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Summary of Safety and Effectiveness

K962344

MYOGLOBIN METHOD FOR THE IMMUNO 1 SYSTEM

Listed below is a comparison of the performance between the Immuno 1 Myoglobin method (T01-3653-51) and a similar device that was granted FDA determination of substantial equivalence: The Behring N Latex Myoglobin Reagents. This reagents are designed to run on the Behring Nephelometer. The information used in this summary of Safety and Effectiveness was extracted from the Myoglobin Method Sheet and from data on file at Bayer Corporation.

Intended Use

This in vitro diagnostic procedure is a solid phase immunoassay intended for the quantitative determination of Myoglobin in human serum or heparin plasma on the Technicon Immuno 1 system. When used in combination with other clinical data such as presenting symptoms and EKG values, measurement of Myoglobin aides in the early phase diagnosis of Myocardial Infarctions.

Assay Description

The method described is an enzyme label sandwich assay using a monoclonal (mouse) capture and a polyclonal (goat) detector antibody. The monoclonal antibody is labelled with fluorescein and the polyclonal antibody labelled with alkaline phosphatase (ALP). The two reagents are the active compounds of the R1 and the R2 reagent, respectively. The solid phase consists of a suspension of magnetizable particles coated with antibody to fluorescein (mIMP reagent). Sample or calibrator, R1and R2 reagent and mIMP reagent are mixed simultaneously and incubated at 37 °C. In the presence of Myoglobin a fluorescein-conjugate= Myoglobin=ALP-conjugate complex is formed and captured by the antiFluorescein antibodies on the magnetic particles. The particles are precipitated by an external magnetic field, washed and para-Nitrophenylphosphate is added as the enzyme substrate. The increase in absorbance due to the formation of p-Nitrophenolate is monitored spectrophotometrically at 405 and 450 nm. The response read is directly proportional to the

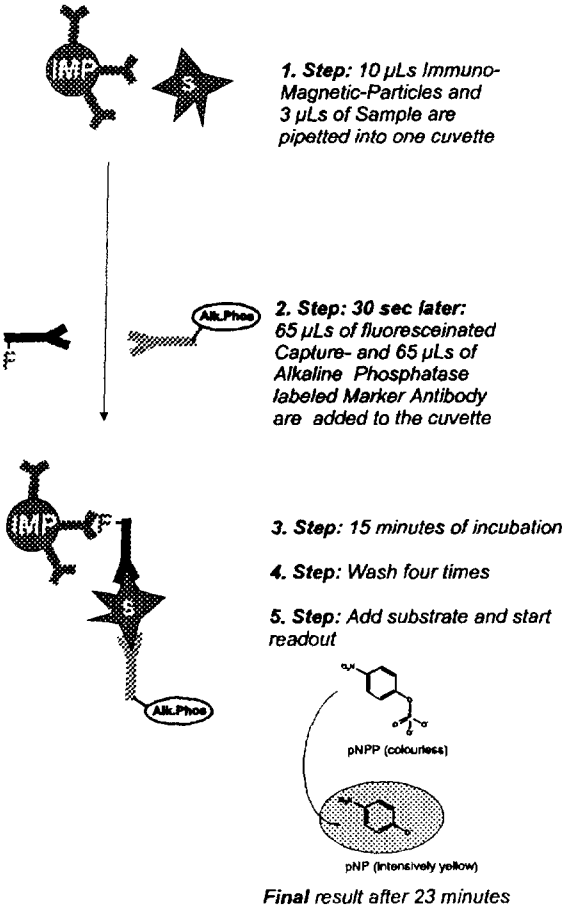
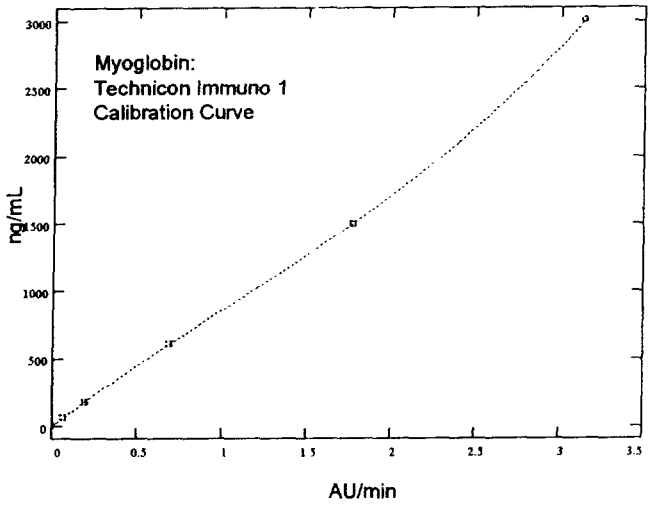


