



08186 Lliçà d'Amunt
Barcelona
Spain

Tel.:+ 34 93 860 90 00
Fax:+ 34 93 860 90 17
e-mail: biokit@biokit.com
www.biokit.com

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SUBJECT Product evaluation

New quantex Myoglobin evaluation

Please find enclosed the report of an external evaluation performed comparing the *New quantex Myoglobin* versus the old one using an **Olympus AU 640**.

This evaluation was performed by Dr. Manfred Haßfeld from Medizinisches Lab. Kyritz (Germany).

Precision (within run & total), detection limit, quantification limit, linearity were compared.

Also a total of 79 samples were evaluated showing a perfect correlation ($r = 1$) between both methods. The slope using the least squares regression was also excellent: 1.028

We hope that this evaluation will help you to demonstrate the good performance of our *New quantex Myoglobin* product.



Final Report

Evaluation of the new quantex Myoglobin product on Olympus AU 640 Analyser

Study Director:

Dr. Manfred Haßfeld
Medizinisches Laboratorium Kyritz
Perleberger Str. 31 A
16866 Kyritz



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1 Introduction

The objective of the study was to evaluate the performance of the new quantex Myoglobin product (MYO new) on a Olympus AU 640 analyser and to compare the results with the current quantex Myoglobin product (MYO old).

The study addressed precision, linearity and quantification limit of the new product. In addition, a method comparison was performed with patient samples.

2 Material

- One lot of reagent and buffer of the current quantex Myoglobin assay with its standards and controls
- One lot of reagent and buffer of the new quantex Myoglobin assay with its standards and controls
- Olympus AU 640 analyser
- patient samples

3 Precision

Two samples of the Biokit control serum, quantex FERRITIN / MYOGLOBIN / IgE control I/II (L1 and L2) with different myoglobin concentrations were used for the precision runs. Additionally, saline solution was measured to determine the detection limit of the assays.

For the precision runs the analyser was calibrated daily in the morning. Series with 4 replicates of each sample were measured once or twice each day. One series of each sample was measured in the morning. On day 3 and 4 a second precision run was performed in the afternoon with fresh material.

3.1 Within Run Precision

In [Table 1](#) the results of the precision runs are summarised.

The CV in the series was found in the lower measuring range (L1) with MYO old between 0,5% and 2,7% and with MYO new between 0,2% and 1,4%. In the higher measuring range (L2) with MYO old CVs between 0,5% and 1,0% were found, with MYO new they ranged between 0,3% and 1,5%.

Both assays fulfilled the acceptance criteria (maximum CVs of 10% in the lower test range and 6% in the higher test range).

3.2 Total Precision

In Table 1 also the values for total precision are given.

Both assays showed similar performance. For MYO old total CVs of 2,7% (lower test range) and 1,4% (higher test range), for MYO new total CVs of 3,1% (lower test range) and 1,1% (higher test range) were found. Both assays were in the limits of the Biokit acceptance criteria (total CV lower than 15% in the lower test range and less than 10% in the higher test range).

3.3 Detection Limit

Using the results of the saline measurements given in Table 1 the detection limit of the two assays was calculated as follows: Mean + 3SD.

For MYO old a detection limit of 5,4 ng/ml was calculated. For MYO new a detection limit of 6,7 ng/ml was found.

4 Linearity

4.1 Linearity on Dilution

Linearity of the new Myoglobin product was tested as follows: Multiple dilutions of a high patient sample were prepared using saline solution. Each dilution was measured twice.

In Table 2 with Figure 1 the results are given. The new product showed linearity on dilution in the tested range between 50 and about 500 ng/ml. The acceptance criteria of Biokit were fulfilled (recovery in the range of +/- 20% of the theoretical value, imprecision under 15%).

4.2 Quantification limit

The quantification limit of the new Myoglobin product was tested as follows: Multiple dilutions of a low patient sample were prepared using saline solution. Each dilution was measured twice.

