Performance Characteristics

1. Analytical performance:

a. Precision/Reproducibility Study

The precision of the Diazyme Cystatin C POC Test was evaluated according to Clinical and Laboratory Standards Institute (CLSI) EP5-A guideline with the following modifications: In the study, three whole blood specimens containing 1.00 mg/L, 2.70 mg/L, and 6.20 mg/L Cystatin C were tested in 2 runs per day with duplicates over 10 working days on three different SMART Analyzers. The results are listed below.

(1) Internal precision study performed at Diazyme Laboratories
Acceptance Criteria: CV≤ 8 % or 0.1 SD for low value samples

Reagent Lot: CC00110

Whole Blood Sample 1: 1.00 mg/L Cystatin C
ProMedDx Whole Blood ID: 11637086
SMART Analyzer: Ab0596

Day	Date	Run 1		Run 2		Mean		
		Result 1	Result 2	Result 1	Result 2	Run 1	Run 2	Daily
1	1/31/2011	1.00	0.93	0.98	1.02	0.97	1.00	0.98
2	2/1/2011	0.97	0.84	0.96	0.97	0.91	0.97	0.94
3	2/2/2011	0.89	1.02	0.99	1.05	0.96	1.02	0.99
4	2/3/2011	1.00	1.00	1.03	0.98	1.00	1.01	1.00
5	2/4/2011	1.00	0.96	0.97	1.10	0.98	1.04	1.01
6	2/7/2011	1.05	1.07	0.99	1.02	1.06	1.01	1.03
7	2/8/2011	1.03	1.00	0.95	0.90	1.02	1.93	0.97
8	2/9/2011	1.05	0.90	1.03	1.05	0.98	1.04	1.01
9	2/10/2011	0.95	1.07	0.92	0.97	1.01	0.95	0.98
10	2/11/2011	0.99	1.07	0.96	0.88	1.03	0.92	0.98

Whole Blood Sample 2: 2.70 mg/L Cystatin C
ProMedDx Whole Blood ID: 11637086 spiked with Cystatin C
SMART Analyzer: Aa0316

Day	Date	Run 1		Run 2		Mean		
		Result 1	Result 2	Result 1	Result 2	Run 1	Run 2	Daily
1	1/31/2011	2.70	2.70	2.71	2.74	2.70	2.73	2.71
2	2/1/2011	2.68	2.66	2.80	2.67	2.67	2.74	2.70
3	2/2/2011	2.72	2.92	2.72	2.75	2.82	2.74	2.78
4	2/3/2011	2.70	2.71	2.76	2.73	2.71	2.75	2.73

5	2/4/2011	2.70	2.90	2.73	2.72	2.80	2.73	2.76
6	2/7/2011	2.79	2.70	2.68	2.62	2.75	2.65	2.70
7	2/8/2011	2.64	2.79	2.78	2.67	2.72	2.73	2.72
8	2/9/2011	2.66	2.81	2.72	2.79	2.74	2.76	2.75
9	2/10/2011	2.71	2.72	2.50	2.55	2.72	2.53	2.62
10	2/11/2011	2.73	2.83	2.74	2.66	2.78	2.70	2.74

Whole Blood Sample 3: 6.20 mg/L Cystatin C
ProMedDx Whole Blood ID: 11637086 spiked by stock Cystatin C
SMART Analyzer: Ae3620

Day	Date	Run 1		Run 2		Mean		
		Result 1	Result 2	Result 1	Result 2	Run 1	Run 2	Daily
1	1/31/2011	6.52	6.49	6.07	6.13	6.51	6.10	6.30
2	2/1/2011	6.16	6.04	6.15	6.14	6.10	6.15	6.12
3	2/2/2011	5.77	5.47	6.49	6.27	5.62	6.38	6.00
4	2/3/2011	6.14	6.06	6.19	6.11	6.10	6.15	6.13
5	2/4/2011	6.30	6.17	6.22	6.02	6.24	6.12	6.18
6	2/7/2011	6.33	6.21	6.30	6.19	6.27	6.25	6.26
7	2/8/2011	6.14	6.11	5.97	6.11	6.13	6.04	6.08
8	2/9/2011	6.23	6.10	6.21	6.15	6.17	6.18	6.17
9	2/10/2011	6.28	6.06	6.01	5.39	6.17	5.70	5.94
10	2/11/2011	6.11	6.06	6.20	6.15	6.09	6.18	6.13

The mean value (Mean), standard deviation, within run imprecision and total imprecision CV mg/L are calculated and summarized in the following tables:

Within Run precision CV%

Total data points	Whole blood 1 1.00 mg/L Cystatin C 40	Whole blood2 2.70 mg/L Cystatin C 40	Whole blood 3 6.20 mg/L Cystatin C 40
Mean (mg/L)	0.988	2.720	6.1305
SD (mg/L)	0.0553	0.0694	0.1353
CV mg/L	5.6%	2.6%	2.2%

Total Precision CV%

Total data points	Whole blood 1 1.00 mg/L Cystatin C 40	Whole blood 2 2.70 mg/L Cystatin C 40	Whole blood 3 6.20 mg/L Cystatin C 40
Mean (mg/L)	0.988	2.720	6.1305
SD (mg/L)	0.0577	0.0780	0.2145
CV mg/L	5.9%	2.9%	3.5%

Conclusion: The results shown indicate good precision and meet acceptance criteria.

(2) Additional precision study at Diazyme

Acceptance Criteria: CV≤ 8 % or 0.1 SD for low value samples

Results:

SMART Analyzer: Aa0316

Sample 1: 11689729 diluted to 0.70 mg/L Cystatin C

		RUN 1		RUN 2				
Day#	Date	Result 1	Result 2	Result 1	Result 2	Mean Run 1	Mean Run 2	Daily mean
1	9/26/2011	0.68	0.72	0.66	0.65	0.70	0.66	0.68
2	9/27/2011	0.65	0.63	0.69	0.7	0.64	0.70	0.67
3	9/28/2011	0.72	0.68	0.63	0.76	0.70	0.70	0.70
4	9/29/2011	0.8	0.81	0.73	0.69	0.81	0.71	0.76
5	10/10/2011	0.74	0.63	0.62	0.67	0.69	0.65	0.67
6	10/11/2011	0.79	0.64	0.60	0.71	0.72	0.66	0.69
7	10/12/2011	0.76	0.78	0.65	0.71	0.77	0.68	0.73
8	10/13/2011	0.65	0.69	0.70	0.71	0.67	0.71	0.69
9	10/14/2011	0.70	0.72	0.69	0.68	0.71	0.69	0.70
10	10/17/2011	0.68	0.71	0.70	0.70	0.70	0.70	0.70

Sample 2: 11689732 natural sample at 1.25 mg/L

		RUN 1		RUN 2				
Day#	Date	Result 1	Result 2	Result 1	Result 2	Mean Run 1	Mean Run 2	Daily mean
1	9/26/2011	1.29	1.23	1.25	1.26	1.26	1.26	1.26
2	9/27/2011	1.23	1.1	1.16	1.25	1.17	1.21	1.19
3	9/28/2011	1.11	1.2	1.31	1.41	1.16	1.36	1.26
4	9/29/2011	1.26	1.21	1.1	1.25	1.24	1.18	1.21
5	10/10/2011	1.24	1.13	1.19	1.29	1.19	1.24	1.21

6	10/11/2011	1.12	1.28	1.22	1.20	1.20	1.21	1.21
7	10/12/2011	1.25	1.25	1.20	1.19	1.25	1.20	1.22
8	10/13/2011	1.22	1.25	1.20	1.19	1.24	1.20	1.22
9	10/14/2011	1.26	1.20	1.22	1.21	1.23	1.22	1.22
10	10/17/2011	1.20	1.18	1.20	1.16	1.19	1.18	1.19

Sample 3: 11689741 spiked to 4.70 mg/L

		RUN 1		RUN 2				
Day#	Date	Result 1	Result 2	Result 1	Result 2	Mean Run 1	Mean Run 2	Daily mean
1	10/18/2011	4.61	4.62	4.3	4.5	4.62	4.40	4.51
2	10/19/2011	4.63	4.71	4.62	4.55	4.63	4.59	4.63
3	10/20/2011	4.83	4.61	4.65	4.7	4.72	4.68	4.70
4	10/21/2011	4.98	5.04	4.65	4.66	4.83	4.66	4.83
5	10/24/2011	4.75	4.52	4.68	4.69	4.64	4.69	4.66
6	10.25/2011	5.06	4.84	4.67	4.74	4.83	4.71	4.83
7	10/26/2011	5.14	4.62	4.69	4.74	4.80	4.72	4.80
8	10/27/2011	4.88	4.80	4.75	4.69	4.78	4.72	4.78
9	10/28/2011	4.69	4.78	4.77	4.68	4.74	4.73	4.73
10	10/31/2011	4.68	4.72	4.65	4.77	4.70	4.71	4.71

The results are summarized in the following table:

Within Run

	Sample 1	Sample 2	Sample 3
No. of Points	40	40	40
Mean (mg/L)	0.696	1.217	4.717
SD (mg/L)	0.0444	0.0563	0.1148
CV	6.4%	4.6%	2.4%

Total

	Sample 1	Sample 2	Sample 3
No. of Points	40	40	40
Mean (mg/L)	0.696	1.217	4.717
SD (mg/L)	0.0478	0.0590	0.1467
CV	6.9%	4.9%	3.1%

Conclusion: The results shown indicate good precision and meet acceptance criteria.

(3) External precision study performed at POL sites

Acceptance Criteria: CV≤ 8 % or 0.1 SD for low value samples

The precision was also evaluated at three (3) physician office laboratories (POL) by intended users such as nurses and office assistants. Three (3) whole blood samples containing Cystatin C levels ranging from low to high were used for the external precision study. At each site, 2 whole blood samples were tested. Each sample was run 4 times per day for 5 days using three SMART Analyzers.