Fig. 1 Schematic reporesentation of the Technicon Immuno 1 Myoglobin Assay

concentration of Myoglobin in a sample. A Cubic Fit Through Zero is used to calculate the dose response curve. The assay is depicted schematically in fig. 1.
The assay has a range of 0 to 3000 ng/mL with a sensitivity of 1.8 ng/mL; six calibrators with Myoglobin concentrations of 0, 60, 180, 600, 1500 and 3000 ng/mL are provided.
A dose response curve is shown in fig. 2.



Data pairs measured:

0 ng/mL:	0.0019 AU/min
60 ng/mL:	0.0645 AU/min
180 ng/mL:	0.1937 AU/min
600 ng/mL:	0.6805 AU/min
1500 ng/mL:	1.7656 AU/min
3000 ng/mL:	3.1391 AU/min

Fig. 2 Calibration Curve of the Technicon Immuno 1 Myoglobin Assay

ASSAY PERFORMANCE

Imprecision

Total imprecision data was obtained by analyzing human serum controls on two Immuno 1 instruments on 20 different days. Two separate lots of reagents and calibrators were used. Both reagent and calibrator combinations on both instruments were tested with two different lots of magnetic particles. The total number of replicates for each level was 160. The calibration was only performed when a new reagent/ calibrator lot or particle lot combination was implemented on a machine.

Table 1: Imprecision of Immuno 1 Myoglobin Assay Data was collected on two Systems over twenty days with four replicates on each day on each system					
Specimen	Total Imprecision (n= 160)			Within run imprecision (mean)	
	Average [ng/mL]	Std Dev [ng/mL]	CV [%]	Std Dev [ng/mL]	CV [%]
Sample 1	14.8	0.8196	5.5	0.3081	2.1
Sample 2	52.6	1.9084	3.6	0.77925	1.5
Sample 3	75.6	2.9831	3.9	1.06855	1.4
Sample 4	131.3	4.6636	3.6	2.28195	1.7
Sample 5	247	10.9436	4.4	4.47045	1.8
Sample 6	278.1	8.2182	3.0	3.53275	1.3
Sample 7	639.7	23.0701	3.6	11.3425	1.8
Sample 8	1557.6	56.46	3.6	21.4224	1.4
Sample 9	2718.9	91.3937	3.4	44.90395	1.7

Correlation with Immuno 1 Myoglobin results with Behring Nephelometer A

A total of 100 serum and plasma samples with Behring Nephelometer (BNA) values in the range of 21 to 2660 ng/mL (BNA) were tested with the Behring Nephelometer A and the Immuno 1 Myoglobin assay. The correlation equation according to Bablock-Passing was

$$y = 1.02 \times x + 1.05$$

(y is *Immuno 1* Myoglobin assay; x is *Behring Nephelometer A* Myoglobin assay).

Calculation of Regression Line :				
Slope (b) :		**1.02**		**Number of Samples :** **100**
Limits :	0.98		1.06	**Sampletype :** **all sample codes**
Intercept (a) :		**1.05**		
Limits :	-3.2		4.5	
Confidence of Correlation :	0.99314			

