

AAO Foundation Award Final Report

Please prepare a report that addresses the following:

Type of Award: Orthodontic Faculty Development Fellowship Award

Name(s) of Principal Investigator(s): Sreedevi Srinivasan
Department of Orthodontics,
The University of Iowa College of Dentistry.

Title of Project: Assessment of jaw growth using biomarkers in saliva and gingival crevicular fluid – a pilot study

Period of AAOF Support: 07-01-14 to 06-30-15

Amount of Funding: \$15,000

Summary/Abstract (250 word maximum)

An understanding of normal and abnormal jaw growth is essential in caring for patients presenting with various malocclusions. The objective of this pilot study is to identify and establish the levels of growth-associated specific proteins, genes and micro-ribonucleic acids (miRNAs), collectively called biomarkers, in gingival crevicular fluid (GCF) and in whole saliva (WS) in a adolescents (males: 12-14 years and females: 11-12 years) and adults (30 to 40 years old) sample population of Caucasian in origin. Methods: IRB approval was obtained (ID#201310820). Subjects completed a demographic questionnaire and a periodontal examination in order to be eligible for the study. Anthropometric measurements including, height, weight and waist circumference was measured using established methods. Bio-impedance was measured using RJL bio-impedance portable device and recorded. GCF was collected using paper strips from 4 sites/tooth around the maxillary and mandibular anterior teeth (canine to canine). Unstimulated whole saliva and cheek swabs were collected using established protocols. A lateral cephalogram and intra-oral photos were taken. A few samples from the growing and non-growing populations were analyzed using the pilot miRNA array. A part of the same samples were analyzed using the RNA- Seq (Life Technologies, NY). Results: So far, we have completed sample collection from 49 adolescents (male: 26; female: 23), 29 adults (male: 13; female: 16). Preliminary results from the miRNA array identified biomarkers in saliva distinctly present in adolescents, in comparison to the adult population. On attempting to validate, we encountered lack of reproducibility in microRNA expression data. Therefore, we opted to determine the expression levels of protein marker related to growth (Insulin-like Growth Factor-1) using multiplex assay. We were able to detect IGF-1 in all ours saliva samples and we are in the process of performing statistical analysis.

Response to the following questions:

Were the original, specific aims of the proposal realized?

This is an ongoing study and the past year was highly productive. We were able to explore micro RNA assay to its depth. But unfortunately, the assay was technique sensitive requiring protocol refinement. We are trying to improve the protocol for collection of saliva samples to aid in better isolation of microRNAs. We plan to present and publish results soon after validation is complete.

Were the results published? a.) If so, was AAOF support acknowledged. b.) If not, are there plans to publish? If not, why not?

It is an ongoing study and therefore, the results are yet to be published. We will proudly acknowledge the support we received from AAOF in all resulting publications.

Have the results of this proposal been presented? a.) If so, when and where? And was AAOF support acknowledged. b.) If not, are there plans to do so? If not, why not?

It is an ongoing study and therefore, the results are yet to be presented. We will proudly acknowledge the support we received from AAOF in all resulting presentations.

To what extent have you used, or how do you intend to use, AAOF funding to further your career?

AAOF funding was an integral resource for this project. We would like to thank the foundation again for their support. The funding was utilized to purchase isolation kits, gift cards for research volunteers and to perform the initial miRNA array. This funding and unwavering support from the AAOF was fundamental for this project.