Results:

Whole blood 4: 11661140

		RUN 1		RUN 2				
Day#	Date	Result 1	Result 2	Result 1	Result 2	Mean Run 1	Mean Run 2	Daily mean
Site 3								
1	05/26/2011	0.95	0.95	0.92	0.94	0.95	0.93	0.94
2	05/27/2011	0.94	0.95	0.91	0.97	0.95	0.94	0.94
3	05/31/2011	0.93	1.00	0.93	1.00	0.97	0.97	0.97
4	06/01/2011	1.09	0.97	1.04	1.08	1.03	1.06	1.05
5	06/02/2011	1.05	1.00	0.97	0.98	1.03	0.98	1.00
Site 2								
1	05/26/2011	0.95	0.91	0.93	0.95	0.93	0.94	0.94
2	05/27/2011	0.96	0.93	0.94	0.97	0.95	0.96	0.95
3	06/31/2011	0.95	0.92	1.05	0.81	0.94	0.93	0.93
4	06/01/2011	0.83	0.84	0.98	0.97	0.84	0.98	0.91
5	06/02/2011	0.90	0.92	0.91	0.92	0.91	0.92	0.91

whole blood 5: 11661151

		RUN 1		RUN 2				
Day#	Date	Result 1	Result 2	Result 1	Result 2	Mean Run 1	Mean Run 2	Daily mean
Site 1								
1	05/25/2011	3.09	3.33	3.08	3.98	3.21	3.53	3.37
2	05/27/2011	3.48	3.33	3.27	3.34	3.41	3.31	3.35
3	05/30/2011	3.27	3.28	3.51	3.20	3.28	3.35	3.32
4	06/01/2011	3.31	3.51	3.19	3.39	3.41	3.29	3.35
5	06/03/2011	3.41	3.36	3.53	3.44	3.38	3.49	3.44
Site 2								
1	05/27/2011	2.99	2.89	3.230	3.150	3.940	3.19	3.56
2	05/31/2011	3.25	3.27	2.780	3.070	3.260	2.93	3.09
3	06/01/2011	2.81	3.29	3.080	3.960	3.050	3.52	3.28
4	06/02/2011	3.09	2.87	3.030	2.890	2.980	2.96	2.97
5	06/03/2011	3.07	3.08	3.030	3.190	3.075	3.11	3.09

whole blood 6: 11661153

		RUN 1		RUN 2				
Day#	Date	Result 1	Result 2	Result 1	Result 2	Mean	Mean	Daily

						Run 1	Run 2	mean
Site 1								
1	05/25/2011	6.05	5.95	6.32	6.62	6.00	6.47	6.24
2	05/27/2011	6.52	5.89	6.39	7.25	6.21	6.82	6.51
3	05/30/2011	6.54	6.12	6.29	6.65	6.33	6.47	6.40
4	06/01/2011	5.82	6.87	6.48	6.15	6.35	6.31	6.33
5	06/03/2011	6.19	6.66	6.08	6.46	6.43	6.27	6.35
Site 3								
1	05/26/2011	5.51	5.66	6.05	5.71	5.59	5.88	5.73
2	05/27/2011	5.81	5.82	5.83	5.95	5.82	5.89	5.85
3	06/31/2011	6.03	6.00	6.07	5.98	6.02	6.03	6.02
4	06/01/2011	5.73	5.75	6.17	5.93	5.74	6.05	5.89
5	06/02/2011	5.88	5.92	5.66	5.93	5.90	5.79	5.84

The results are summarized in the following table:

Site 1 precision:

Replicate	Whole blood Sample 5	Whole blood Sample 6
1	3.09	6.05
2	3.48	6.52
3	3.27	6.54
4	3.31	5.82
5	3.41	6.19
6	3.33	5.95
7	3.33	5.89
8	3.28	6.12
9	3.51	6.87
10	3.36	6.66
11	3.08	6.32
12	3.27	6.39
13	3.51	6.29
14	3.19	6.48
15	3.53	6.08
16	3.98	6.62
17	3.34	7.25
18	3.2	6.65
19	3.39	6.15
20	3.44	6.46
mean	3.365	6.365
SD	0.1940	0.3531
CV	**5.8%**	**5.6%**

Site 2 precision:

Replicate	Whole blood Sample 4	Whole blood Sample 5
1	0.95	2.99

2	0.96	3.25
3	0.95	2.81
4	0.83	3.09
5	0.90	3.07
6	0.91	2.89
7	0.93	3.27
8	0.92	3.29
9	0.84	2.87
10	0.92	3.08
11	0.93	3.23
12	0.94	2.78
13	1.05	3.08
14	0.98	3.03
15	0.91	3.03
16	0.95	3.15
17	0.97	3.07
18	0.81	3.96
19	0.97	2.89
20	0.92	3.19
mean	0.927	3.101
SD	0.0546	0.2500
CV	**5.9%**	**8.0%**

Site 3 precision:

Replicate	Whole blood Sample 4	Whole blood Sample 6
1	0.95	5.51
2	0.94	5.81
3	0.93	6.03
4	1.09	5.73
5	1.05	5.88
6	0.95	5.66
7	0.95	5.82
8	1.00	6.00
9	0.97	5.75
10	1.00	5.92
11	0.92	6.05
12	0.91	5.83
13	0.93	6.07
14	1.04	6.17
15	0.97	5.66
16	0.94	5.71
17	0.97	5.95
18	1.00	5.98
19	1.08	5.93
20	0.98	5.93

mean	0.979	5.870
SD	0.0520	0.1652
CV	**5.3%**	**2.8%**

Conclusion: For the three whole blood samples containing Cystatin C from 0.95 mg/L to 6.11 mg/L, the results indicated good precision and CV% met precision criteria.

(4) Additional Precision Study

Acceptance Criteria: CV≤ 8 % or 0.1 SD for low value samples

The additional precision was evaluated at three physician office laboratories (POL) by intended users such as nurses and office assistances to test systemic and random error on three Diazyme Cys C SMART assay.

Results:

Sample 7: 11689739 diluted to 0.58 mg/L Cystatin C

Day#	Date	RUN 1 Result 1	RUN 1 Result 2	RUN 2 Result 1	RUN 2 Result 2	Mean Run 1	Mean Run 2	Daily mean
Site 1								
1	10/31/2011	0.55	0.57	0.64	0.51	0.57	0.58	0.57
2	11/1/2011	0.57	0.66	0.54	0.57	0.59	0.56	0.59
3	11/2/2011	0.56	0.61	0.52	0.65	0.59	0.59	0.59
4	11/3/2011	0.57	0.48	0.51	0.52	0.52	0.52	0.52
5	11/4/2011	0.63	0.59	0.64	0.55	0.62	0.60	0.60
Site 3								
1	10/31/2011	0.51	0.6	0.58	0.53	0.56	0.56	0.56
2	11/1/2011	0.56	0.58	0.48	0.62	0.57	0.54	0.55
3	11/2/2011	0.47	0.58	0.5	0.63	0.53	0.57	0.55
4	11/3/2011	0.59	0.6	0.65	0.55	0.60	0.60	0.60
5	11/4/2011	0.58	0.53	0.56	0.59	0.56	0.58	0.57

Sample 8: 11689742 spiked to 1.40 mg/L Cystatin C

Day#	Date	RUN 1 Result 1	RUN 1 Result 2	RUN 2 Result 1	RUN 2 Result 2	Mean Run 1	Mean Run 2	Daily mean
Site 2								
1	11/15/2011	1.41	1.42	1.51	1.5	1.42	1.51	1.46
2	11/16/2011	1.41	1.33	1.45	1.41	1.37	1.43	1.40
3	11/17/2011	1.42	1.43	1.48	1.45	1.45	1.47	1.45
4	11/18/2011	1.24	1.39	1.41	1.26	1.33	1.34	1.33
5	11/19/2011	1.3	1.28	1.45	1.44	1.37	1.45	1.37
Site 3								
1	11/15/2011	1.42	1.29	1.44	1.26	1.36	1.35	1.35

2	11/16/2011	1.22	1.47	1.35	1.15	1.35	1.25	1.30
3	11/17/2011	1.32	1.44	1.24	1.31	1.38	1.28	1.33
4	11/18/2011	1.29	1.38	1.18	1.51	1.34	1.35	1.34
5	11/19/2011	1.31	1.36	1.30	1.30	1.34	1.30	1.32

Sample 9: 11689740 spiked to 4.90 mg/L Cystatin C

Day#	Date	Result 1	RUN 1 Result 2 Run 1	RUN 2 Result 1 Run 2	RUN 2 Result 2 mean	Mean	Mean	Daily
Site 1								
1	10/31/2011	4.75	4.7	5.03	5.73	4.73	5.38	5.05
2	11/1/2011	5.16	4.73	4.98	5.46	5.08	5.22	5.08
3	11/2/2011	5.12	5.07	5.03	5.32	5.10	5.18	5.14
4	11/3/2011	4.91	4.96	4.59	5.68	5.04	5.14	5.04
5	11/4/2011	4.95	5.03	4.86	5.78	4.99	5.32	5.16
Site 2								
1	10/31/2011	4.91	4.94	4.99	5.02	4.97	5.01	4.97
2	11/1/2011	4.8	4.61	4.94	4.95	4.83	4.95	4.83
3	11/2/2011	5.09	4.97	4.93	4.85	4.96	4.89	4.96
4	11/3/2011	4.83	4.89	4.74	4.85	4.86	4.80	4.83
5	11/4/2011	4.63	4.89	4.77	4.71	4.76	4.74	4.75

The results are summarized in the following table:

Site 1 precision:

Replicate	Whole blood Sample 7	Whole blood Sample 9
1	0.55	4.75
2	0.57	5.16
3	0.64	5.12
4	0.51	4.91
5	0.57	4.95
6	0.66	4.7
7	0.54	4.73
8	0.57	5.07
9	0.56	4.96
10	0.61	5.03
11	0.52	5.03
12	0.65	4.98
13	0.57	5.03
14	0.48	4.59
15	0.51	4.86
16	0.52	5.73
17	0.63	5.46
18	0.59	5.32
19	0.64	5.68

20	0.55	5.78
mean	0.572	5.092
SD	0.0523	0.3420
CV	**9.1%**	**6.7%**

Site 2 precision:

Replicate	Whole blood Sample 8	Whole blood Sample 9
1	1.41	4.91
2	1.41	4.8
3	1.42	5.09
4	1.24	4.83
5	1.30	4.63
6	1.42	4.94
7	1.33	4.61
8	1.43	4.97
9	1.39	4.89
10	1.28	4.89
11	1.51	4.99
12	1.45	4.94
13	1.48	4.93
14	1.41	4.74
15	1.45	4.77
16	1.50	5.02
17	1.41	4.95
18	1.45	4.85
19	1.26	4.85
20	1.44	4.71
mean	1.400	4.866
SD	0.0776	0.1261
CV	**5.5%**	**2.6%**

Site 3 precision:

Replicate	Whole blood Sample 7	Whole blood Sample 8
1	0.51	1.42
2	0.56	1.22
3	0.47	1.32
4	0.59	1.29
5	0.58	1.31
6	0.6	1.29
7	0.58	1.47
8	0.58	1.44
9	0.6	1.38
10	0.53	1.36
11	0.58	1.44
12	0.48	1.35

13	0.5	1.24
14	0.65	1.18
15	0.56	1.3
16	0.53	1.26
17	0.62	1.15
18	0.63	1.31
19	0.55	1.51
20	0.59	1.3
mean	0.565	1.327
SD	0.0488	0.0956
CV	**8.7%**	**7.2%**

Conclusion: For the three whole blood samples containing Cystatin C from 0.58 mg/L to 4.90 mg/L, the results indicated good precision and CV% met precision criteria.

b. Linearity/assay reportable range:

Acceptance criteria:
Expected vs. recovered plot: $r^2 > 0.95$, Slope = 1.0+/-0.05
Or EP evaluator analysis with 10% allowable total error

Reagent lot: CC00210
Analyzer: SMART equipment # Ab0596

A set of eleven levels of linearity materials were prepared by diluting a whole blood sample containing 8 mg/L of Cystatin C (Cys C) with saline according to Clinical and Laboratory Standards Institute EP6-A and were tested with the Diazyme Cystatin C POC Test in triplicate on the SMART Analyzer.