In Table 3 with Figure 2 the results are given. The new product showed linearity on dilution in the tested range between 6 and 56 ng/ml. The acceptance criteria of Biokit were fulfilled with all dilutions (recovery in the range of +/- 20% of the theoretical value, imprecision under 15%). Thus, in this study the quantification limit of the new Myoglobin assay on AU 640 was very close to the detection limit and may be given with 7 ng/ml.

5 Method Comparison

Serum samples of 79 patients were collected and stored at -20°C prior to analysis. All samples were measured in parallel with MYO old and MYO new. Samples with results exceeding the highest calibrator were flagged automatically by the instrument. These samples were manually diluted with saline to fit in the calibration curve and were re-analysed. The results were corrected with the appropriate dilution factor. In Figures 3-5 the results of the comparison are given graphically. Linear correlation of the two assays was observed. This was confirmed mathematically: In Method Comparison the results of all patient samples as well as the result of the regression and correlation analysis are listed. The evaluation of the data was done by the Passing and Bablok method. The Biokit acceptance criteria were fulfilled (slope: 0.80 – 1.20; intercept: <20% of the mean of the x-Values; coefficient of correlation >0.980).

6 Conclusion

The new quantex Myoglobin product showed similar precision characteristics as the current product on our Olympus AU 640 analyser. Within run precision as well as total precision were excellent. Method comparison showed that results of patient samples were slightly lower with the new product than with the old one. Nevertheless, the coefficient of correlation ($r = 1.0$) indicated a very good correlation of the two tests.

With the new quantex Myoglobin product patient samples may be measured with high precision and reproducibility even in the low normal range.

Kyritz, 06.07.2004



Dr. Manfred Haßfeld
Medizinisches Laboratorium Kyritz

Table 1: Precision

Day #	MYO old [ng/ml]			MYO new [ng/ml]		
	L 1	L 2	NaCl	L 1	L 2	NaCl
1	57,1	197,3	0,8	60,7	216,2	-0,8
	57,5	196,0	0,8	61,5	216,0	-1,8
	56,7	198,6	-1,1	61,7	214,9	-0,3
	57,9	196,6		59,9	216,6	
	mean = CV % =	57,3 0,9	197,1 0,6		61,0 1,3	215,9 0,3
2	55,9	194,5	0,0	62,1	215,3	0,2
	56,6	196,7	0,8	62,8	214,4	-0,2
	56,6	193,6	-2,3	62,4	215,1	0,0
	55,9	194,5	-0,4	60,9	208,4	-5,7
	mean = CV % =	56,3 0,7	194,8 0,7		62,1 1,3	213,3 1,5
3	54,4	190,8	1,1	63,9	214,8	1,1
	54,8	192,5	1,1	64,5	213,5	1,9
	54,8	191,4	0,0	63,0	210,2	-3,2
	53,7	190,2	0,4	64,1	212,8	-9,0
	mean = CV % =	54,4 0,9	191,2 0,5		63,9 1,0	212,8 0,9
	58,4	195,8	3,2	65,0	216,3	1,9
	58,0	197,3	4,0	65,0	214,1	1,7
	58,7	196,1	3,6	65,0	213,7	0,9
	58,4	197,6	3,2	64,7	217,2	-3,9
	mean = CV % =	58,4 0,5	196,7 0,5		64,9 0,2	215,3 0,8
4	57,8	197,3	-0,8	59,4	212,2	-0,4
	58,2	201,1	-0,4	60,6	212,9	0,6
	58,2	201,8	-0,8	60,3	210,5	0,8
	57,3	199,0	-1,2	59,8	213,1	0,4
	mean = CV % =	57,8 0,8	199,8 1,0		60,0 0,9	212,2 0,6
	55,7	196,2	-0,8	59,6	212,5	0,4
	56,9	198,0	0,0	61,0	210,1	0,6
	56,1	200,4	-1,7	59,1	210,1	0,8
	56,9	197,6	-2,5	60,3	210,7	0,6
	mean = CV % =	56,4 1,1	198,1 0,9		60,0 1,4	210,9 0,5
5	54,9	197,3	1,4	60,3	212,0	-0,2
	56,1	196,6	0,6	60,5	211,8	-0,5
	53,0	198,3	-0,2	60,5	210,0	-0,7
	56,1	197,6	-0,6	60,5	212,0	0,5
	mean = CV % =	55,0 2,7	197,5 0,4		60,5 0,2	211,5 0,5
Total mean =	56,5	196,5	0,3	61,8	213,1	-0,5
Total CV % =	2,7	1,4		3,1	1,1	
SD =			1,7			2,4

Table 2 with Figure 1: Linearity on dilution

Multiple dilutions of a high patient sample were prepared using saline solution. Samples were measured twice on AU 640 with MYO new.

