







diluent and added to PLT-139 as per assay procedure. The percent (%) recovery on individual samples is represented in the following table.

Sample	Dilution Factor	Expected Conc. (pg/mL)	Observed Conc. (pg/mL)	% Recovery
Antigen	Neat	296.0	NA	NA
	1:2	148.0	145.70	98%
	1:4	74.0	68.79	93%
	1:8	37.0	38.17	103%
Sample-1	1:5	177.87	NA	NA
	1:10	88.93	97.33	109%
	1:20	44.47	48.66	109%
Sample-2	1:5	191.45	NA	NA
	1:10	95.73	81.44	85%
	1:20	47.86	42.61	89%
	1:40	23.93	21.55	90%

#### Recovery:

Known amounts of Oxyntomodulin were added to five K<sub>2</sub>EDTA samples containing different levels of endogenous Oxyntomodulin. The concentration of Oxyntomodulin was determined before and after the addition of exogenous Oxyntomodulin and the percent recovery was calculated.

Sample	Endogenous Conc. (pg/mL)	Expected Conc. (pg/mL)	Observed Conc. (pg/mL)	% Recovery
1	14.60	28.37	27.97	99%
		42.14	40.33	96%
		55.91	51.95	93%
2	0.001	14.50	15.55	107%
		29.00	29.02	100%
		43.50	43.01	99%
3	0.001	14.50	13.26	91%
		29.00	26.43	91%
		43.50	38.01	87%
4	12.76	26.62	26.54	100%
		40.48	39.23	97%
		54.35	50.39	93%
5	23.96	37.26	37.78	101%
		50.56	49.07	97%
		63.86	58.82	92%

#### Analytical Specificity:

Monoclonal antibody pair used in the assay detects human, bovine, equine, canine, rabbit, goat, sheep, mouse, squirrel and monkey Oxyntomodulin. Cross-reactivity to other closely related analyte is represented in the table below.

Cross-Reactant	Concentration	% Cross-reactivity
Glucagon (1-29)	1000 ng/mL	Non-Detectable
GLP-1 (7-36)	1000 ng/mL	Non-Detectable
GLP-1 (9-36)	1000 ng/mL	Non-Detectable
GLP-2 (1-34)	1000 ng/mL	Non-Detectable
GRPP	1000 ng/mL	Non-Detectable
MPGF	1000 ng/mL	Non-Detectable
Glicentin	300 pg/mL	< 7.4%
Insulin	1000 pg/mL	Non-Detectable
C-peptide	1000 pg/mL	Non-Detectable
Thyroglobulin	1000 pg/mL	Non-Detectable
Pro-glucagon KO serum (n=3)	NA	Non-Detectable
Wild Type Mice Serum (n=3)	103-246 pg/mL	100%
Oxyntomodulin (1-37)	100 pg/mL	100%

#### Interference:

When potential interferents (hemoglobin, triglycerides, bilirubin and biotin) were added at least at two times their physiological concentration to control

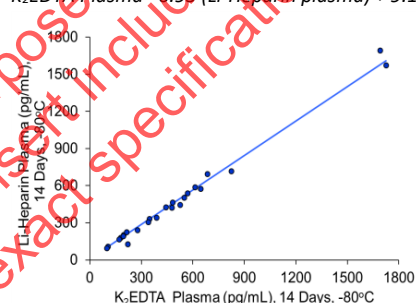
sample, Oxyntomodulin concentration were within  $\pm 10\%$  of the control as represented in the following table.