Level 10: 0 mL saline + 1.0mL of 8.0 mg/L Cys-C
Level 9: 0.1mL saline + 0.9mL of 8.0 mg/L Cys-C
Level 8: 0.2mL saline + 0.8mL of 8.0 mg/L Cys-C
Level 7: 0.3mL saline + 0.7mL of 8.0 mg/L Cys-C
Level 6: 0.4mL saline + 0.6mL of 8.0 mg/L Cys-C
Level 5: 0.5mL saline + 0.5mL of 8.0 mg/L Cys-C
Level 4: 0.6mL saline + 0.4mL of 8.0 mg/L Cys-C
Level 3: 0.7mL saline + 0.3mL of 8.0 mg/L Cys-C
Level 2: 0.8mL saline + 0.2 mL of 8.0 mg/L Cys-C
Level 1: 0.9mL saline + 0.1mL of 8.0 mg/L Cys-C
Level 0: 1.0mL saline + 0mL of 8.0 mg/L Cys-C

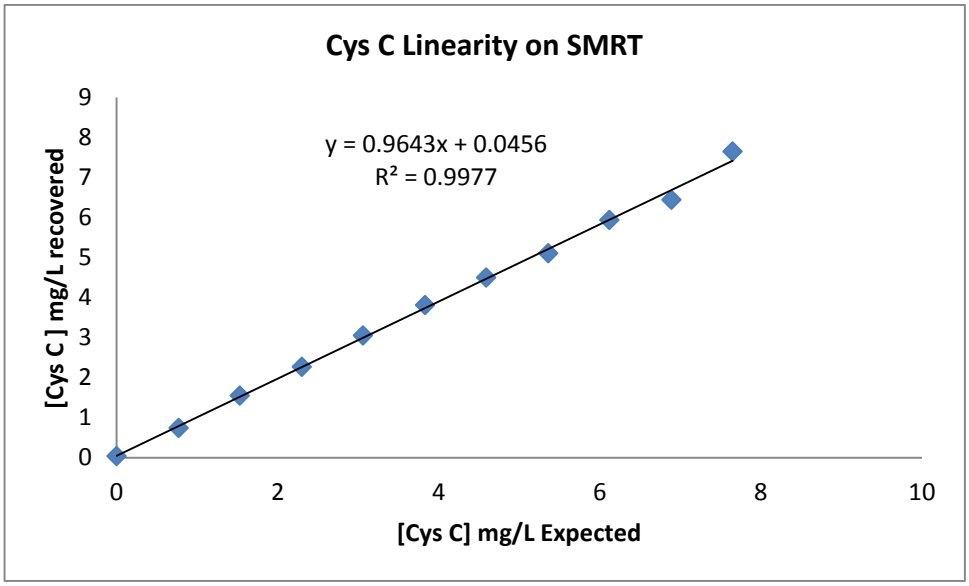
Data is summarized below:

Reagent lot: CC00110 Calibrator lot: CCS00110A

Analyzer: Ab0596

Dilution	Mean Recovery (mg/L)	Exp Recovery (mg/L)	Error (mg/L)	%Error
Level 0	7.65	7.65	0.000	0.00
Level 1	6.44	6.89	0.453	7.04
Level 2	5.94	6.12	0.180	3.03
Level 3	5.11	5.36	0.250	4.89
Level 4	4.50	4.59	0.093	2.08
Level 5	3.81	3.83	0.023	0.61
Level 6	3.05	3.06	0.013	0.44
Level 7	2.27	2.30	0.033	1.47
Level 8	1.55	1.53	-0.020	-1.29
Level 9	0.74	0.77	0.027	3.59
Level 10	0.04	0.00	-0.040	n/a

The mean of the obtained results are plotted against the theoretical values.



Results:
The recovered Cystatin C values were plotted expected values and an appropriate line fitted by standard linear regression resulted in: $y = 0.9643 x + 0.0456$; $R^2 = 0.9977$.

Data was also analyzed with EP evaluator software and results are shown in the next page. Analysis indicated that the device performance is linear in the range of 0.04 to 7.65 mg/L. Allowable systematic error (Sea) was 4.0%.

Conclusion: Linearity data and the LOQ data support Analytical Measuring Range (AMR) of 0.30 to 7.65 mg/L.

EP Evaluator

Cys-C

Research and Development -- Diazyme Laboratories

Instrument: Smart

Linearity

	Assigned	Pct	N	Est	Mean	Residual	Linearity
11	0.000	0%	3	0.039	0.040	0.001	Pass
10	0.765	10%	3	0.771	0.743	-0.028	Pass
9	1.530	20%	3	1.504	1.550	0.046	Pass
8	2.295	30%	3	2.236	2.267	0.030	Pass
7	3.060	40%	3	2.969	3.047	0.078	Pass
6	3.825	50%	3	3.701	3.807	0.105	Pass
5	4.590	60%	3	4.434	4.497	0.063	Pass
4	5.355	70%	3	5.167	5.110	-0.057	Pass
3	6.120	80%	3	5.899	5.940	0.041	Pass
2	6.885	90%	3	6.632	6.437	-0.195	Pass
1	7.650	100%	3	7.364	7.650	0.286	Pass

See User's Specifications for Pass/Fail criteria

Linearity Summary

	Overall
Slope	0.958
Intercept	0.039
Obs. Err.	3.7%
N	11

LINEAR within Allowable Systematic Error of 4.0%

Experimental Results

11	0.08	0.04	0.00
10	0.74	0.70	0.79
9	1.60	1.47	1.58
8	2.20	2.29	2.31
7	3.09	2.98	3.07
6	3.77	3.80	3.85
5	4.54	4.41	4.54
4	5.09	5.09	5.15
3	5.98	5.89	5.95
2	6.42	6.42	6.47
1	7.57	7.68	7.70

X: Excluded from calculations

User's Specifications

Allowable Total Error:	10.0%
Systematic Error Budget:	40%
Allowable Systematic Error:	4.0%

Supporting Data

Analyst:	Yan
Date:	22 Feb 2011
Value Mode:	Pct-Assigned
Units:	mg/L
Lot Number:	00210
Comment:	

Analytical Claim

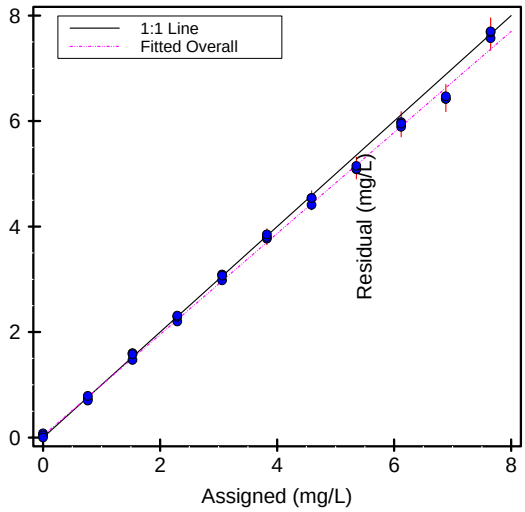
The Linearity of Cys-C was analyzed on Smart over a measured range of 0.040 to 7.650 mg/L. Allowable systematic error (SEa) was 4.0%. The results are LINEAR.

Accepted by: _____ _____

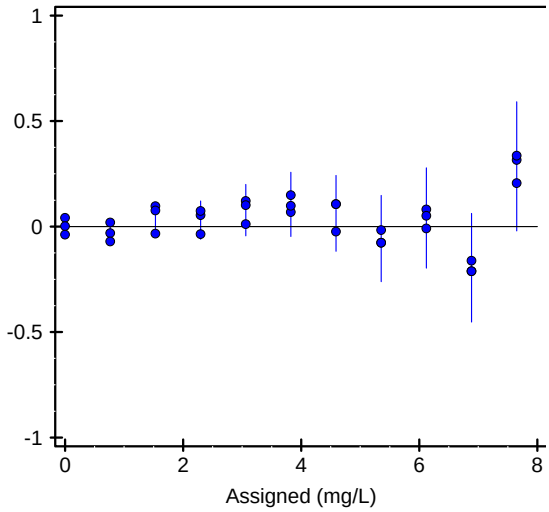
Signature Date

Linearity

Scatter Plot



Residual Plot



c. Stability/shelf life:

A. Reagent Stability

Acceptance criteria: Minimum of 7 days of stress at 37ºC and control recovery within expected ranges (≤ 15% control recovery change when Cys C ≥ 1.0 mg/L or 0.2 mg/L change when Cys C < 1.0 mg/L from Day 0 value).

Two lots of the Diazyme Cystatin C POC Test reagents were used for this study. The reagents from each lot were filled in SMART cartridges and kept in incubators at 37ºC and 2-8ºC. Two levels of Whole Blood Cystatin C controls containing 0.92 mg/L and 2.73 mg/L Cystatin C stored at 2-8ºC (unstressed) and were tested in duplicates with the stressed reagents. At the indicated time, the Diazyme Cystatin C POC Test kits were removed from storage and tested with the two levels of the Cystatin C controls above. The results are summarized in the following table:

Reagent lot#: CC00110
Reagent lot#: CC00210
Smart Analyzer: Ab0596

Accelerated Stability (37ºC)

[Cys-C]	Lot #	0 Day	2 days	4days	6days	8days	10days
0.92 mg/L	Lot 1: CC00110	0.93	0.97	0.88	0.77	0.96	0.83
	Lot 2: CC00210	0.91	0.94	0.92	0.92	0.88	0.86
Mean		0.92	0.96	0.90	0.85	0.92	0.85
Recovery%		100%	104%	98%	92%	100%	92%
2.73 mg/L	Lot 1: CC00110	2.69	2.68	2.65	2.65	2.67	2.70
	Lot 2:CC00210	2.76	2.67	2.73	2.95	2.95	2.75
Mean		2.73	2.68	2.69	2.80	2.81	2.73
Recovery%		100%	98%	99%	103%	103%	100%

The results listed in table above demonstrated reagents were stable for about 10 days at 37ºC. According to the Arrhenius law based stress models, the shelf-life of the Diazyme Cystatin C Assay kit is conservatively determined to be 12 months when stored at 2-8ºC per labeling. The real time stability study is ongoing.

Real time stability studies

Acceptance criteria: Minimum of 12 months of stress at 2-8ºC and control recovery within expected ranges (≤ 15% control recovery change when Cys C ≥ 1.0 mg/L or 0.2 mg/L change when Cys C < 1.0 mg/L from Day 0 value).

Reagent Lot #: CC00110
Control Lot #: Validation Lot 3

Smart Analyzer: Ab0596

[Cys-C]	Lot #	0 Months	3 Months	6 Months	9 Months	12 Months
1.15 mg/L	Lot: CC00110	1.18 1.04	1.11 1.19	1.12 1.15	1.16 1.13	
Mean		1.11	1.15	1.14	1.15	
Recovery%		100.0%	103.6%	102.7%	103.6%	
2.64 mg/L	Lot: CC00110	2.60 2.68	2.56 2.79	2.80 2.66	2.68 2.70	
Mean		2.64	2.68	2.73	2.69	
Recovery%		100.0%	101.5%	103.4%	101.9%	

The real time stability study is ongoing.

B. Control stability

Acceptance criteria: Minimum of 7 days of stress at 37°C and control recovery within expected ranges (≤ 15% control recovery change when Cys C ≥ 1.0 mg/L or 0.2 mg/L change when Cys C < 1.0 mg/L from Day 0 value)

To determine the shelf life of the Cystatin C POC Test Controls, stress model tests were performed. Representative number of vials from one lot of the Diazyme Cystatin C controls (level 1 and level 2) was subject to stress in an incubator at 37°C. At predetermined times, vials were removed from storage, reconstituted and tested in duplicates with Diazyme Cystatin C POC Test reagents stored at 2-8°C (unstressed) .