Dilution [%]	Run 1 [ng/ml]	Run 2 [ng/ml]	Mean [ng/ml]	Target [ng/ml]	Recovery [%]	CV %
0	-0,6	0,6	0,0	0,0		
10	56,1	57,2	56,7	49,4	+14,8	1,4
20	110,3	111,7	111,0	98,9	+12,2	0,9
30	165,2	166,1	165,7	148,2	+11,8	0,4
40	214,4	215,9	215,2	197,6	+8,9	0,5
50	272,6	269,4	271,0	247,0	+9,7	0,8
60	345,3	337,2	341,3	296,4	+15,1	1,7
70	392,6	397,5	395,1	345,8	+14,3	0,9
80	431,4	433,5	432,5	395,2	+9,4	0,3
90	466,7	470,5	468,6	444,6	+5,4	0,6
100	494,2	493,8	494,0	494,0	+0,0	0,1

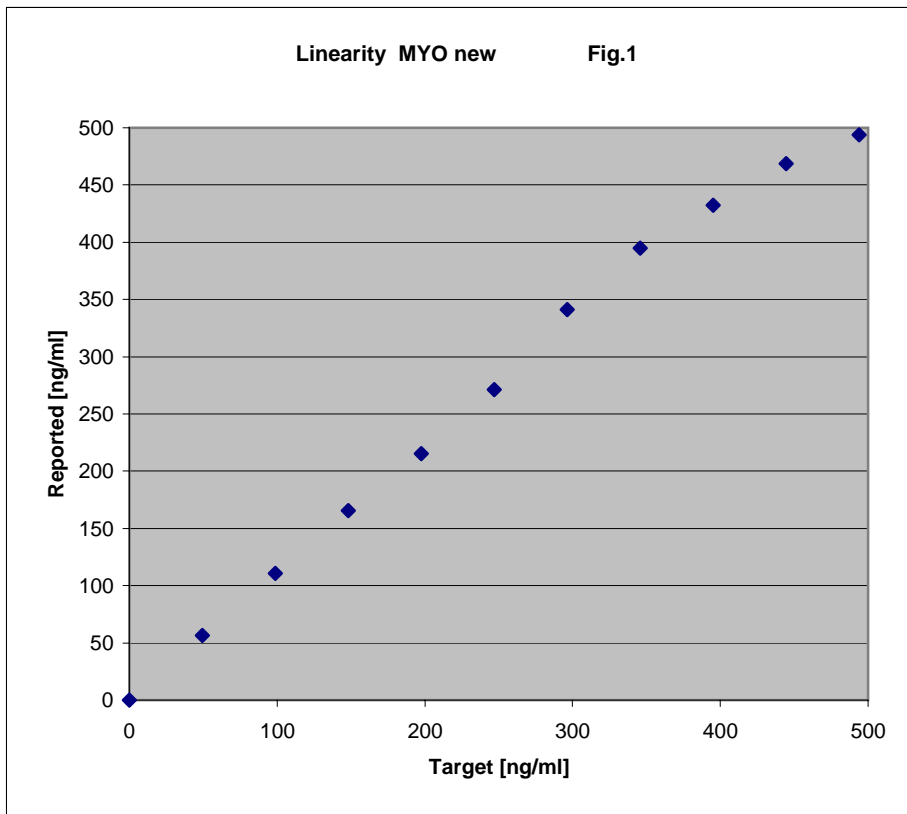
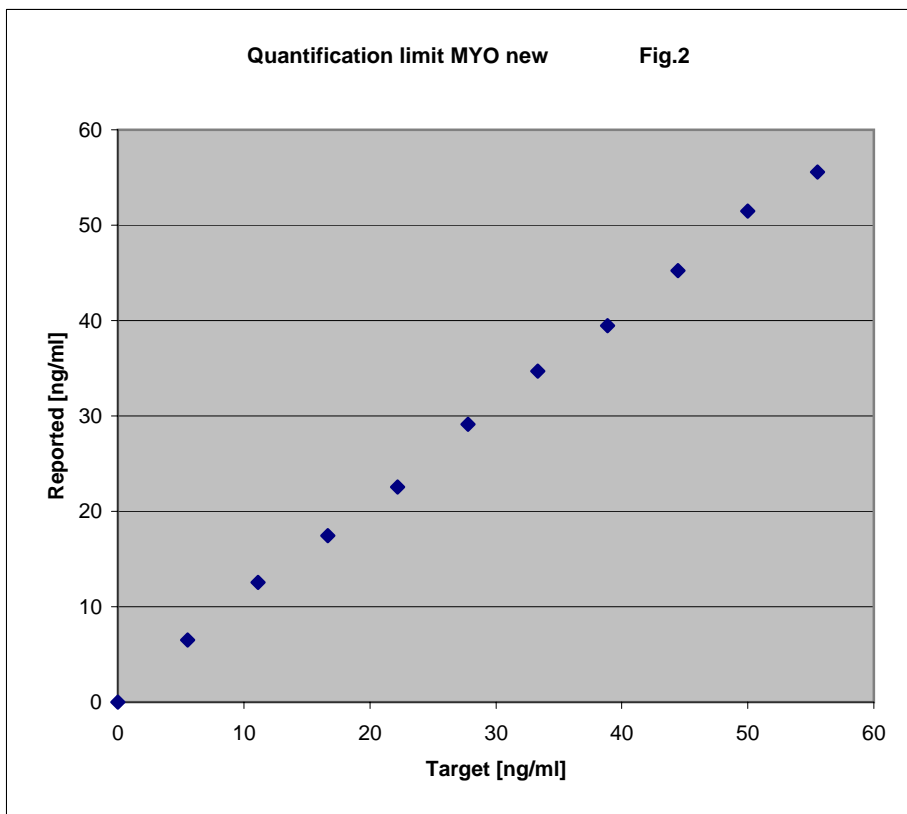