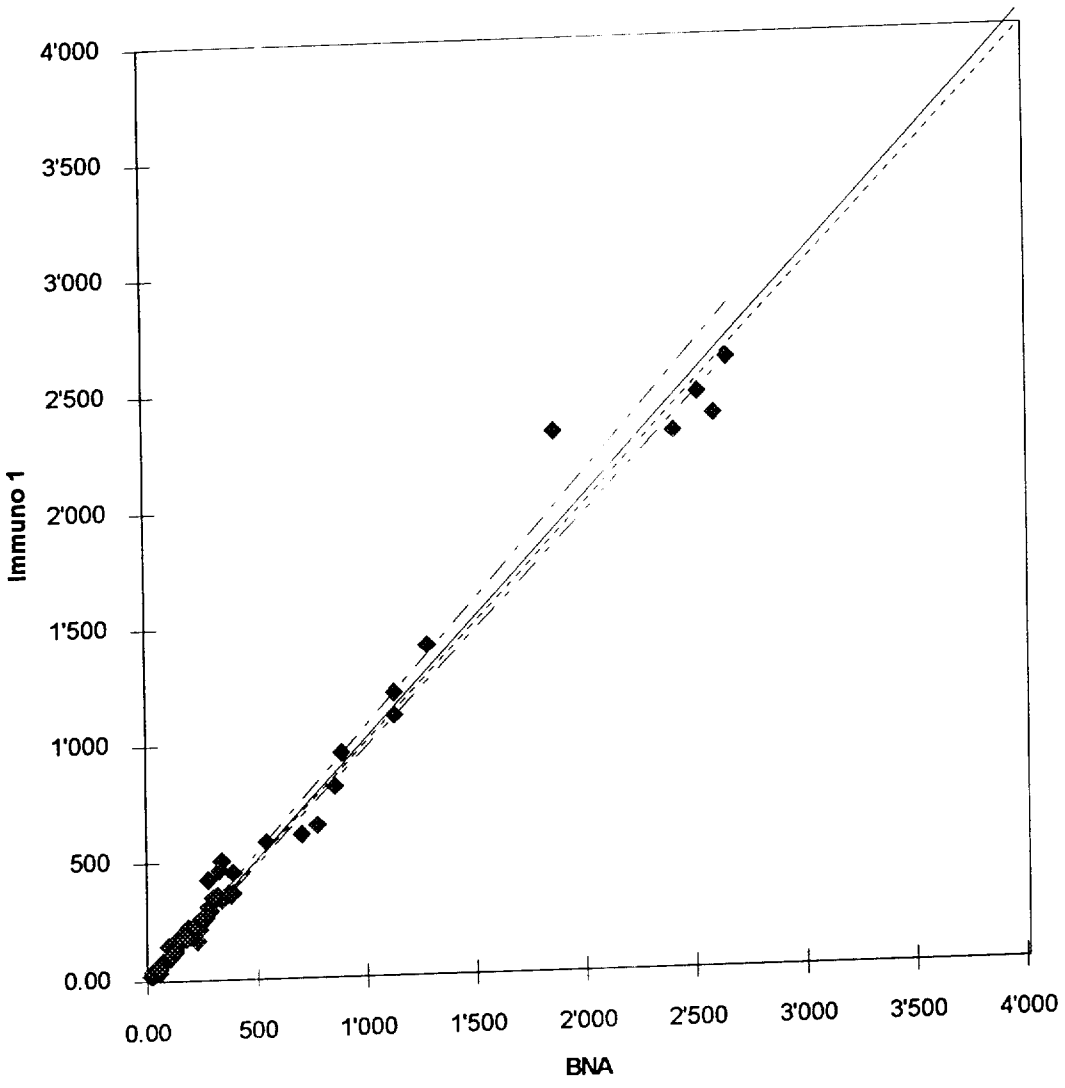


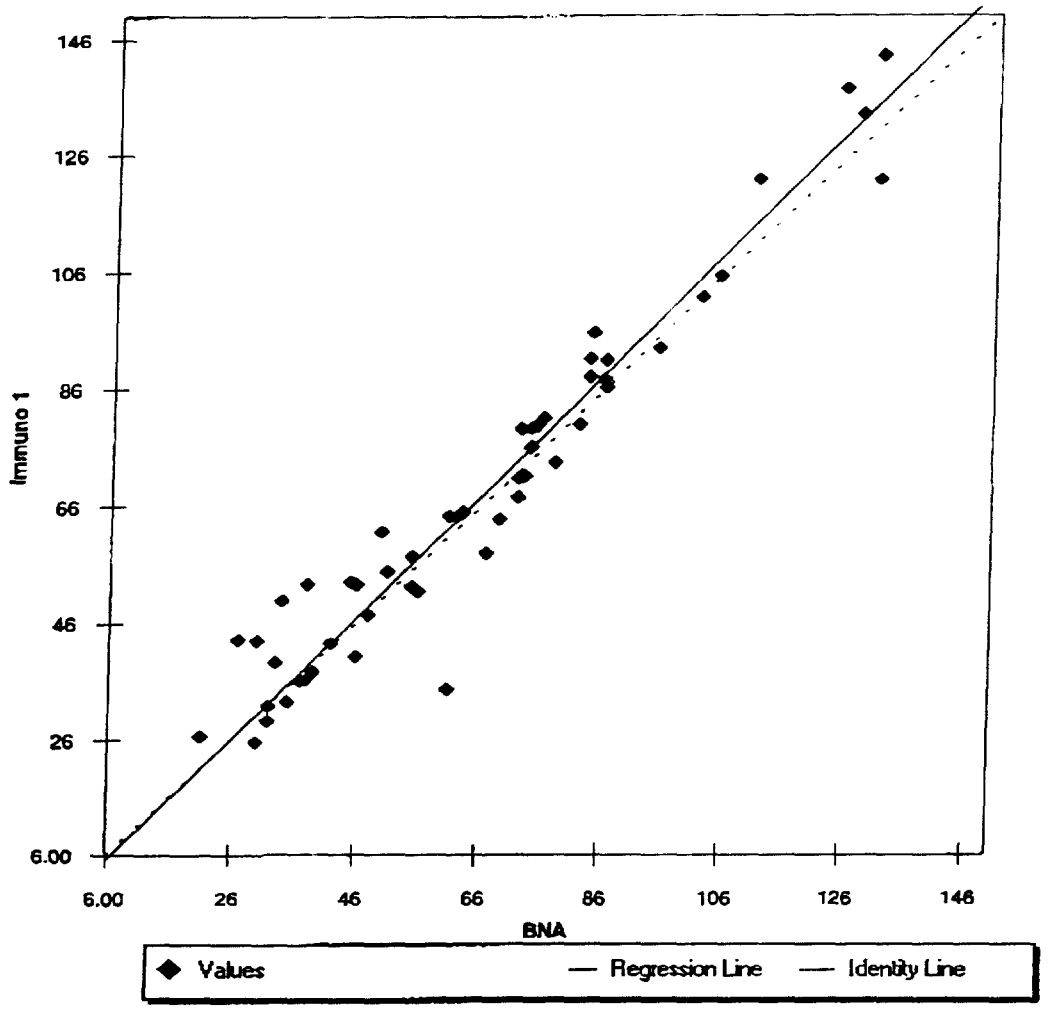
Fig. 3 Correlation Plot of Immuno 1 Myoglobin Results versus Behring Nephelometer A results

TEST.XLG (METHOD.XLD)

Method Comparison acc. to Bablok-Passing

Methods: Immuno 1 Myoglobin vs. Behring N-Latex Reagents (BNA)

Serum and Plasma Samples



Calculation of Regression Line :

Slope (b) :		**1.04110**	
Limits :	0.96763		1.12364
Intercept (a) :		**-0.98356**	
Limits :	-5.79055		4.21007
Confidence of Correlati		0.96814	

Number of Samples : **54**

Sampletype : **all sample codes**

Interference

For all interference measurements a +2 Pool and a -2 Pool was prepared. The +2 Pool was made from a solution of Myoglobin in serum with a concentration of approximately 180 µg/l (double of the concentration at the medical decision point) being diluted 1+1 with a solution of the potentially interfering substance in Myoglobin-stripped serum at a concentration twice as high as required. This yields a Myoglobin concentration at the medical decision level with the required interferant-concentration. The -2 Pool was made up from the same Myoglobin solution, but this time being diluted 1+1 with stripped serum containing no interferant. So two solutions of exactly identical Myoglobin concentration were obtained, one containing no interferent, the other with the required high concentration. The 0-Pool was obtained mixing equivalent amounts of the +2- and the -2-Pool while the -1- and +1-Pool were prepared from equal quantities of the 0-Pool and the -2- respectively the +2-Pool.