Reagent lot: CC00310

Control lot: Validation Lot 1

Control	Expected Value (mg/L)	Expected Range (mg/L)
Level 1	0.72	0.72 ± 0.2
Level 2	2.11	2.11± 0.31

Analyzer: Ab0596

The results are summarized in the following tables:

Accelerated Stability (37°C)

Cystatin C, mg/L	Lot #	Day 0	Day 4	Day 6	Day 8	Day 10
Level 1	Lot 1	0.70 0.73	0.65 0.63	0.63 0.61	0.55 0.61	0.61 0.59
Mean		0.72	0.64	0.62	0.58	0.60

Level 2	Lot 1	2.15 2.07	2.13 2.10	2.05 2.03	2.07 1.94	1.99 1.85
Mean		2.11	2.12	2.04	2.01	1.92

The recovery results obtained demonstrated that the whole blood based controls were stable for at least 7 days when stressed at 37ºC and recovered within assigned ranges. According to the Arrhenius law based stress models, the shelf-life of the lyophilized Diazyme Cystatin C controls is at least 12 months when stored at 2-8ºC per labeling.

Real time stability studies

Acceptance criteria: Minimum of 12 months of stress at 2-8ºC and control recovery within expected ranges (≤ 15% control recovery change when Cys C ≥ 1.0 mg/L or 0.2 mg/L change when Cys C < 1.0 mg/L from Day 0 value).

Control lot: Validation Lot 2

Control	Expected Value (mg/L)	Expected Range (mg/L)
Level 1	1.15	1.15±0.17
Level 2	2.11	2.73± 0.41

Control lot: Validation Lot 3

Control	Expected Value (mg/L)	Expected Range (mg/L)
Level 1	0.72	1.15 ± 0.17
Level 2	2.11	2.64±0.40

Reagent Lot: CC002110
SMART Analyzer: Ab0596

[Cys-C]	Lot #	0 Months	3 Months	6 Months	9 Months	12 Months
1.15 mg/L	Validation Lot 2	1.15 1.14	1.20 1.18	1.15 1.20	1.21 1.15	
Mean		1.15	1.19	1.18	1.15	
Recovery%		100.0%	%	%	100.0%	
2.73 mg/L	Validation Lot 2	2.60 2.66	2.84 2.79	2.75 2.69	2.69 2.71	
Mean		2.64	2.82	2.72	2.70	
Recovery%		100.0%	106.8%	103.0%	102.3%	

Reagent Lot: CC002110
SMART Analyzer: Aa0316

[Cys-C]	Lot #	0 Months	3 Months	6 Months	9 Months	12 Months

1.15 mg/L	Validation Lot 3	1.17 1.14	1.16 1.12	1.10 1.17	1.17 1.15	
Mean		1.16	1.14	1.14	1.15	
Recovery%		100.0%	98.3%	98.3%	99.1%	
2.64 mg/L	Validation Lot 3	2.62 2.68	2.85 2.79	2.88 2.83	2.86 2.85	
Mean		2.65	2.82	2.86	2.84	
Recovery%		100.0%	106.4%	107.9%	107.2%	

C. Analyte stability

Acceptance criteria: Three whole blood specimens were tested for analyte Cystatin C stability. The samples were tested in replicates of 4 daily and stored at 2-8°C between run. The sample is to recover within ≤10% of the point measure on day one.

Reagent lot: CC00210
SMART Analyzer: Ab0596

Sample 1

Day	Rep				Mean	Recovery
	1	2	3	4		
1	0.62	0.61	0.61	0.57	0.60	100.0%
2	0.61	0.59	0.62	0.60	0.61	101.7%
3	0.54	0.46	0.60	0.60	0.55	91.7%
4	0.62	0.58	0.55	0.53	0.57	95.0%
5	0.60	0.62	0.58	0.60	0.60	100.0%

Sample 2

Day	Rep				Mean	Recovery
	1	2	3	4		
1	1.20	1.21	1.18	1.20	1.20	100.0%
2	1.22	1.19	1.20	1.20	1.20	100.2%
3	1.25	1.22	1.19	1.20	1.22	101.3%
4	1.20	1.26	1.21	1.20	1.22	101.5%
5	1.10	1.18	1.20	1.19	1.17	97.3%

Sample 3

Day	Rep	Mean	Recovery

	1	2	3	4		
1	5.10	5.00	4.98	5.05	5.03	100.0%
2	5.03	4.99	5.12	4.92	5.02	99.7%
3	4.98	4.82	5.10	5.02	4.98	99.0%
4	5.27	5.17	5.13	5.20	5.19	103.2%
5	5.16	5.05	5.10	5.07	5.10	101.3%

Conclusion: Results indicate specimen is stable for at least 5 days.

d. Limit of Detection LOB, LOD, LOQ

Acceptance criteria:

LOB = the average of 57th and 58th highest values for true blanks

LOD = LOB + (1.645* SD of Low samples)

LOQ is determined as the lowest sample run that displayed CV% < 20%

The LOB, LOD and LOQ of Diazyme Cystatin C POC Test are determined according to CLSI EP17-A: Protocols for Determination of Limits of Detection and Limits of Quantitation; Approved Guideline described as follows:

Samples: Whole blood samples were from ProMedDx, LLC and came with an IRB certification that protocols used to collect samples and informed consent were IRB approved.

True blanks: 7.5% BSA solution in phosphate buffered saline (PBS).
Low Samples - Five whole blood samples diluted 1:100 with 7.5% BSA solution.

(1) Limit of Blank (LOB)

To calculate the limit of blank (LOB) of the Diazyme Cystatin C SMART Whole Blood Assay, the True Blank Sample (7.5% BSA) was tested with 20 replicates daily for three days. LOB was calculated as the mean of the 57th and 58th highest values for the true blanks.

Reagent lot #: CC00110
Smart Analyzer: Ab0596

Day	Replicate	[Cystatin C] (mg/L)
Day 1	1	0.03
	2	0.00
	3	0.03
	4	0.00

Rank	[Cystatin C] mg/L
1	0.00
2	0.00
3	0.00
4	0.00

	5	0.01	5	0.00
	6	0.00	6	0.00
	7	0.04	7	0.00
	8	0.00	8	0.00
	9	0.00	9	0.00
	10	0.03	10	0.00
	11	0.00	11	0.00
	12	0.01	12	0.00
	13	0.00	13	0.00
	14	0.00	14	0.00
	15	0.01	15	0.00
	16	0.01	16	0.00
	17	0.00	17	0.00
	18	0.01	18	0.00
	19	0.01	19	0.00
	20	0.00	20	0.00
Day 2	1	0.00	21	0.00
	2	0.00	22	0.00
	3	0.00	23	0.00
	4	0.03	24	0.00
	5	0.00	25	0.00
	6	0.03	26	0.00
	7	0.00	27	0.00
	8	0.00	28	0.00
	9	0.01	29	0.00
	10	0.00	30	0.00
	11	0.00	31	0.00
	12	0.00	32	0.00
	13	0.01	33	0.00
	14	0.00	34	0.00
	15	0.00	35	0.00
	16	0.00	36	0.00
	17	0.04	37	0.00
	18	0.02	38	0.00
	19	0.03	39	0.01
	20	0.00	40	0.01
Day 3	1	0.00	41	0.01
	2	0.05	42	0.01
	3	0.00	43	0.01
	4	0.00	44	0.01
	5	0.00	45	0.01
	6	0.00	46	0.01
	7	0.00	47	0.02
	8	0.00	48	0.02
	9	0.00	49	0.03

	10	0.00	50	0.03
	11	0.05	51	0.03
	12	0.04	52	0.03
	13	0.00	53	0.03
	14	0.00	54	0.03
	15	0.00	55	0.04
	16	0.06	56	0.04
	17	0.02	**57**	**0.04**
	18	0.00	**58**	**0.05**
	19	0.00	59	0.05
	20	0.00	60	0.06

Limit of Blank (LOB) = the average of 57th and 58th highest values for true blanks = (0.04 mg/L+ 0.05 mg/L)/2 = 0.045 mg/L

Conclusion: LOB = 0.045 mg/L

(2) Cystatin C Limit of Detection (LOD)

To calculate the limit of detection (LOD) of the Diazyme Cystatin C POC Test, five Low Samples were tested with 4 replicates daily for three days. LOD = LOB + (1.645* SD of Low samples).

Reagent lot #: CC00210
Smart Analyzer: Ab0596

Day	Sample	Replicate	[Cystatin C] mg/L
1	1	1	0.06
		2	0.05
		3	0.06
		4	0.14
	2	1	0.05
		2	0.09
		3	0.03
		4	0.08
	3	1	0.00
		2	0.06
		3	0.09
		4	0.05
	4	1	0.02
		2	0.00
		3	0.00
		4	0.02
	5	1	0.12
		2	0.04

		3	0.09
		4	0.02
2	1	1	0.06
		2	0.00
		3	0.10
		4	0.00
	2	1	0.05
		2	0.08
		3	0.01
		4	0.08
	3	1	0.00
		2	0.06
		3	0.04
		4	0.08
	4	1	0.00
		2	0.00
		3	0.02
		4	0.00
	5	1	0.07
		2	0.00
		3	0.04
		4	0.04
3	1	1	0.10
		2	0.00
		3	0.12
		4	0.05
	2	1	0.00
		2	0.00
		3	0.04
		4	0.10
	3	1	0.07
		2	0.05
		3	0.12
		4	0.04
	4	1	0.04
		2	0.02
		3	0.02
		4	0.00
	5	1	0.00
		2	0.00
		3	0.00
		4	0.00
Mean	0.0429		
StDev	0.0391		

Therefore LOD = LOB + (1.645* SD of Low samples) = 0.045 mg/L + (1.645 × 0.0391 mg/L) = 0.109 mg/L.

Conclusion: The LOD is 0.11 mg/L

(3) Limit of Quantitation (LOQ)

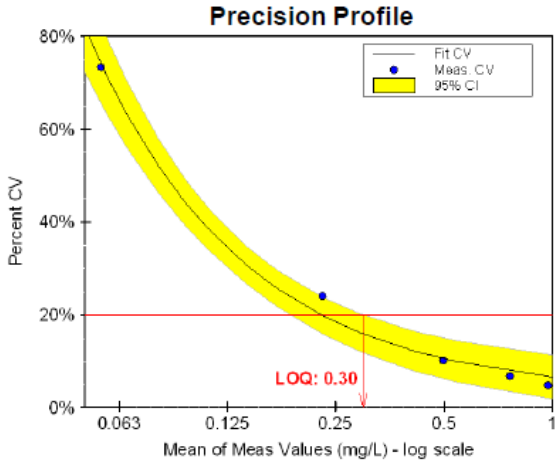
To calculate the LOQ of the Diazyme Cystatin C POC Test, five patient whole blood samples from ProMedDx were diluted with 7.5% BSA to targeted concentrations: 0.1, 0.25, .50, 1.0 mg/L. The diluted samples were tested with the Diazyme Cystatin C SMART reagent on SMART analyzers in 5 runs with 4 replicates per run (20 replicates total per sample). EP Evaluator software 8 was used to estimate the LOQ. See attached file for EP evaluator results.