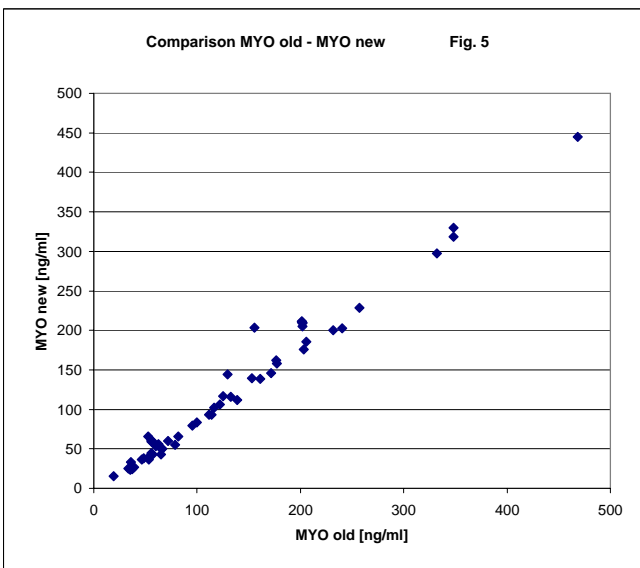
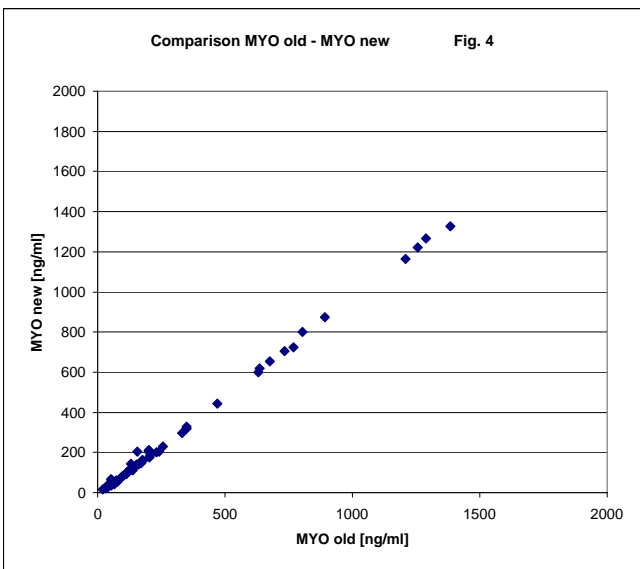
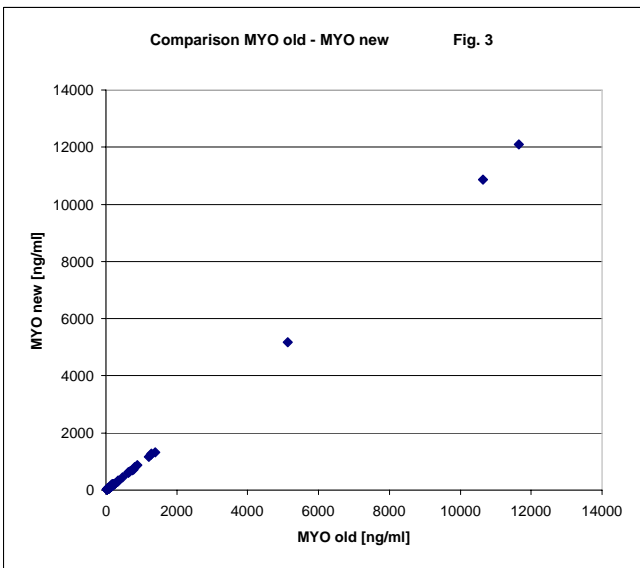
Table 3 with Figure 2: Quantification limit