Please mail hard copy to AAOF and also send electronically
(as a Word document and e-mail attachment) to
aaofevp@aaortho.org

File Name: C:\Users\sheym\Desktop\SRINI_1.rbx

Analyte: IGF-1 (21)

Acquisition Date: 10-Feb-2016, 04:17 PM

Reader Serial Number: LX10005244401

Plate ID:

RP1 PMT (Volts): 595.12

RP1 Target: 3658

Analyte	Type	Well	Sample #	Outlier	FI	FI - Bkgd	Std Dev	%CV	Conc in Range	Obs Conc	Exp Conc	(Obs/Exp) * 100	Dilution
IGF-1 (21)	B	A3,A4		0	4.5	4.5	0.71	15.71					1
IGF-1 (21)	S1	A1,A2		0	15194	15189.5	240.42	1.58	87	87	87	100	1
IGF-1 (21)	S2	B1,B2		0	6801.5	6797	75.66	1.11	29	29	29	100	1
IGF-1 (21)	S3	C1,C2		0	1049.5	1045	53.74	5.12	9.67	9.67	9.67	100	1
IGF-1 (21)	S4	D1,D2		0	164	159.5	6.36	3.88	3.22	3.22	3.22	100	1
IGF-1 (21)	S5	E1,E2		0	32.8	28.3	1.06	3.24	1.08	1.08	1.07	100	1
IGF-1 (21)	S6	F1,F2		0	13	8.5	1.41	10.88	0.36	0.36	0.36	100	1
IGF-1 (21)	S7	G1,G2		1	---	---					0.12		1
IGF-1 (21)	S8	H1,H2		1	---	---					0.04		1
IGF-1 (21)	C1	B3,B4		0	1390	1385.5	4.24	0.31	11.39	11.39	23	50	1
IGF-1 (21)	C2	C3,C4		0	49.3	44.8	1.06	2.15	1.47	1.47	1.8	82	1
IGF-1 (21)	X1	D3,E3,F3	1014	0	4.8	0.3	0.76	15.8	OR <	OR <			3
IGF-1 (21)	X2	D4,G3,H3	1091	0	9.8	5.3	1.61	16.35	OR <	*0.24			3
IGF-1 (21)	X3	E4,F4,G4	1058	0	3.8	-0.7	1.44	37.65	OR <	OR <			3
IGF-1 (21)	X4	A5,B5,H4	1088	0	5	0.5	2	40	OR <	OR <			3
IGF-1 (21)	X5	C5,D5,E5	1099	0	247.8	243.3	183.33	73.97	12.4	12.4			3
IGF-1 (21)	X6	F5,G5,H5	1023	0	4.5	0	0.87	19.25	OR <	OR <			3
IGF-1 (21)	X7	A6,B6,C6	1017	0	13.8	9.3	8.84	63.88	1.22	1.22			3
IGF-1 (21)	X8	D6,E6,F6	1012	0	4.3	-0.2	0.58	13.32	OR <	OR <			3
IGF-1 (21)	X9	A7,G6,H6	1098	0	366.2	361.7	165.32	45.15	15.64	15.64			3
IGF-1 (21)	X10	B7,C7,D7	1096	0	1695	1690.5	2768.72	163.35	38.34	38.34			3
IGF-1 (21)	X11	E7,F7,G7	1095	0	6.7	2.2	2.08	31.22	OR <	OR <			3
IGF-1 (21)	X12	A8,B8,H7	1025	0	220.7	216.2	107.96	48.93	11.56	11.56			3
IGF-1 (21)	X13	C8,D8,E8	1030	0	4	-0.5	1	25	OR <	OR <			3
IGF-1 (21)	X14	F8,G8,H8	1073	0	3	-1.5	0	0	OR <	OR <			3
IGF-1 (21)	X15	A9,B9,C9	1063	0	2.7	-1.8	0.58	21.65	OR <	OR <			3
IGF-1 (21)	X16	D9,E9,F9	1045	0	34.2	29.7	40.78	119.36	3.34	3.34			3
IGF-1 (21)	X17	A10,G9,H9	1031	0	3.3	-1.2	1.15	34.64	OR <	OR <			3
IGF-1 (21)	X18	B10,C10,D10	1053	0	380.3	375.8	229.27	60.28	16	16			3