	Rep	Sample 1 Cys-C 1.0 mg/L	Sample 2 Cys-C 0.75 mg/L	Sample 3 Cys-C 0.50 mg/L	Sample 5 Cys-C 0.25 mg/L	Sample 6 Cys-C 0.1 mg/L
Run 1	1	1.01	0.72	0.53	0.26	0.13
	2	0.92	0.74	0.35	0.30	0.06
	3	1.00	0.73	0.53	0.26	0.10
	4	0.95	0.64	0.49	0.24	0.08
Run 2	5	0.92	0.82	0.48	0.07	0.01
	6	0.88	0.78	0.59	0.24	0.08
	7	0.96	0.73	0.54	0.25	0.01
	8	0.98	0.77	0.52	0.31	0.10
Run 3	9	1.01	0.84	0.51	0.21	0.08
	10	1.02	0.75	0.48	0.23	0.02
	11	0.99	0.80	0.54	0.23	0.06
	12	0.98	0.72	0.43	0.15	0.00
Run 4	13	0.96	0.79	0.52	0.27	0.08
	14	0.96	0.82	0.45	0.23	0.08
	15	1.00	0.79	0.46	0.24	0.06
	16	0.97	0.79	0.51	0.20	0.08
Run 5	17	0.94	0.74	0.48	0.24	0.00
	18	0.99	0.77	0.52	0.30	0.00
	19	1.10	0.79	0.47	0.20	0.00
	20	0.92	0.75	0.52	0.17	0.08

EP Evaluator — **Cys C**

Research and Development -- Diazyme Laboratories — **Instrument: SMART**

Sensitivity-Limit of Quantitation



Analytical Claim

Cys C was analyzed by SMART to determine the LOQ (lowest concentration for which CV is less than a target of 20%).

Specimens with mean measured concentration ranging from 0.056 to 0.973 mg/L were assayed. A curve was fit to estimate the relationship between Mean and CV. Based on the fitted model, the LOQ is 0.30 mg/L. This is the point where the upper 95% confidence interval for the curve has a CV of 20%.

Sample	Target Conc.	N	Mean	SD	Meas. CV(%)	Fitted CV(%)	95% CI for Fitted Low	95% CI for Fitted High
level 5	.1	20	0.056	0.041	73.4	74.1	65.5	82.8
level 4	.25	20	0.230	0.055	24.1	19.9	15.9	23.9
Estimated LOQ		–	**0.30**	–	–	**15.9**	**11.8**	**20.0**
level 3	.5	20	0.496	0.050	10.2	10.6	6.2	15.1
level 2	.75	20	0.762	0.052	6.8	7.9	3.2	12.5
level 1	1.0	20	0.973	0.047	4.9	6.7	2.0	11.4

x: Excluded because either Mean or SD was zero.

Conclusion: The LOQ is 0.30 mg/L

e. Analytical specificity

Common endogenous substance interference

Acceptance criteria: < 10% deviation from un-spiked samples

To determine the level of interference from the substances normally present in whole blood, the Diazyme Cystatin C POC Test was used to test two whole blood samples with "low" and "high" Cystatin C concentration spiked with various concentrations of substances following Clinical and Laboratory Standards Institute EP7-A "Interference Testing in Clinical Chemistry": dose-response guidelines.

To ensure a suitable degree of precision, each sample spiked with interference substances was tested in triplicates.

The interference substances examined and their concentrations tested are listed in the following table:

Interference Substances	C1	C2	C3	C4	C5	
Ascorbic acid	0 mg/dL	17.6 mg/dL	44 mg/dL	88 mg/dL	176 mg/dL	
Ascorbic acid	0 mg/dL	4.4 mg/dL	8.8 mg/dL	10 mg/dL	13.2 mg/dL	
Bilirubin	0 mg/dL	10mg/dL	20mg/dL	30mg/dL	40 mg/dL	
Conjugated Bilirubin	0 mg/dL	10mg/dL	20mg/dL	30mg/dL	40 mg/dL	
Triglycerides	0 mg/dL	250mg/dL	500mg/dL	750mg/dL	1000 mg/dL	
Rheumatoid Factor	0 IU/ml	200 IU/ml	400 IU/ml	600 IU/ml	800 IU/ml	1000IU/ml

The stock solutions for interference testing were prepared as follows:

Interference Substances	Stock solution	Preparation Method
Ascorbic acid	0.5M	Dissolve 88mg in 1 mL of cold water; Keep the stock on ice before testing
Ascorbic acid	0.05M	Dissolve 8.8mg in 1 mL of cold water; Keep the stock on ice before testing
Bilirubin	40 mg/mL	Dissolve 40mg bilirubin unconjugated in 1 mL 0.1N NaOH
Conjugated Bilirubin	40 mg/mL	Dissolve 40mg bilirubin unconjugated in 1 mL water
Triglycerides	1000 mg/mL	Neat solution
RF Positive Serum Control	1500 IU/mL	Prepared from Human Plasma
RF Negative Serum	0 IU/mL	Prepared from Human Plasma

Reagent lot #: CC00310
Smart Analyzer: Ab0596

Bilirubin					
	0 mg/dL	10 mg/dL	20 mg/dL	30 mg/dL	40 mg/dL
Sample 1 [Cys-C] mg/L	0.89	0.93	0.94	0.89	0.89
	0.89	0.94	0.95	0.83	0.91
	0.85	0.88	0.82	0.88	0.93
Mean	0.88	0.92	0.90	0.87	0.91
Recovery %	100%	104.6%	103.0%	98.9%	103.8%
Sample 2	2.56	2.41	2.56	2.62	2.53

[Cys-C] mg/L	2.45	2.43	2.66	2.56	2.62
	2.61	2.50	2.41	2.57	2.41
Mean	2.54	2.45	2.54	2.58	2.52
Recovery %	100%	96.3%	100.1%	101.7%	99.2%

Conjugated Bilirubin					
	0 mg/dL	10 mg/dL	20 mg/dL	30 mg/dL	40 mg/dL
Sample 1 [Cys-C] mg/L	0.89	0.82	1.00	1.04	0.97
	0.89	0.79	0.87	0.90	0.90
	0.85	0.99	0.87	0.96	0.94
Mean	0.88	0.87	0.91	0.96	0.94
Recovery%	100%	98.1%	103.4%	109.8%	106.0%
Sample 2 [Cys-C] mg/L	2.56	2.54	2.77	2.53	2.53
	2.46	2.76	2.64	2.48	2.67
	2.74	2.56	2.55	2.65	2.45
Mean	2.59	2.62	2.65	2.55	2.55
Recovery %	100%	101.3%	102.6%	98.7%	98.6%

Ascorbic Acid					
	0 mg/dL	17.6 mg/dL	44 mg/dL	88 mg/dL	176 mg/dL
Sample 2 [Cys-C] mg/L	2.59	2.65	2.84	3.04	3.16
	2.47	2.52	2.71	2.96	3.09
	2.63	2.68	2.79	2.97	3.07
Mean	2.56	2.62	2.78	3.00	3.11
Recovery %	100%	102.15	108.5%	117.2%	121.2%

	0 mg/dL	4.4 mg/dL	8.8 mg/dL	10.0 mg/dL	13.2 mg/dL
Sample 1 [Cys-C] mg/L	0.89	0.81	0.91	0.89	1.04
	0.85	0.85	0.87	0.93	0.99
	0.83	0.88	0.88	0.95	0.91
Mean	0.86	0.85	0.89	0.92	0.98
Recovery %	100%	98.8%	103.5%	107.8%	114.4%

Triglycerides					
	0 mg/dL	250 mg/dL	500 mg/dL	750 mg/dL	1000 mg/dL
Sample 1 [Cys-C] mg/L	0.85	0.92	0.94	0.90	0.90
	0.88	0.91	0.77	0.93	0.84
	0.79	0.86	0.89	0.77	0.87
Mean	0.84	0.90	0.87	0.87	0.87
Recovery%	100%	106.7%	103.2%	103.2%	103.6%
Sample 2 [Cys-C] mg/L	2.63	2.83	2.78	2.67	2.57
	2.71	2.82	2.81	2.56	2.44
	2.41	2.77	2.57	2.44	2.44
Mean	2.58	2.81	2.72	2.56	2.48

Recovery %	100%	108.6%	105.3%	99.0%	96.1%

Rheumatoid Factor						
	0 IU/mL	200 IU/mL	400 IU/mL	600 IU/mL	800 IU/mL	1000 IU/mL
Sample 1 [Cys-C] mg/L	0.90	0.91	0.94	0.86	0.78	0.86
	0.83	0.94	0.97	0.75	0.91	0.90
	0.85	0.88	0.85	0.86	0.84	0.79
Mean	0.86	0.91	0.92	0.82	0.84	0.85
Recovery %	100%	105.8%	107.0%	95.7%	98.1%	98.8%
Sample 2 [Cys-C] mg/L	2.50	2.41	2.43	2.53	2.55	2.84
	2.63	2.56	2.84	2.44	2.76	2.48
	2.48	2.55	2.43	2.81	2.81	2.93
Mean	2.54	2.51	2.57	2.59	2.71	2.75
Recovery %	100%	98.8%	101.2%	102.2%	106.7%	108.4%

Conclusion: The common interfering substances had no significant interference up to the concentrations summarized below.

Interference	Concentration
Triglyceride	1000 mg/dL
Ascorbic Acid	10 mg/dL
Bilirubin	40 mg/dL
Bilirubin Conjugated	40 mg/dL
Rheumatoid Factor	1000 IU/mL

Additionally, hemoglobin Interference study was performed

Acceptance Criteria: Recovery $\leq \pm$ 10%
The interference of hemoglobin on the SMART Cystatin C Assay is tested at the following concentrations: 0.0 g/dL, 5.0 g/dL, 7.0 g/dL, and 10.0 g/dL.

To determine the level of interference from the substances normally present in whole blood, the Diazyme Cystatin C POC Test was used to test two whole blood samples with "low" and "high" Cystatin C concentration spiked with various concentrations of substances following Clinical and Laboratory Standards Institute EP7-A "Interference Testing in Clinical Chemistry": dose-response guidelines.

Low Level					
Concentration	Rep 1	Rep 2	Rep 3	Mean	Recovery
0.0 g/dL	0.91	0.92	0.89	0.91	100%
5.0 g/dL	0.93	0.91	0.88	0.91	100%
7.0 g/dL	0.93	0.87	0.80	0.87	96%
10.0 g/dL	0.80	0.79	0.98	0.86	94%
High Level					

Concentration	Rep 1	Rep2	Rep3	Mean	Recovery
0.0 g/dL	2.28	2.30	2.24	2.27	100%
5.0 g/dL	2.31	2.34	2.53	2.39	105%
7.0 g/dL	2.21	2.21	2.34	2.25	99%
10.0 g/dL	2.37	2.19	2.21	2.26	99%

Based on the recovery of the analyte, the acceptance criterion is met up to 10.0 g/dL of hemoglobin.

f. Dilution Study

To accurately determine the levels of Cys C > 7.7 mg/L, we recommend the users to perform a 1:1 of the sample with saline if the result of Cys C > 7.7 mg/L is obtained on the SMART analyzer.