Multiple dilutions of a low patient sample were prepared using saline solution. Samples were measured twice on AU 640 with MYO new.

Dilution %	Run 1 [ng/ml]	Run 2 [ng/ml]	Mean [ng/ml]	Target [ng/ml]	Recovery [%]	CV %
0	-0,6	0,6	0,00	0,00		
10	6,4	6,6	6,50	5,56	+16,9	2,2
20	13,3	11,8	12,55	11,11	+13,0	8,5
30	17,1	17,8	17,45	16,67	+4,7	2,8
40	22,1	23,0	22,55	22,22	+1,5	2,8
50	29,5	28,8	29,15	27,78	+4,9	1,7
60	34,6	34,8	34,70	33,33	+4,1	0,4
70	39,1	39,8	39,45	38,89	+1,4	1,3
80	45,4	45,1	45,25	44,44	+1,8	0,5
90	51,4	51,6	51,50	50,00	+3,0	0,3
100	55,9	55,2	55,55	55,55	+0,0	0,9



Figures 3-5:



Method Comparison

Method X: Myo old ng/ml	Instrument: AU 640
Method Y: Myo new ng/ml	Instrument: AU 640
Sample Size: 79	

Descriptive Statistics

	X	Y	Y-X	(Y - X)% of X
Median	116,200	101,800	-15,800	-13,0
Mean	609,619	601,549	-8,070	-12,7
Minimum	19,500	15,500	-57,000	-35,2
Maximum	11.646,000	12.085,200	439,200	30,9
68% Median Distance	106,250	99,750	10,200	10,5
Standard Deviation	1.837,560	1.889,045	59,003	11,6

Differences

Medians	-12,392
Means	-1,324

Regression and Correlation Analysis

Coefficients of Correlation: $r = 1,0$ $\tau = 0,957$

	slope b	intercept a	lower limit	upper limit
Structural Relationship Model:				
Passing/Bablok (P/B)	0,972 *	-11,101 *		
95% Confidence Region for b (P/B)			0,960	0,982
95% Confidence Region for a (P/B)			-12,385	-9,095
Std. Principal Component (SPC)	1,028 *	-25,150		