- Bilirubin

Bilirubin: -2 Pool: 0 mg/ dL
+2 Pool 25 mg/ dL

Results:

Pool	conc. (meas.)	% of -2 pool
2-	90.6	100.0
1-	90.6	100.0
0	91.3	100,8
1+	91.5	101
2+	90.9	100.3

- Albumin

Albumin: -2 Pool: 0 mg/mL
+2 Pool: 6.5 g/dL

Results:

Pool	conc (meas.)	% of -2 Pool
2-	87.1	100.0
1-	87.1	100.0
0	87.9	100.9
1+	88.1	101.1
2+	88.4	101.5

- Hemoglobin

Hemoglobin: -2 Pool: 0 mg/ mL
+2 Pool: 1 g/ dL

Results:

Pool	conc. (meas)	% of -2 Pool
2-	88	100.0
1-	89.3	101.5
0	89.8	102
1+	88.1	100.1
2+	89.3	101.5

- Gamma Globulins

BGG: -2 Pool: 0 mg/dL
+2 Pool: 5.3 g/ dL

Results:

Pool	conc. (meas.)	% of -2 Pool
2-	82,7	100,0
1-	84,1	101,7
0	84,6	102,3
1+	78,6	95
2+	85,6	103,5

- Triglycerides

Triglyceride Supertrate: -2 Pool: 0 g/ dL
+2 Pool: 1.3 g/ dL (calculated for Triglycerides)

Results:

Pool	conc. (meas.)	% of -2 Pool
2-	94,9	100,0
1-	90,4	95,3
0	90	94.8
1+	89	93.8
2+	99,2	104.5

- Heparin

Heparin: -2 Pool: 0 IU/ mL (Serum)
+2 Pool: 65 IU/mL (0.5 mg/mL)

Results:

Pools	conc. (meas.)	% of -2 Pool
2-	85,9	100,0
1-	85,8	99,9
0	85,8	99,9
1+	86,4	100,6
2+	87,2	101,5

- Citrate

Trisodium Citrate, Dihydrate: -2 Pool: 0 mg/ml
+2Pool: 50 mg/mL

Results:

Pools	conc. (meas.)	% of -2 Pool
2-	92,4	100,0
1-	90,8	98,3
0	91,5	99
1+	91,1	98,6
2+	88,7	96

- Urea and Creatine

Urea and Creatine: -2 Pool: no Urea, no Creatine
+2 Pool: 200 mg/ dL Urea, 2.5 mg/dL Creatine

Results:

Pools	conc. (meas.)	% of -2 Pool
2-	127,7	100,0
1-	129,2	101,2
0	127,9	100,2
1+	127,5	99,8
2+	128,4	100,5

- Rheumatory Factor

Rheumatory Factor: -2 Pool: 0 IU/ mL
+2 Pool: 567 IU/ mL

Results:

Pool	conc. (meas.)	% of -2 Pool
2-	95,9	100,0
1-	96,1	100,2
0	98,1	102,3
1+	98,8	103
2+	99,6	103,9

Linearity

All control to check linearity pools were generated in a way, that a serum sample with a high Myoglobin level (+2 Pool) and a low sample (-2 Pool) were mixed in a ratio of 1 + 1. The 0 Pool thus generated was furtherly mixed with the same amount of the +2 respectively the -2 Pool to have the +1 and -1 Pool. By that procedure five equally spaced controls covering the whole assay range are generated. For data analysis a linear regression was calculated from the result of the -2, -1 and 0 Pools of each sample series. The expected results and the deviation from the measured values were calculated from the equation. The result is shown in the table below.

Pool (Sample)	measured	calculated	dev (%)
-2 (A)	18.7	19.5	-3.9
-1 (A)	656.7	655.2	0.2
0 (A)	1290.2	1291	-0.1
1 (A)	1855.7	1926.7	-3.7
2 (A)	2481.8	2562.5	-3.1
-2 (B)	19.1	24.2	-21
-1 (B)	659.9	649.7	1.6
0 (B)	1270.2	1275.3	-0.4
1 (B)	1862.7	1900.8	-2
2 (B)	2439.2	2526.4	-3.5
-2 (C)	19.1	20.4	-6.5
-1 (C)	664.9	662.2	0.4
0 (C)	1302.7	1304	-0.1
1 (C)	1891.6	1945.8	-2.8
2 (C)	2510.9	2587.6	-3

Sample Dilution

For testing Sample Dilution of clinical serum and plasma samples a dilution series with Immuno 1 Sample Diluent B and Immuno 1 Myoglobin Calibrator Level 1 was run. The recovery of the undiluted sample was set to 100 %