To demonstrate this procedure's validity, 3 whole blood samples (spiked) containing Cys C level near the upper detection limit of 7.7 mg/L and one sample containing 1.47 mg/L Cys C were diluted 1:1 with saline and tested on the SMART analyzer in comparison without dilution. The result of the diluted samples was then multiplied by the dilution factor (DF) of 2 to show the recovery.

Acceptance criteria: < 10.0% deviation from the original value of undiluted sample.

The table below summarizes the results:

	Undiluted sample			Diluted Sample					
Sample ID	Run1	Run2	Avg	Run1	Run2	Avg	DF	value	% Recov
11714688*	7.57	7.46	7.52	3.45	3.68	3.57	2	7.13	94.9%
11714665*	6.92	6.76	6.84	3.49	3.56	3.53	2	7.05	103.1%
11714653*	7.72	8.02	7.87	4.62	4.01	4.32	2	8.63	109.7%
11708695	1.42	1.52	1.47	0.72	0.8	0.76	2	1.52	103.4%

*denotes spiked sample

Conclusion: The diluted samples were recovered within 10% of the undiluted samples when applying the dilution factor of 2.

2. Comparison studies:

a. Method comparison with predicate device:

To demonstrate accuracy, the candidate device was tested with individual samples and the results compared to predicate device (k093680) using CLSI EP9-A2: *Method Comparison and Bias Estimation Using patient samples* as a guideline. The method comparison study was performed internally at Diazyme laboratories and externally at three POL sites.

(1) Internal method comparison

Paired human whole blood-serum samples (venous whole blood and plasma from the same individual) were tested for comparison. The whole blood samples were tested with the Diazyme Cystatin C POC Test on SMART analyzer and the correspondent plasma samples were tested with predicate Assay (k093680) on Hitachi 917.

The paired individual human whole blood-plasma samples used for this study were from a certified commercial source, ProMedDx, LLC and came with an IRB certification that protocols, informed consent, used to collect samples were IRB approved.

Data plotting:
Passing & Bablok regression, Scatter plot of Diazyme Cystatin C POC Test Y versus comparison method Diazyme Hitachi 917 Cystatin C X and Bias plot where the differences between the Y (Diazyme Cystatin C SMART method) and X (Diazyme Cystatin C Hitachi 917 method) were plotted against X values.

Acceptance Criteria: Slope = 0.90~1.10; $r^2 \geq 0.95$.

A total of fifty five (55) EDTA whole blood specimens were tested with Diazyme Cystatin C POC Test. The correspondent plasma samples were tested with Diazyme Cystatin C on Hitachi 917 analyzer.

The comparison results with individual patient demographic information are tabulated in the following table:

Diazyme SMART equipment #: Ab0596

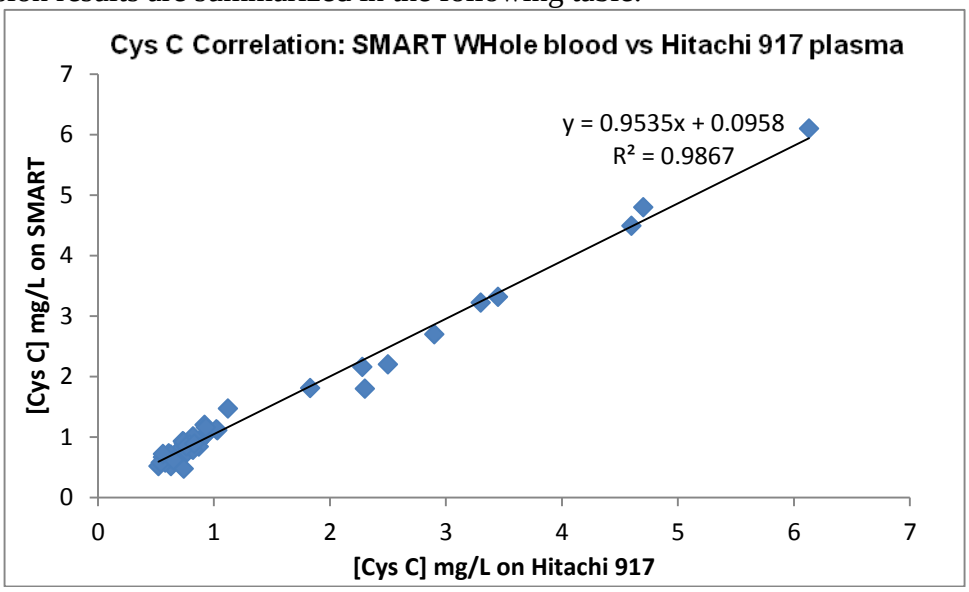
Reagents Lot: CC00110
Results are listed in the following table:

	Sample ID	Gender	Age	Plasma [Cys-C] mg/L on Hitachi 917	Whole blood [Cys-C] mg/L on SMART
1	11637053	M	50	0.87	0.94
2	11637054	M	49	0.77	0.89
3	11637055	M	47	0.52	0.52
4	11637056	M	31	0.63	0.52
5	11637057	M	63	0.92	1.02
6	11637058	F	45	0.82	0.86
7	11637059	F	35	0.66	0.65
8	11637060	M	26	0.80	0.88
9	11637061	F	53	0.71	0.72
10	11637062	M	22	0.58	0.58

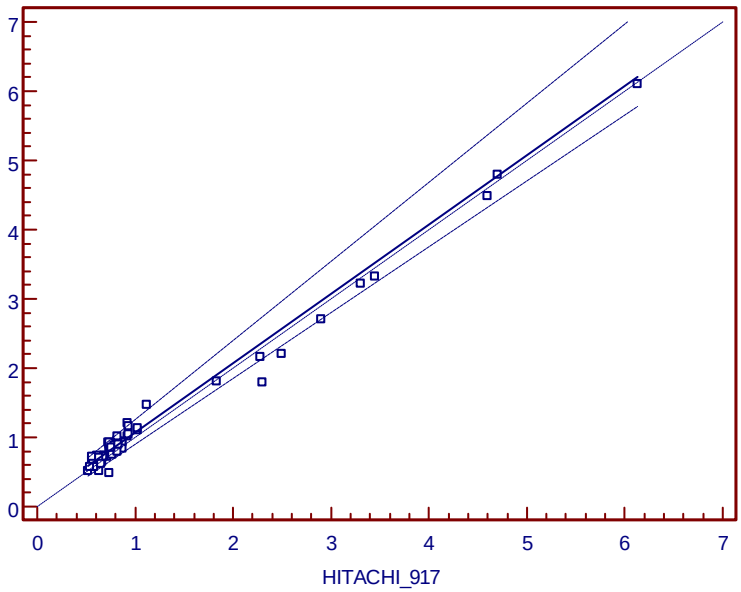
11	11637063	M	43	0.56	0.67
12	11637064	M	44	0.82	0.95
13	11637065	M	29	0.74	0.48
14	11637066	F	44	0.68	0.73
15	11637067	M	60	0.74	0.86
16	11637068	M	24	0.92	1.20
17	11637069	M	48	0.81	0.80
18	11637070	M	43	0.75	0.83
19	11637071	M	27	0.93	1.04
20	11637072	M	44	1.03	1.11
21	11637073	M	31	1.02	1.13
22	11637074	F	34	0.74	0.90
23	11637075	M	31	0.75	0.85
24	11637076	M	23	0.82	0.89
25	11637077	M	25	0.87	0.92
26	11637078	M	28	0.57	0.68
27	11637079	M	38	0.93	1.16
28	11637080	M	47	0.83	0.93
29	11637081	M	47	0.54	0.58
30	11637082	M	34	0.68	0.74
31	11637083	M	22	0.67	0.72
32	11637084	M	24	1.12	1.47
33	11637085	M	65	0.77	0.75
34	11637086	M	30	0.87	0.84
35	11637087	F	48	0.82	1.01
36	11637088	M	45	0.73	0.93
37	11637089	F	49	0.61	0.73
38	11637090	M	40	0.63	0.71
39	11637091	M	29	0.74	0.92
40	11637092	F	31	0.65	0.62
41	11637093	M	46	0.79	0.88
42	11637094	M	51	0.56	0.72
43	11637095	M	19	0.82	0.79
44	11637096	M	18	0.76	0.85
45	11637097	M	29	0.83	0.89
46	Spiked 1	N/A	N/A	2.28	2.16
47	Spiked 2	N/A	N/A	3.45	3.32
48	Spiked 3	N/A	N/A	4.70	4.80
49	Spiked 4	N/A	N/A	6.13	6.10
50	11686231	F	37	3.3	3.22
51	11686251	M	65	1.83	1.81
52	11671905	F	46	2.3	1.8
53	11686224	M	47	2.5	2.2
54	11686258	M	70	2.9	2.7
55	11686228	F	70	4.6	4.49

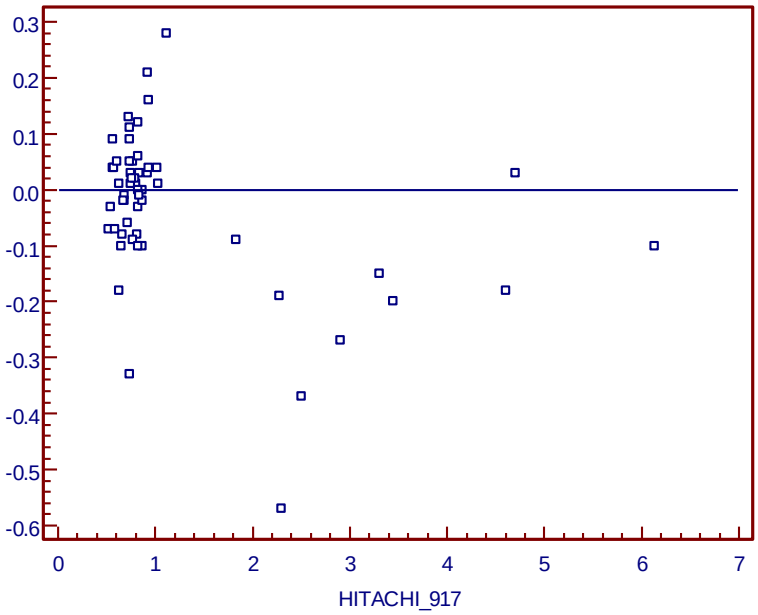
Cystatin C concentrations in whole blood obtained with Diazyme Cystatin C POC Test were plotted against Cystatin C concentrations in corresponding plasma obtained with predicate assay on Hitachi 917.

Regression results are summarized in the following table:



Passing & Bablok regression is shown below:





Variable X : HITACHI_917
Variable Y : SMART_POC

Sample size = 55

HITACHI_917
Lowest value = 0.5200
Highest value = 6.1300
Arithmetic mean = 1.2431
Median = 0.8100
Standard deviation = 1.1722
Standard error of the mean = 0.1581

SMART_POC
Lowest value = 0.4800
Highest value = 6.1000
Arithmetic mean = 1.2811
Median = 0.8900
Standard deviation = 1.1252
Standard error of the mean = 0.1517

-- REGRESSION EQUATION ----------

Y = 0.0700 + 1.0000 X

Intercept A : 0.0700
95% CI : -0.0471 to 0.1130

Slope B : 1.0000
95% CI : 0.9506 to 1.1429

Cusum test for linearity
Significant deviation from linearity (P<0.05)

Cusum test for linearity
No significant deviation from linearity (P>0.10)

Summary

N	55
Slope	0.9535
Intercept	0.0958
R^2	0.9867

Conclusion: Method Comparison data showed good correlation and met the method comparison acceptance criteria.