IGF-1 (21)	X19	E10,F10,G10	1015	0	5	0.5	1	20	OOB <	OOB <	3
IGF-1 (21)	X20	A11,B11,H10	1085	0	7.2	2.7	1.61	22.43	OOB <	OOB <	3
IGF-1 (21)	X21	C11,D11,E11	1027	0	16.7	12.2	12.66	75.97	1.63	1.63	3
IGF-1 (21)	X22	F11,G11,H11	1028	0	5	0.5	1	20	OOB <	OOB <	3
IGF-1 (21)	X23	A12,B12,C12	1069	0	47	42.5	2.65	5.63	4.26	4.26	3
IGF-1 (21)	X24	D12,E12,F12	1079	0	86.8	82.3	54.43	62.69	6.47	6.47	3

*** = Value not available; --- = Designated as an outlier

*Value = Value extrapolated beyond standard range

OOB = Out of Range; OOB> = Out of Range Above; OOB< = Out of Range Below

Exp Conc = Expected Concentration; Obs Conc = Observed Concentration

Conc in Range = Unknown sample concentrations within range where standards recovery is 70-130%

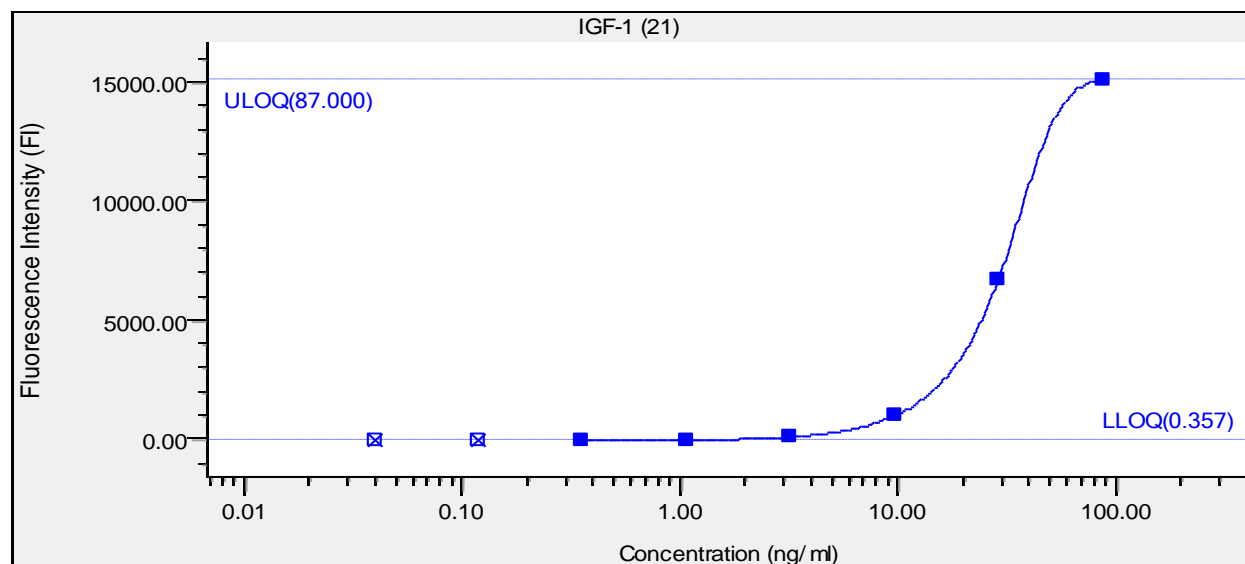
Conc In Range = 0.357 to 87.000 (equivalent to 8.5 to 15189.5 FI - Bkgd)

Concentration Units = ng/ml

Regression Type: Logistic - 5PL

Std. Curve: $FI = 5.07477 + (15337.5 - 5.07477) / ((1 + (Conc / 45.6972)^{-5.4087}))^{0.320364}$

FitProb. = 0.9529, ResVar. = 0.0035



■ Standard □ Partial Outlier ⊠ Outlier

File Name: Z:\SrinivasanS\BioPlex\Raw Data\srinivasan plate 2.rbx

Analyte: IGF-1 (21)

