n.d.
	GLP-1 (7-37)	n.d.	n.d.
	GRPP	n.d.	n.d.
	Mini-glucagon	n.d.	n.d.
	MPGF	n.d.	n.d.
Insulin	Insulin receptor (s)	n.d.	n.d.
	Proinsulin	n.d.	n.d.
	Insulin – Aspart	n.d.	n.d.
	Insulin – Glargine	n.d.	n.d.
	Insulin - Lispro	n.d.	n.d.
C-Peptide	GLP-1 (9-36)	n.d.	n.d.
	Insulin	n.d.	n.d.
	Proinsulin	n.d.	19.9%

Assay	Potential Interferent	% Interference	% Cross-reactivity
Ghrelin (active)	C-Peptide	n.d.	n.d.
	Desghrelin	n.d.	n.d.
	GLP-1 (7-36)	n.d.	n.d.
	GLP-1 (9-36)	n.d.	n.d.
	Glucagon	n.d.	n.d.
	Insulin	n.d.	n.d.
	Leptin	n.d.	n.d.
	PYY (1-36)	n.d.	n.d.
	PYY (3-36)	n.d.	n.d.
	Ghrelin (1-10)	n.d.	n.d.
	NPY	n.d.	n.d.
	proGhrelin	n.d.	n.d.
Ghrelin (total)	C-Peptide	n.d.	n.d.
	Octanoylated Ghrelin	131.4%	73.7%
	GLP-1 (7-36)	n.d.	n.d.
	GLP-1 (9-36)	n.d.	n.d.
	Glucagon	n.d.	n.d.
	Insulin	n.d.	n.d.
	Leptin	n.d.	n.d.
	PYY (1-36)	n.d.	n.d.
	PYY (3-36)	n.d.	n.d.
	Ghrelin (1-10)	n.d.	n.d.
	NPY	n.d.	n.d.
	proGhrelin	n.d.	n.d.
Leptin	IL-6	n.d.	n.d.
	Resistin	n.d.	n.d.
	ApoJ	n.d.	n.d.
	CNTF	n.d.	n.d.
	IL-11	n.d.	n.d.
	IL-12	n.d.	n.d.
	IL-6	n.d.	n.d.
	Leptin R	76.8%	n.d.
	LIF	n.d.	n.d.
	Oncostatin M	n.d.	n.d.

n.d. = not detected

CONCLUSIONS

Twenty-two new human and twelve new mouse and rat metabolic assays have been developed for use on the MSD U-PLEX platform. These assays demonstrate sensitive and accurate measurement of both traditional and emergent biomarkers associated with diabetes, obesity, and metabolic syndromes. Several of these assays, including GLP-1 (total) and glucagon, feature best-in-class sensitivity and specificity. These assays can be combined with 65 existing human or 46 existing mouse U-PLEX assays in a species-specific manner, enabling researchers and drug developers to simultaneously measure novel combinations of biomarkers associated with metabolic-related diseases.

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