(2) External method comparison

Protocol: Clinical and Laboratory Standards Institute **EP9-A2** - Method Comparison and Bias Estimation Using Patient Samples: Approved Guideline-Second Edition (2002)

Acceptance Criteria: Slope = 0.90~1.10; $r^2 \geq 0.95$

Method comparison was also performed at three (3) POL sites by total of three (3) intended users. Sixty (60) paired human whole blood-plasma samples (venous whole blood and plasma from the same individual) were tested for comparison blood samples are used for the external method comparison at three POL sites. At each site 20 whole blood samples were tested using SMART analyzers. The corresponding sixty (60) plasma specimens were tested on Hitachi 917 with predicate device (k093680) at Diazyme Laboratories. The paired human whole blood-plasma samples used for this study were from a certified commercial source, ProMedDx, LLC and came with an IRB certification that protocols, informed consent, used to collect samples were IRB approved.

The test results are listed below:

Whole blood samples on SMART vs. paired plasma Hitachi 917

Sample ID	Gender	Age	Paired Plasma [Cys C] on Hitachi 917	Paired Whole blood [Cys C] on SMART
Site 1				

11661098	F	41	0.80	0.89
11661097	M	64	1.00	1.07
11661099	M	41	0.67	0.86
11661100	F	30	0.61	0.80
11661101	M	37	0.84	0.94
11661102	M	21	0.81	0.89
11661103	M	36	0.90	0.95
11661104	M	23	0.72	0.85
11661105	M	24	0.75	0.56
11661106	F	50	0.90	0.94
11661107	M	34	0.96	0.96
11661108	M	60	1.48	1.95
11661109	M	48	0.76	0.66
11661110	M	26	0.60	0.58
11661111	M	47	0.81	0.70
11661112	F	46	0.88	1.09
11661113	M	29	0.75	0.86
11661114	M	23	0.70	0.93
11661155*	N/A	N/A	5.03	5.49
11661156*	N/A	N/A	5.64	5.54
Site 2				
11661150	M	27	1.06	0.90
11661136	F	47	0.67	0.69
11661135	M	27	0.82	0.66
11661134	F	27	0.51	0.71
11661133	M	22	0.82	0.66
11661152*	N/A	N/A	4.18	4.17
11661141	M	53	0.78	0.83
11661137	M	39	0.83	0.76
11661146	F	21	0.64	0.90
11661157*	N/A	N/A	7.71	6.81
11661139	M	57	0.92	0.89
11661138	F	33	0.64	0.65
11661145	F	53	0.87	0.75
11661142	M	30	0.76	0.77
11661144	M	30	1.09	1.11
11661143	F	58	1.35	1.36
11661148	M	34	0.72	0.77
11661149	F	34	0.84	1.04
11661147	M	32	0.95	0.86
11661140	F	49	1.00	0.81
Site 3				
11661154	M	54	7.07	6.75
11661132	F	48	0.90	0.91
11661131	M	40	0.69	0.81

11661130	M	39	1.08	1.21
11661127	M	25	0.81	0.83
11661129	M	24	0.97	0.96
11661128	M	25	0.67	0.69
11661126	F	42	0.75	0.77
11661125	M	32	1.04	0.93
11661120	F	62	1.32	1.49
11661124	M	30	0.78	0.78
11661121	M	47	0.80	0.82
11661122	M	26	0.83	0.79
11661123	M	26	1.10	1.11
11661115	M	53	1.00	1.01
11661116	M	38	0.70	0.80
11661117	M	51	0.67	0.79
11661118	M	38	1.01	1.01
11661119	F	33	0.99	0.95
11661153*	N/A	N/A	5.63	5.71

*: Cystatin C spiked samples

Additional 20 individual samples were tested at each POL site. The paired human whole blood-plasma samples (a tube of whole blood and a tube of plasma from the same individual) used for this study are from a certified commercial source, ProMedDx, LLC with an IRB certification that informed consent and protocols used to collect samples are IRB approved.

The test results are listed below:
Whole blood samples on SMART vs paired plasma Hitachi 917

Sample ID	Gender	Age	Paired Plasma [Cys C] on Hitachi 917	Paired Whole blood [Cys C] on SMART
Site 1				
11689708	M	37	0.62	0.77
11689709	M	36	0.69	0.73
11689710	M	31	0.62	0.86
11689711	M	58	0.71	0.88
11689712	M	34	0.68	0.71
11689713	M	27	0.79	1.08
11689714	M	48	0.83	0.96
11689715*	N/A	N/A	6.72	6.66
11689716	M	29	0.72	0.85
11689717	M	42	0.59	0.59

11691887	M	52	0.83	0.99
11691888	M	34	0.84	0.91
11691889	M	46	0.72	0.84
11691890	M	21	0.85	1.11
11691892	M	51	0.65	0.81
11691893	M	55	0.66	0.65
11691895	M	52	0.63	0.74
11691896	F	43	0.58	0.62
11686224	M	47	2.5	2.69
11671905			2.3	2.25
Site 2				
11689698	M	23	0.67	0.57
11689700	M	56	0.64	0.57
11689701	M	25	0.67	0.59
11689702	M	33	0.76	0.72
11689703	M	49	0.74	0.65
11689704	M	54	0.75	0.83
11689706	M	46	1.07	1.05
11689707	M	27	0.67	0.67
11691897	F	41	0.72	0.84
11691898	M	50	0.72	0.78
11691899	F	25	0.62	0.57
11691900	M	27	0.66	0.6
11691901	M	34	0.85	0.88
11691902	M	51	0.72	0.72
11691903	M	29	0.86	0.86
11691904	M	28	0.68	0.58
11691905	M	24	0.83	0.94
11691906	M	32	0.88	0.77
11686258	M	70	2.9	2.95
11686228	F	70	4.6	4.27
Site 3				
11689718	M	29	0.79	0.69
11689719	F	33	0.77	0.69
11689720	M	44	0.7	0.65
11689721	M	52	0.77	0.7
11689722	M	26	0.87	0.74
11689723	M	26	0.62	0.71
11689724	F	31	0.74	0.72
11689726	M	39	0.78	0.78
11689727	M	23	0.65	0.65

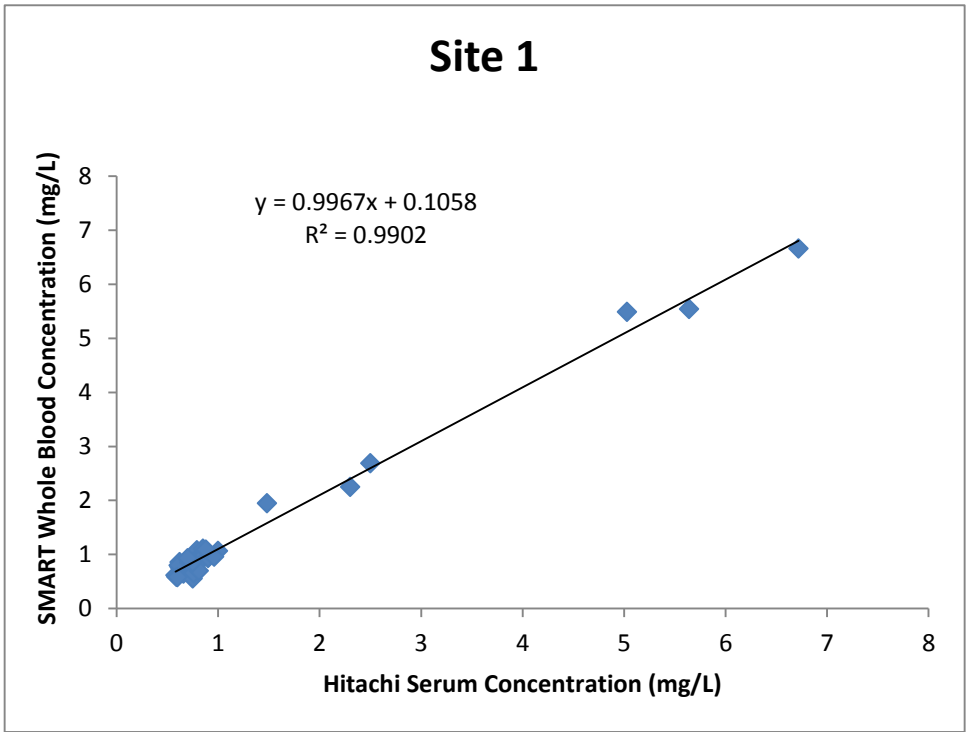
11691907	M	51	0.73	0.7
11691908	M	40	0.53	0.52
11691909	M	56	0.91	0.9
11691910	M	57	0.77	0.61
11691912	M	54	0.7	0.79
11691913	M	45	0.94	0.93
11691914	M	41	0.69	0.79
11691915	M	28	0.57	0.58
11691916	M	23	0.74	0.85
11686231	F	37	3.3	2.9
11686251	M	65	1.83	1.79

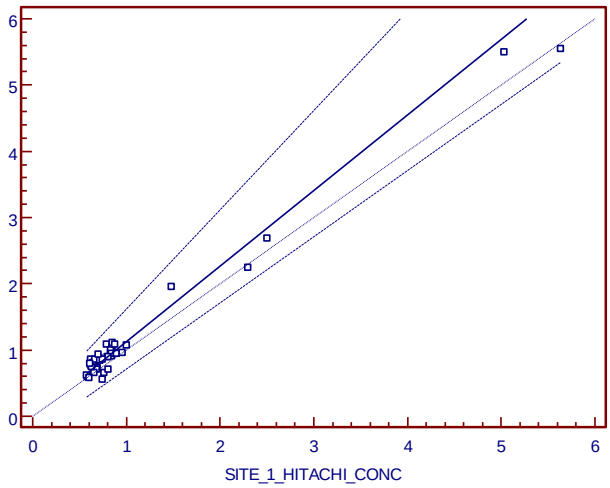
* Denotes Spiked Sample

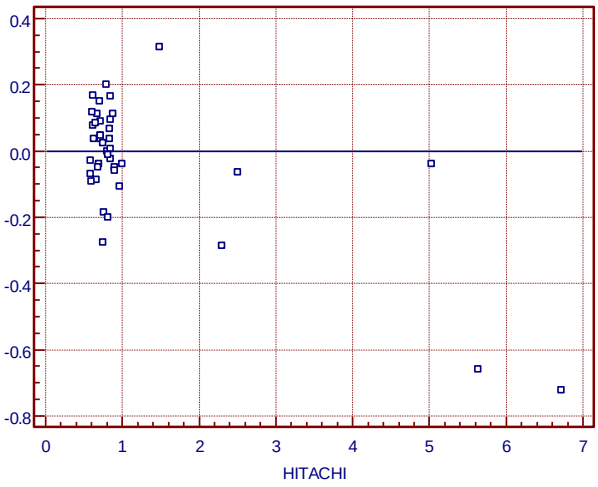
One hundred and twenty (120) serum specimens and 120 whole blood samples (including 6 natural samples above 2.0 mg/L) were used for external method comparison at three POL sites. Each site ran 40 whole blood samples using SMART analyzers. The corresponding plasma specimens were tested on Hitachi 917 with predicate device (k093680) at Diazyme Laboratories.