Acquisition Date: 13-Apr-2016, 01:29 PM

Reader Serial Number: LX10009023402

Plate ID:

RP1 PMT (Volts): 642.25

RP1 Target: 3659

Analyte	Type	Well	Sample#	Outlier	FI	FI - Bkgd	Std Dev	%CV	Conc in Range	Obs Conc	Exp Conc	(Obs/Exp) * 100	Dilution	
IGF-1 (21)	B	A3,A4			0	4.5	4.5	1.41	31.43				1	
IGF-1 (21)	S1	A1,A2			0	10985.3	10980.8	2325.32	21.17	80.89	80.89	87	93	1
IGF-1 (21)	S2	B1,B2			2	4979	4974.5	0	0	29.43	29.43	29	101	1
IGF-1 (21)	S3	C1,C2			2	725	720.5	0	0	9.52	9.52	9.67	98	1
IGF-1 (21)	S4	D1,D2			2	108.5	104	0	0	3.06	3.06	3.22	95	1
IGF-1 (21)	S5	E1,E2			2	28	23.5	0	0	1.26	1.26	1.07	117	1
IGF-1 (21)	S6	F1,F2			0	7.8	3.3	1.77	22.81	0.32	0.32	0.36	90	1
IGF-1 (21)	S7	G1,G2			1	---	---					0.12		1
IGF-1 (21)	S8	H1,H2			1	---	---					0.04		1
IGF-1 (21)	C1	B3,B4			0	1082	1077.5	86.27	7.97	12.05	12.05	23	52	1
IGF-1 (21)	C2	C3,C4			0	34.8	30.3	1.06	3.05	1.47	1.47	2.3	64	1
IGF-1 (21)	X1	D3,D4,D5	1033		0	62.5	58	15.26	24.41	6.5	6.5			3
IGF-1 (21)	X2	E3,E4,E5	1034		0	10.2	5.7	2.02	19.88	1.49	1.49			3
IGF-1 (21)	X3	F3,F4,F5	1036		0	8.5	4	1.8	21.21	1.15	1.15			3
IGF-1 (21)	X4	G3,G4,G5	1038		0	14.3	9.8	3.06	21.31	2.18	2.18			3
IGF-1 (21)	X5	H3,H4,H5	1056		0	21.8	17.3	10.15	46.5	3.13	3.13			3
IGF-1 (21)	X6	A5,B5,C5	1057		0	12.3	7.8	5.77	46.81	1.87	1.87			3
IGF-1 (21)	X7	A6,A7,A8	1052		0	12	7.5	1	8.33	1.82	1.82			3
IGF-1 (21)	X8	B6,B7,B8	1055		0	28.2	23.7	10.13	35.96	3.79	3.79			3
IGF-1 (21)	X9	C6,C7,C8	1060		0	12.5	8	1.8	14.42	1.9	1.9			3
IGF-1 (21)	X10	D6,D7,D8	1072		0	8.7	4.2	3.06	35.25	1.18	1.18			3
IGF-1 (21)	X11	E6,E7,E8	1074		0	13.2	8.7	2.36	17.95	2	2			3
IGF-1 (21)	X12	F6,F7,F8	1075		0	15.8	11.3	2.75	17.39	2.39	2.39			3
IGF-1 (21)	X13	G6,G7,G8	1026		0	23	18.5	8.54	37.15	3.26	3.26			3
IGF-1 (21)	X14	H6,H7,H8	1044		0	18.3	13.8	3.51	19.16	2.71	2.71			3
IGF-1 (21)	X15	A9,A10	1046		0	112.8	108.3	27.93	24.77	9.4	9.4			3
IGF-1 (21)	X16	B9,B10	1048		0	58.5	54	64.35	109.99	6.23	6.23			3
IGF-1 (21)	X17	C9,C10	1049		0	12.5	8	6.36	50.91	1.9	1.9			3