Interferents	Analyte Conc.	Unspiked Sample Value (pg/mL)	Spiked Sample Value (pg/mL)	% Difference
Hemoglobin	1.35 mg/mL	26.38	26.60	0.8
		48.77	47.25	-3.1
		83.62	83.62	0.0
Triglycerides	10.0 mg/mL	25.44	23.07	-9.3
		46.67	45.78	-1.9
		81.65	78.17	-4.2
Bilirubin	0.60 mg/mL	22.05	22.22	0.8
		42.49	40.21	-5.3
		69.27	68.13	-1.6
Biotin	1200 ng/mL	5.23	5.30	1.4
	600 ng/mL	5.74	5.23	-8.9
	200 ng/mL	5.59	5.30	-5.2

#### Sample Type:

Twenty-four matched K<sub>2</sub>EDTA and Lithium-heparin plasma specimens in the range of 100-1600 pg/mL were compared in AnshLabs Oxyntomodulin ELISA assay (AL-139). Passing Bablok analysis of the results yielded the following Regression:

$$K_2EDTA\ Plasma = 0.93 (Li-Heparin\ plasma) + 9.13, (r=0.99; P<0.0001)$$

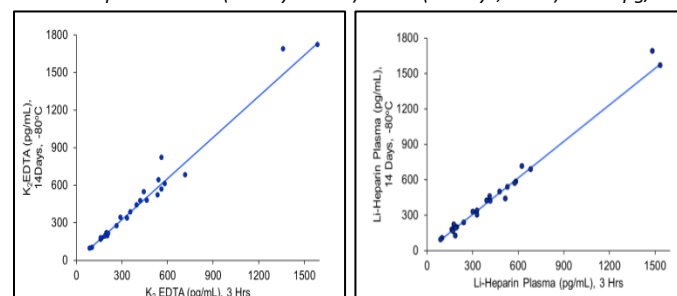


#### Plasma Stability:

Twenty-five K<sub>2</sub>EDTA and Twenty-four Lithium-heparin plasma specimens in the range of 100-1600 pg/mL were aliquoted stored at -80°C for 14 days and compared to freshly drawn plasma samples in Oxyntomodulin ELISA assay (AL-139). Passing Bablok analysis of the results yielded the following Regression:

$$K_2EDTA\ Plasma\ (Freshly\ Drawn) = 1.09\ (14\ Days,\ -80^\circ C) - 2.99\ pg/mL$$

$$Lithium-Heparin\ Plasma\ (Freshly\ Drawn) = 1.03\ (14\ Days,\ -80^\circ C) + 0.66\ pg/mL$$

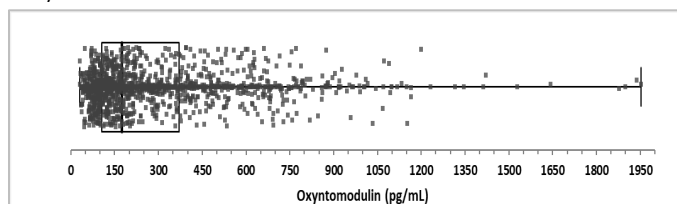


#### Expected Ranges:

Seven matched Fasting and non-fasting (30-45 mins after meal) K<sub>2</sub>EDTA and Lithium-heparin plasma specimens from participants were analyzed in Ansh Labs Oxyntomodulin assay (AL-139) and their mean and 95% confidence interval were calculated and are represented below:

Sample Type	n	Mean (pg/mL)	95% CI
Li-Heparin (Fasting)	7	268.50	123.58-413.41
Li-Heparin (Non-Fasting)	7	682.23	238.81-1125.66
K <sub>2</sub> EDTA (Fasting)	7	283.29	125.41-441.16
K <sub>2</sub> EDTA (Non-Fasting)	7	721.50	285.88-1157.12

Expected Oxyntomodulin concentration in undifferentiated population (diabetic and non-diabetic) was calculated by evaluating 1534 samples in Ansh Labs Oxyntomodulin ELISA. The frequency distribution was calculated using Analyse-It® for Microsoft Excel and is shown below.



n	Oxyntomodulin (pg/mL)			
	Mean	Median	Range	95% CI
1534	277.7	175.7	29.9 - 1953.8	49.5 - 948.1
Quantile		Oxyntomodulin (pg/mL)		
0.100		77.4		
0.200		97.0		
0.300		118.1		
0.400		141.7		
0.500		175.7		
0.600		222.6		
0.700		309.9		
0.800		441.4		
0.900		635.9		

NOTE: It is recommended that each laboratory should determine the reference range(s) for its own patient population. The results of this assay should be used in conjunction with other relevant and applicable clinical information.

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