Dilution of Clinical Serum Samples with Immuno 1 Sample Diluent B					
sample content %	**Identification**	**AU/min**	**conc.(meas.)**	**conc.(calc)**	**recovery (%)**
100	Serum Sample A	1.1004	880.5	880.5	100
75		0.7783	623.3	660.4	106.0
50		0.4671	426.6*	440.3	103.2
25		0.2536	205.1	220.1	107.3
10		0.1013	81.6	88.1	108
0		0.0027	0.6	0.0	-
100	Serum Sample B	2.7817	2420.1	2420.1	100
75		2.2541	1881.2	1815.1	96.5
50		1.5296	1233	1210.1	98.1
25		0.7516	602.1	605.0	100.5
10		0.2922	236.2	242.0	102.5
0		0.002	0.1	0.0	-
100	Serum Sample C	3.1881	2887.9	2887.9	100
75		2.5717	2197.4	2165.9	98.6
50		1.7897	1456.1	1444.0	99.2
25		0.9565	765.2	722.0	94.4
10		0.3813	307.7	288.8	93.9
0		0.0021	0.2	0.0	-
100	Serum Sample D	2.7268	2360.9	2360.9	100
75		2.0877	1724.3	1770.7	102.7
50		1.4969	1205.5	1180.5	97.9
25		0.7662	613.7	590.2	96.2
10		0.2915	235.6	236.1	100.2
0		0.0023	0.3	0.0	-
100	Serum Sample E	2.6725	2302.7	2302.7	100
75		2.0673	1705.4	1727.0	101.3
50		1.456	1171.4	1151.4	98.3
25		0.7215	578.2	575.7	99.6
10		0.2689	217.5	230.3	105.9
0		0.002	0.1	0.0	-

Dilution of Serum Samples with Myoglobin Calibrator Level 1					
Sample content %	**Identification**	**AU/min**	**conc. (meas.)**	**conc. (calc)**	**dev. (%)**
100	Serum Sample A	1.0525	859.7	859.7	100
75		0.8022	659.3	644.8	97.8
50		0.5132	426.3	429.9	100.8
25		0.2648	222.1	214.9	96.8
10		0.0976	81	86.0	106.2
0		0.0019	-1.3	0.0	-
100	Serum Sample B	2.7167	2344.3	2344.3	100
75		2.1286	1767.2	1758.2	99.5
50		1.4933	1217.3	1172.2	96.3
25		0.7267	598.8	586.1	97.9
10		0.2955	247.5	234.4	94.7
0		0.003	-0.3	0.0	-
100	Serum Sample C	3.0948	2765.1	2765.1	100
75		2.4005	2023.7	2073.8	102.5
50		1.7318	1417.3	1382.6	97.6
25		0.9207	754.1	691.3	91.7
10		0.3701	309.3	276.5	89.4
0		0.0018	-1.4	0.0	-
100	Serum Sample D	2.8607	2499.2	2499.2	100
75		2.1077	1748.1	1874.4	107.2
50		1.4938	1217.7	1249.6	102.6
25		0.751	618.3	624.8	101.1
10		0.2835	237.7	249.9	105.1
0		0.0019	-1.3	0.0	-
100	Serum Sample E	2.7182	2345.6	2345.6	100
75		2.1273	1766	1759.2	99.6
50		1.4916	1215.9	1172.8	96.5
25		0.7647	629.3	586.4	93.2
10		0.297	248.8	234.6	94.3
0		0.0021	-1.1	0.0	-

Dilution of Plasma Samples with Immuno 1 Sample diluent B					
Sample content [%]	Identification	AU/min	conc. (meas)	conc (calc)	dev (%)
100	Plasma Sample AA	1.44	1224.1	1224.1	100
75		1.0586	898	918.075	102.2