Linear Model:

Least Squares Regr.:	1,028	-25,075
Theil Regression	0,971	-10,653

Dispersion of Residuals:

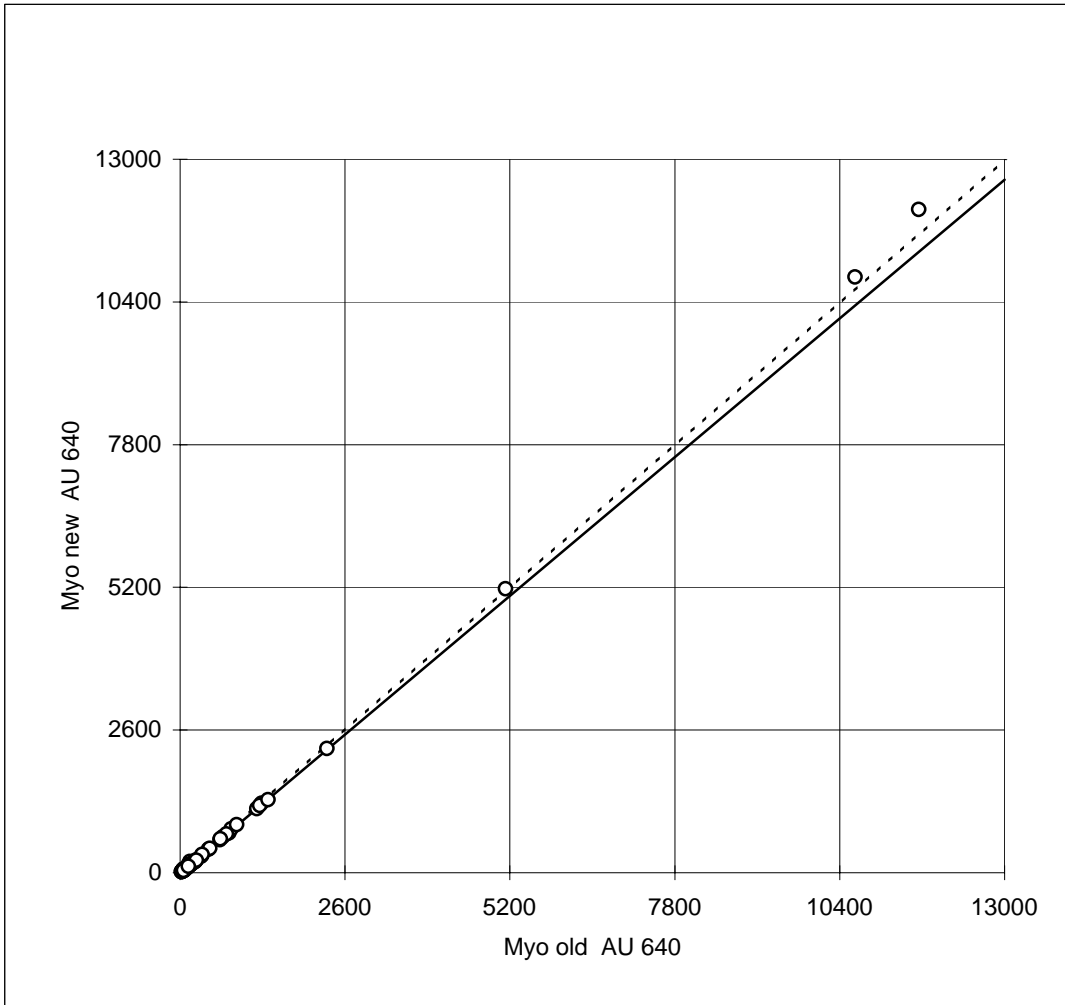
Passing/Bablok Regression:	md(68) = 6,501	md(95) = 33,058
Std. Principal Component		SE = 20,515

Data Assessment

Cusum test for linearity shows no significant deviation from linearity.