IGF-1 (21)	X18	D9	1007	0	8	3.5	0	0	1.03	1.03	3
IGF-1 (21)	X19	E9	1011	0	12.5	8	0	0	1.9	1.9	3
IGF-1 (21)	X20	F9	1043	0	97	92.5	0	0	8.57	8.57	3
IGF-1 (21)	X21	G9	1029	0	57.5	53	0	0	6.16	6.16	3
IGF-1 (21)	X22	H9	1032	0	19.5	15	0	0	2.86	2.86	3

*** = Value not available; --- = Designated as an outlier

*Value = Value extrapolated beyond standard range

OOB = Out of Range; OOB> = Out of Range Above; OOB< = Out of Range Below

Exp Conc = Expected Concentration; Obs Conc = Observed Concentration

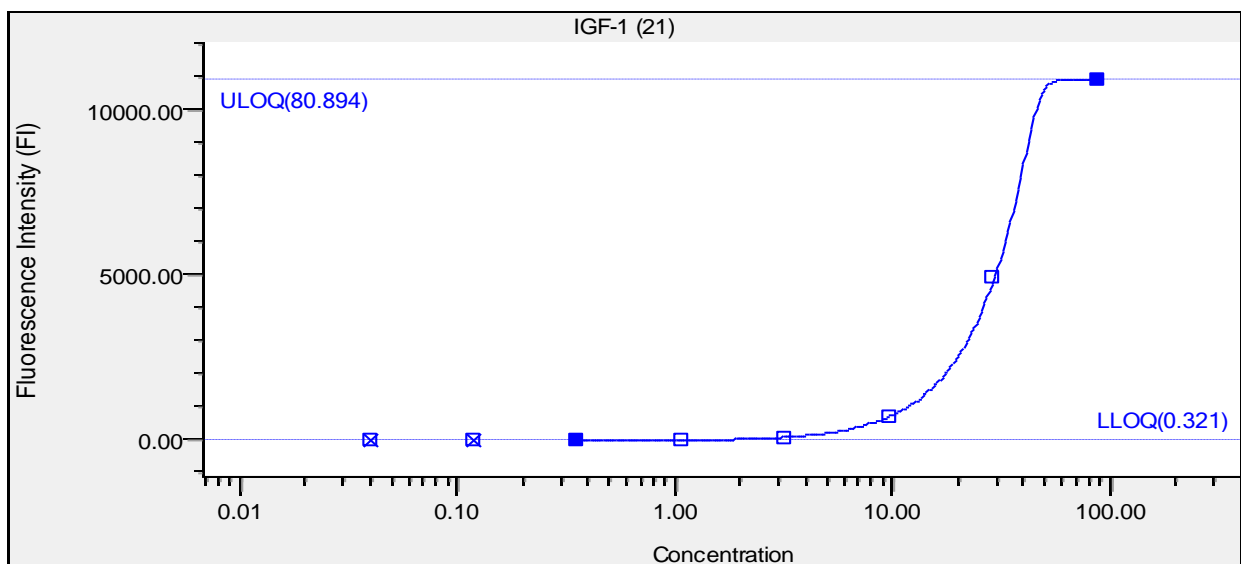
Conc in Range = Unknown sample concentrations within range where standards recovery is 70-130%

Conc In Range = 0.321 to 80.894 (equivalent to 3.3 to 10980.8 FI - Bkgd)

Regression Type: Logistic - 5PL

Std. Curve: $FI = 1.08343 + (10980.8 - 1.08343) / ((1 + (Conc / 46.7261)^{-17.1328})^{0.0999998})$

FitProb. = 0.0028, ResVar. = 8.9462



■ Standard □ Partial Outlier ⊠ Outlier

File Name: Z:\SrinivasanS\BioPlex\Raw Data\160419 plate 4.rbx

Analyte: IGF-1 (21)

Acquisition Date: 19-Apr-2016, 02:53 PM

Reader Serial Number: LX10009023402

Assay Lot: K9347

Plate ID:

RP1 PMT (Volts): 634.68

RP1 Target: 3659

Analyte	Type	Well	Sample #	Outlier	FI	FI - Bkgd	Std Dev	%CV	Conc in Range	Obs Conc	Exp Conc	(Obs/Exp) * 100	Dilution	
IGF-1 (21)	B	A3,A4			0	5.3	5.3	1.77	33.67				1	
IGF-1 (21)	S1	A1,A2			0	11404.5	11399.3	2102.94	18.44	88.6	88.6	87	102	1
IGF-1 (21)	S2	B1,B2			2	3771.5	3766.3	0	0	27.6	27.6	29	95	1
IGF-1 (21)	S3	C1,C2			2	1089	1083.8	0	0	11.04	11.04	9.67	114	1
IGF-1 (21)	S4	D1,D2			0	127.8	122.5	42.07	32.93	2.91	2.91	3.22	90	1
IGF-1 (21)	S5	E1,E2			2	34.5	29.3	0	0	1.25	1.25	1.07	116	1
IGF-1 (21)	S6	F1,F2			0	10.3	5	3.18	31.04	0.32	0.32	0.36	89	1
IGF-1 (21)	S7	G1,G2			1	---	---					0.12		1
IGF-1 (21)	S8	H1,H2			1	---	---					0.04		1
IGF-1 (21)	C1	B3,B4			0	1304.5	1299.3	51.62	3.96	12.47	12.47	23	54	1
IGF-1 (21)	C2	C3,C4			0	46.5	41.3	4.95	10.64	1.54	1.54	2.3	67	1
IGF-1 (21)	X1	D3,D4,D5	1010		0	11.8	6.5	2.47	21.06	1.28	1.28			3
IGF-1 (21)	X2	E3,E4,E5	1068		0	27.3	22.1	12.34	45.15	3.15	3.15			3
IGF-1 (21)	X3	F3,F4,F5	1064		0	59	53.8	0	0	5.39	5.39			3
IGF-1 (21)	X4	G3,G4,G5	1065		0	51.8	46.6	17.35	33.48	4.95	4.95			3
IGF-1 (21)	X5	H3,H4,H5	1084		0	64.2	58.9	58.55	91.24	5.69	5.69			3
IGF-1 (21)	X6	A5,B5,C5	1081		0	5.3	0.1	2.02	37.89	OOB <	OOB <			3
IGF-1 (21)	X7	A6,A7,A8	1067		0	9	3.8	0	0	OOB <	*0.60			3
IGF-1 (21)	X8	B6,B7,B8	1048		0	32.5	27.3	6.36	19.58	3.59	3.59			3
IGF-1 (21)	X9	C6,C7,C8	1059		0	53.5	48.3	30.41	56.83	5.06	5.06			3
IGF-1 (21)	X10	D6,D7,D8	1029		0	105	99.8	7.07	6.73	7.73	7.73			3
IGF-1 (21)	X11	E6,E7,E8	1049		0	15.8	10.6	7.65	48.34	1.92	1.92			3
IGF-1 (21)	X12	F6,F7,F8	1066		0	9.5	4.3	2.78	29.3	OOB <	*0.76			3
IGF-1 (21)	X13	G6,G7,G8	1032		0	13.3	8	2.47	18.68	1.54	1.54			3
IGF-1 (21)	X14	H6,H7,H8	1057		0	172	166.8	73.37	42.66	10.45	10.45			3
IGF-1 (21)	X15	A9,A10,A11	1042		0	17.8	12.6	13.75	77.11	2.17	2.17			3
IGF-1 (21)	X16	B9,B10,B11	1070		0	12	6.8	2.78	23.2	1.32	1.32			3
IGF-1 (21)	X17	C9,C10,C11	1037		0	14.3	9.1	1.26	8.78	1.71	1.71			3