Sample content [%]	Identification	AU/min	conc. (meas)	conc (calc)	dev (%)
50		0.6935	591.2	612.05	103.5
25		0.3398	292.3	306.025	104.7
10		0.1379	118.7	122.41	103.1
0		0.0024	0.1	0	-
100	Plasma Sample BB	2.6022	2332.7	2332.7	100
75		2.0189	1747.1	1749.525	100.1
50		1.3546	1150.3	1166.35	101.4
25		0.6843	583.5	583.175	99.9
10		0.2568	221.3	233.27	105.4
0		0.0023	-0.1	0	-
100	Plasma Sample CC	2.5736	2302.1	2302.1	100
75		1.9753	1705.9	1726.575	101.2
50		1.361	1155.8	1151.05	99.6
25		0.6662	568.3	575.525	101.3
10		0.2714	233.9	230.21	98.4
0		0.0022	-0.2	0	-
100	Plasma Sample DD	1.2854	1090.7	1090.7	100
75		0.9627	817.3	818.025	100.1
50		0.635	542.1	545.35	100.6
25		0.3104	267.3	272.675	102
10		0.1269	109.1	109.07	100
0		0.002	-0.4	0	-
100	Plasma Sample EE	1.62	1382.2	1382.2	100
75		1.2144	1030.1	1036.65	100.6
50		0.8063	685.9	691.1	100.8
25		0.3932	337.8	345.55	102.3
10		0.1604	138.2	138.22	100
0		0.0022	-0.1	0	-

Dilution of Plasma Samples with Immuno 1 Calibrator Level 1					
Sample content %	Identification	AU/min	conc (meas)	conc (calc)	dev (%)
100	Plasma Sample AA	1.4613	1216.1	1216.1	100
75		1.1144	929.1	912.075	98.2
50		0.814	683.6	608.05	88.9
25		0.3588	307	304.025	99
10		0.1268	108.8	121.61	111.8
0		0.0025	-0.1	0	-

Dilution of Plasma Samples with Immuno 1 Calibrator Level 1					
Sample content %	Identification	AU/min	conc (meas)	conc (calc)	dev (%)
100	Plasma Sample BB	2.724	2392.9	2392.9	100
75		2.1735	1843.6	1794.675	97.3
50		1.4542	1210.1	1196.45	98.9
25		0.7596	639.2	598.225	93.6
10		0.283	242.9	239.29	98.5
0		0.0023	-0.2	0	-
100	Plasma Sample CC	2.7637	2435.5	2435.5	100
75		2.1392	1811.5	1826.625	100.8
50		1.4943	1243.9	1217.75	97.9
25		0.7273	612.7	608.875	99.4
10		0.2993	256.7	243.55	94.9
0		0.0023	-0.3	0	-
100	Plasma Sample DD	1.3432	1117.6	1117.6	100
75		1.0524	878.4	838.2	95.4
50		0.6883	580.7	558.8	96.2
25		0.3372	288.8	279.4	96.7
10		0.1365	117.3	111.76	95.3
0		0.0021	-0.4	0	-
100	Plasma Sample EE	1.6146	1345.7	1345.7	100
75		1.2409	1033	1009.275	97.7
50		0.8323	698.6	672.85	96.3
25		0.3987	340.5	336.425	98.8
10		0.1619	139.1	134.57	96.7
0		0.0021	-0.4	0	-

Hook Effect

Samples with Myoglobin concentrations up to 1 Million ng/mL were assayed with the Technicon Immuno 1 Myoglobin Assay. The assay will not erroneously compute raw data to concentrations within the calibration range of the assay as long as the Myoglobin content in the sample is less than 150,000 ng/mL. In the following figure there is a graphical representation of the expected concentrations against the reported for antigen levels between 500 ng/mL and 1 Million ng/mL.