* indicates significant difference (rejection of null-hypothesis, $\gamma = 0.05$ for slope and intercept from P/B and slope for SPC).

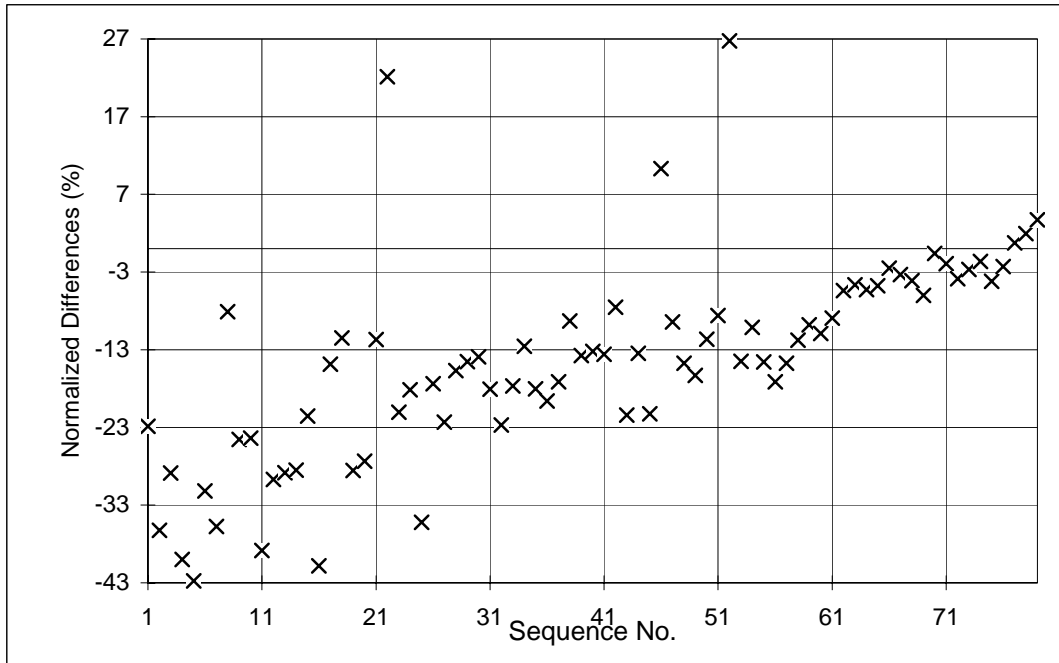
Method Comparison



Statistics	Method X: Myo old ng/ml	Method Y: Myo new ng/ml
N	79	79
Mean	609,619	601,549
Median	116,200	101,800
Minimum	19,500	15,500
Maximum	11.646,000	12.085,200
Range	11.626,500	12.069,700

Method Comparison

Difference Plot
(Normalized Differences)