IGF-1 (21)	X18	D9,D10,D11	1077	0	23	17.8	15.66	68.09	2.74	2.74	3
IGF-1 (21)	X19	E9,E10,E11	1050	0	460.5	455.3	0	0	19.06	19.06	3
IGF-1 (21)	X20	F9,F10,F11	1087	0	***	***		***	***	***	3
IGF-1 (21)	X21	G9,G10,G11	1083	0	99.8	94.6	59.52	59.62	7.5	7.5	3
IGF-1 (21)	X22	H9,H10,H11	1076	0	18	12.8	1.73	9.62	2.19	2.19	3
IGF-1 (21)	X23	A12,B12,C12		0	5	-0.3	1.73	34.64	OOB <	OOB <	3
IGF-1 (21)	X24	D12,E12,F12		0	1.8	-3.4	0.29	15.75	OOB <	OOB <	3

*** = Value not available; --- = Designated as an outlier

*Value = Value extrapolated beyond standard range

OOB = Out of Range; OOB> = Out of Range Above; OOB< = Out of Range Below

Exp Conc = Expected Concentration; Obs Conc = Observed Concentration

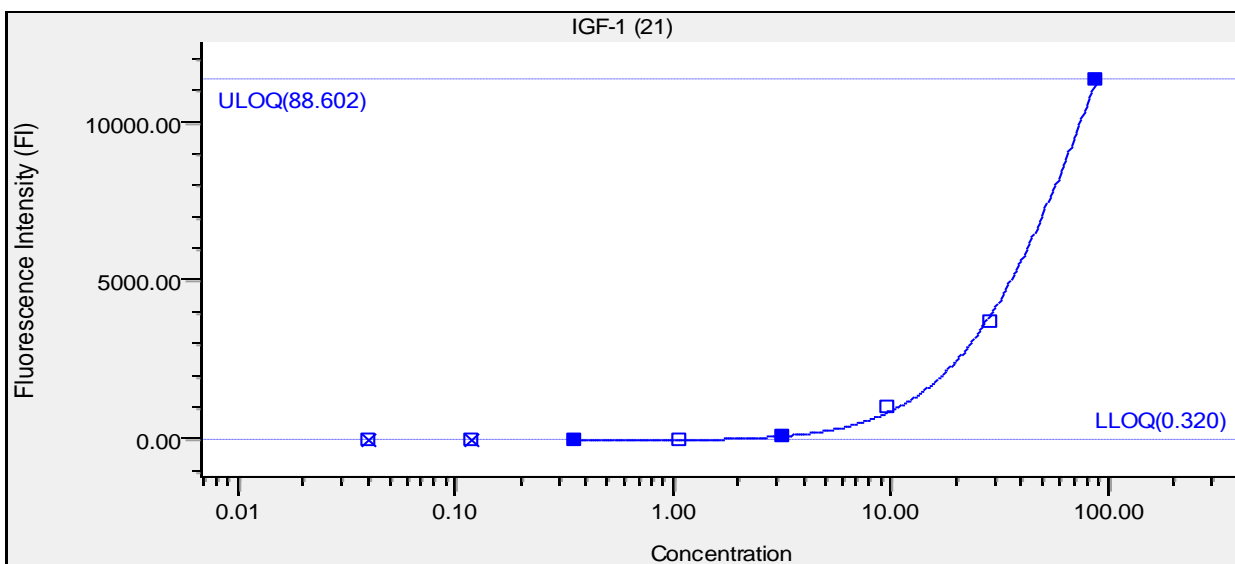
Conc in Range = Unknown sample concentrations within range where standards recovery is 70-130%

Conc In Range = 0.320 to 88.602 (equivalent to 5.0 to 11399.3 FI - Bkgd)

Regression Type: Logistic - 5PL

Std. Curve: $FI = 2.86294 + (29704.6 - 2.86294) / ((1 + (Conc / 50.7068)^{-0.92003})^2)^{2.04238}$

FitProb. = 0.0000, ResVar. = 28.1183



■ Standard □ Partial Outlier ⊠ Outlier