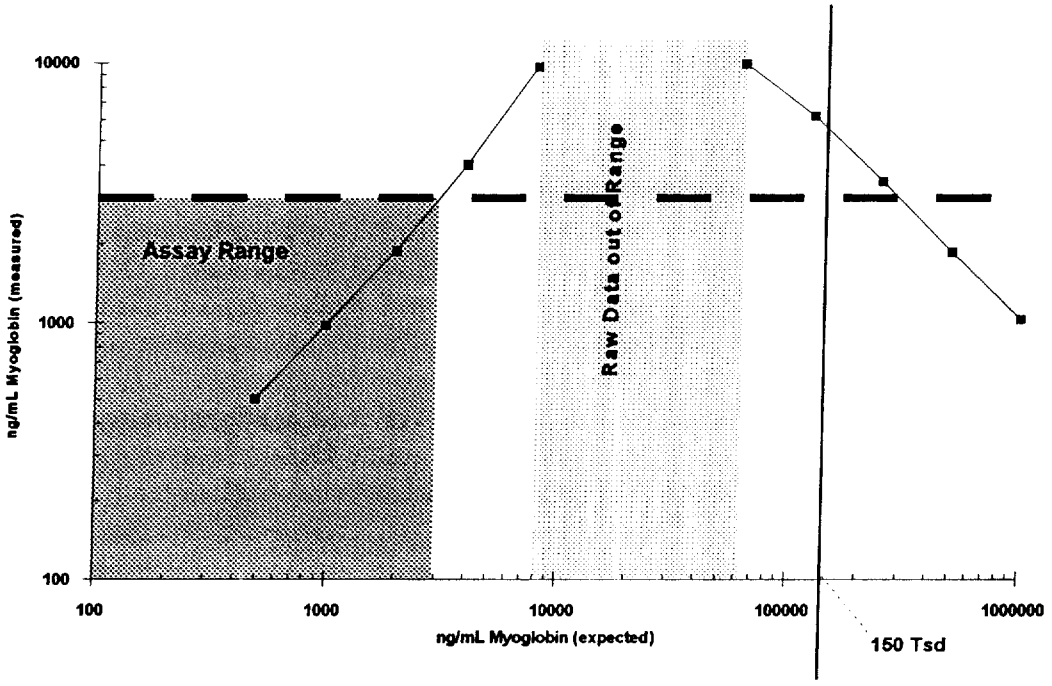


Fig. 4 Concentrations reported by the Immuno 1 Myoglobin assay against expected concentrations. There are no results reported if they above the dashed line in *Fig. 4*.

Recovery

Known amounts of Myoglobin Solution were spiked into four clinical samples, two serum samples, two plasma samples. For serum the recoveries were ranging from 96.5 to 105.2 %; for plasma the recoveries are between 97.7 and 103%. For the recoveries of spiked antigen in plasma it is important to also use Myoglobin containing plasma as the spiking material. Serum- of buffer-based material may lead to deviations from the expected.

Serum (PEY 2795)			
Sample	**Expected**	**found**	**recovery**
Serum Sample α	39.1	39.1	100.0
	77.2	78.1	101.2
	651	672.6	103.3
	1224	1250.1	102.1
	1798	1757.7	97.8
	2371	2377.2	100.3
Serum Sample β	38.5	38.5	100.0
	77.2	77.4	100.3
	651	685	105.2
	1224	1223.6	100.0
	1798	1735.1	96.5
	2371	2331.8	98.3

Plasma (PEY 2795)			
Sample	**expected**	**found**	**recovery**
Plasma Sample γ	24.8	24.8	100.0
	77	78.1	101.4
	843.45	860.6	102.0
	1609.9	1624.3	100.9
	2376.35	2326.6	97.9
	3142.8	3180.4	101.2
Plasma Sample δ	31.3	31.3	100.0
	83.5	81.6	97.7
	758.45	780.9	103.0
	1433.4	1459.2	101.8
	2108.35	2078	98.6
	2783.3	2782	100.0

Expected Values

Samples from 77 non AMI individuals were assayed and gave the distribution of results shown in *Fig. 4*.
It was found that 98% of of the values were 88 ng/mL or less.

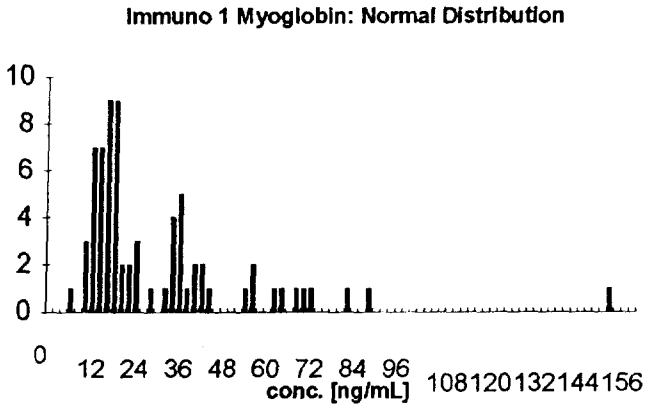


Fig. 5 Technicon Immuno 1 Myoglobin Assay: Normal Distribution

Minimum Detectable Concentration

The minimum detectable concentration was measured in 32 different runs on four different days using two different lots of reagents, calibrators and magnetic particles. The L1 calibrator containing no Myoglobin was measured 576 times all together. Calculated from the mean zero absorption plus two standard deviations the minimum detectable dose was determined as 1.8 ng/mL.