P/B Regression
 $Y = 0,972 * X - 11,101$
N = 79, r = 1,000

Method Comparison

Serial Number	Sample Values			(Y - X)% of X	Normalized Difference
	X	Y	Y - X		
1	138,6	112,0	-26,6	-19,2	-21,2
2	176,3	161,8	-14,5	-8,2	-8,6
3	769,8	724,8	-45,0	-5,8	-6,0
4	171,9	146,0	-25,9	-15,1	-16,3
5	33,3	24,9	-8,4	-25,2	-28,9
6	630,0	600,6	-29,4	-4,7	-4,8
7	65,7	49,3	-16,4	-25,0	-28,5
8	62,5	55,6	-6,9	-11,0	-11,7
9	805,2	800,4	-4,8	-0,6	-0,6
10	52,6	65,7	13,1	24,9	22,1
11	35,4	23,6	-11,8	-33,3	-40,0
12	64,9	42,9	-22,0	-33,9	-40,8
13	78,5	55,0	-23,5	-29,9	-35,2
14	240,5	202,6	-37,9	-15,8	-17,1
15	46,2	36,1	-10,1	-21,9	-24,5
16	35,9	33,1	-2,8	-7,8	-8,1
17	153,2	139,4	-13,8	-9,0	-9,4
18	38,9	27,1	-11,8	-30,3	-35,8
19	19,5	15,5	-4,0	-20,5	-22,9
20	53,5	36,1	-17,4	-32,5	-38,8
21	36,7	26,8	-9,9	-27,0	-31,2
22	348,3	318,5	-29,8	-8,6	-8,9
23	72,1	60,1	-12,0	-16,6	-18,2
24	121,8	106,3	-15,5	-12,7	-13,6
25	155,4	203,4	48,0	30,9	26,8
26	99,8	83,6	-16,2	-16,2	-17,7
27	55,6	44,8	-10,8	-19,4	-21,5
28	59,9	53,4	-6,5	-10,9	-11,5
29	66,1	50,2	-15,9	-24,1	-27,3
30	54,8	41,0	-13,8	-25,2	-28,8
31	448,3	428,0	-20,3	-4,5	-4,6
32	111,4	101,5	-9,9	-8,9	-9,3
33	115,0	100,2	-14,8	-12,9	-13,8
34	26,4	18,3	-8,1	-30,7	-36,2
35	86,9	75,6	-11,3	-13,0	-13,9
36	248,5	214,4	-34,1	-13,7	-14,7
37	115,4	97,2	-18,2	-15,8	-17,1
38	51,8	38,4	-13,4	-25,9	-29,7
39	75,7	63,6	-12,1	-16,0	-17,4
40	255,8	231,9	-23,9	-9,3	-9,8
41	84,9	73,4	-11,5	-13,5	-14,5
42	70,9	57,4	-13,5	-19,0	-21,0
43	58,6	50,5	-8,1	-13,8	-14,8
44	100,6	80,1	-20,5	-20,4	-22,7
45	80,5	68,8	-11,7	-14,5	-15,7
46	125,4	116,3	-9,1	-7,3	-7,5
47	105,8	93,3	-12,5	-11,8	-12,6
48	129,8	143,9	14,1	10,9	10,3
49	113,8	93,5	-20,3	-17,8	-19,6
50	160,9	138,8	-22,1	-13,7	-14,7
51	675,6	653,4	-22,2	-3,3	-3,3

Method Comparison

Sample Values					
Serial Number	X	Y	Y - X	(Y - X)% of X	Normalized Difference
52	2.314,2	2.262,0	-52,2	-2,3	-2,3
53	733,2	703,8	-29,4	-4,0	-4,1
54	1.288,8	1.267,8	-21,0	-1,6	-1,6
55	5.132,8	5.171,2	38,4	0,7	0,7
56	10.644,4	10.855,0	210,6	2,0	2,0
57	11.646,0	12.085,2	439,2	3,8	3,7
58	332,1	297,7	-34,4	-10,4	-10,9
59	116,2	101,8	-14,4	-12,4	-13,2
60	111,8	93,3	-18,5	-16,5	-18,0
61	203,4	175,9	-27,5	-13,5	-14,5
62	468,6	444,5	-24,1	-5,1	-5,3
63	134,9	108,8	-26,1	-19,3	-21,4
64	95,4	79,6	-15,8	-16,6	-18,1
65	177,4	157,9	-19,5	-11,0	-11,6
66	634,8	619,2	-15,6	-2,5	-2,5
67	1.209,6	1.164,0	-45,6	-3,8	-3,8
68	1.255,8	1.222,8	-33,0	-2,6	-2,7
69	1.385,4	1.328,4	-57,0	-4,1	-4,2
70	891,0	874,2	-16,8	-1,9	-1,9
71	232,0	200,5	-31,5	-13,6	-14,6
72	81,7	65,3	-16,4	-20,1	-22,3
73	205,6	185,8	-19,8	-9,6	-10,1
74	348,3	330,1	-18,2	-5,2	-5,4
75	37,2	24,1	-13,1	-35,2	-42,7
76	256,9	228,3	-28,6	-11,1	-11,8
77	48,3	37,8	-10,5	-21,7	-24,4
78	56,9	42,7	-14,2	-25,0	-28,5
79	132,3	115,6	-16,7	-12,6	-13,5