CYS C concentrations obtained with Diazyme Cys C SMART at each POL site are plotted against that obtained with Diazyme Enzymatic Cys C on Hitachi 917. The results are shown below:

Site 1 results







Variable X : SITE_1_HITACHI_CONC
Variable Y : SITE_1_SMART_CONC

Sample size = 40

SITE_1_HITACHI_CONC
Lowest value = 0.5800
Highest value = 5.6400
Arithmetic mean = 1.0818
Median = 0.7750
Standard deviation = 1.0651
Standard error of the mean = 0.1684

SITE_1_SMART_CONC
Lowest value = 0.5600
Highest value = 5.5400
Arithmetic mean = 1.1898
Median = 0.8850

Standard deviation = 1.0889
Standard error of the mean = 0.1722

-- REGRESSION EQUATION ----------

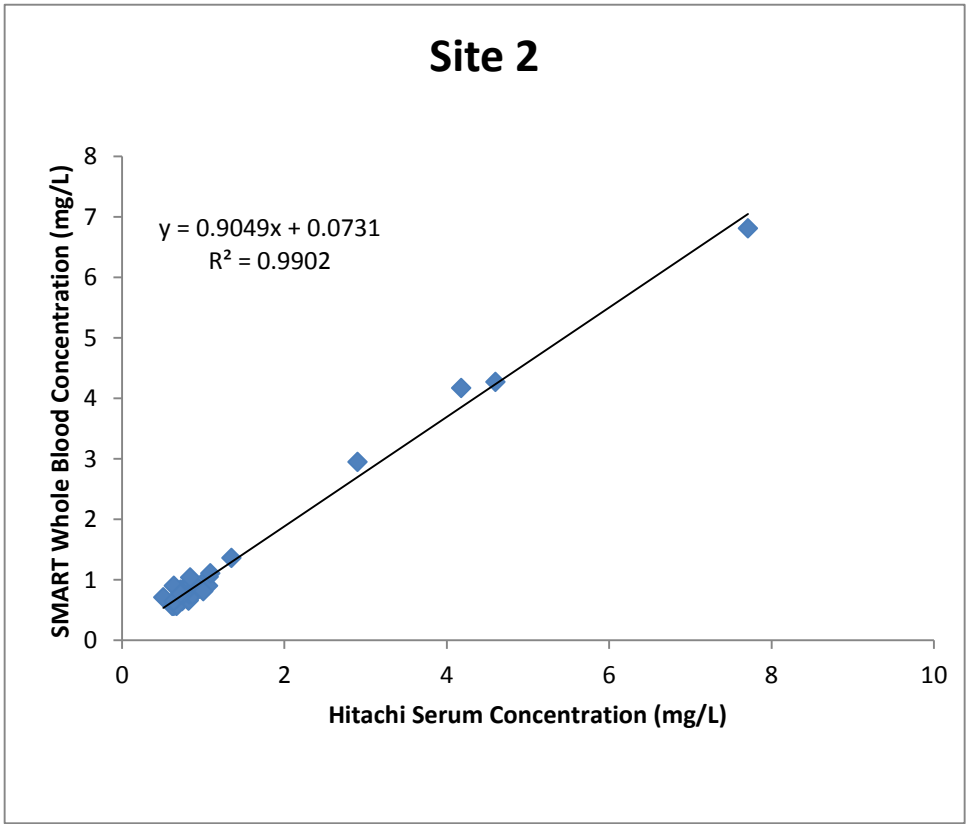
Y = -0.0221 + 1.1429 X

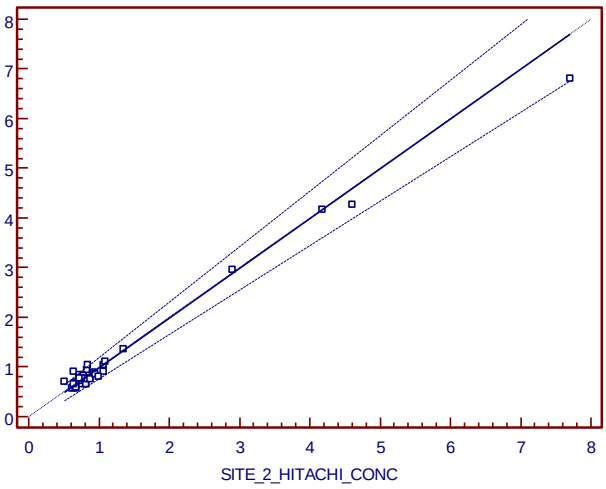
Intercept A : -0.0221
95% CI : -0.2900 to 0.1100

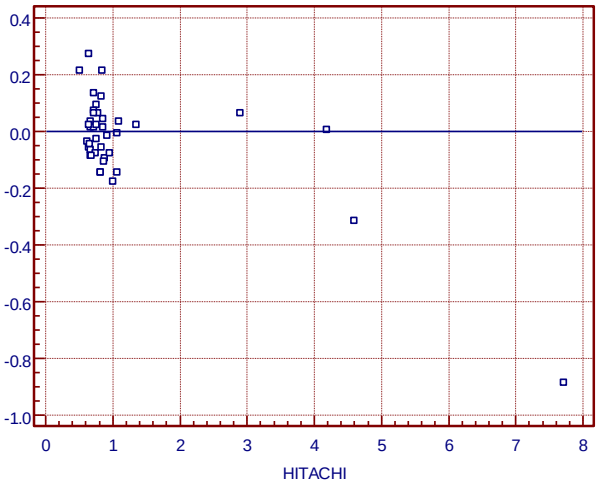
Slope B : 1.1429
95% CI : 1.0000 to 1.5000

Cusum test for linearity
No significant deviation from linearity (P>0.05)

Site 2 results:







Variable X : SITE_2_HITACHI_CONC
Variable Y : SITE_2_SMART_CONC

Sample size = 40

SITE_2_HITACHI_CONC
Lowest value = 0.5100
Highest value = 7.7100
Arithmetic mean = 1.2043
Median = 0.8000
Standard deviation = 1.3629
Standard error of the mean = 0.2155

SITE_2_SMART_CONC
Lowest value = 0.5700
Highest value = 6.8100
Arithmetic mean = 1.1628
Median = 0.7750
Standard deviation = 1.2394

Standard error of the mean = 0.1960

-- REGRESSION EQUATION ----------

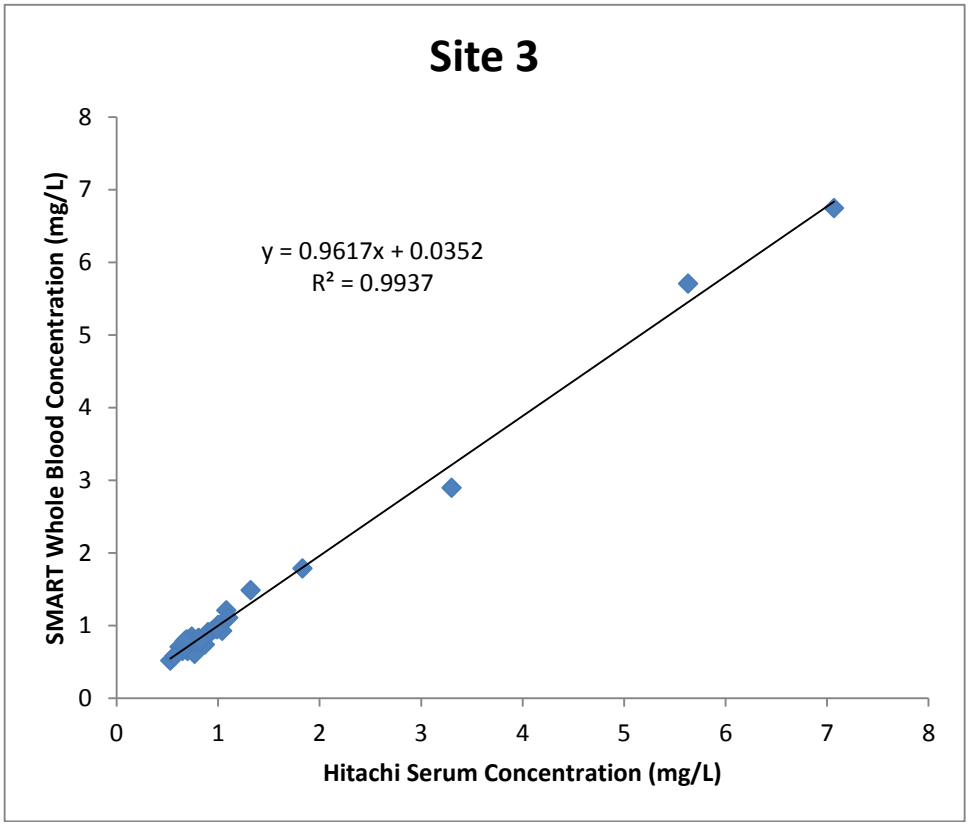
Y = -0.0150 + 1.0000 X

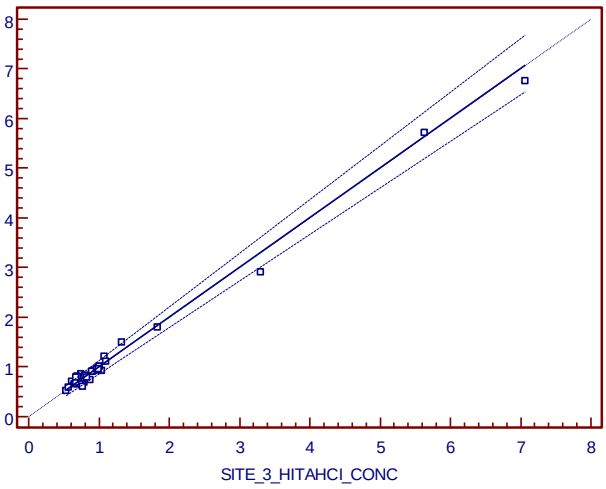
Intercept A : -0.0150
95% CI : -0.1362 to 0.0766

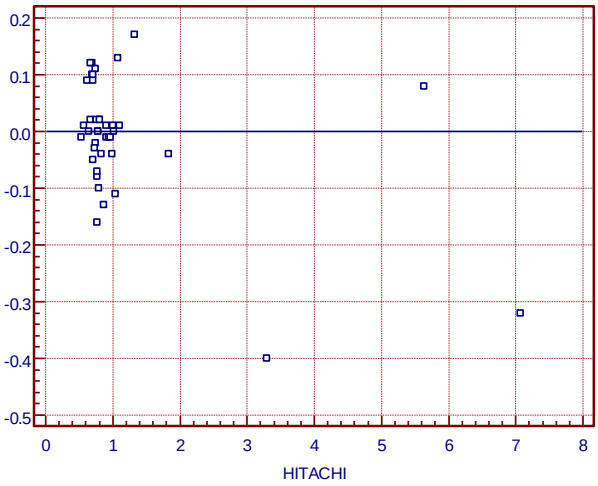
Slope B : 1.0000
95% CI : 0.8947 to 1.1154

Cusum test for linearity
No significant deviation from linearity (P>0.10)

Site 3 results:







Variable X : SITE_3_HITAHCI_CONC
Variable Y : SITE_3_SMART_CONC

Sample size = 40

SITE_3_HITAHCI_CONC
Lowest value = 0.5300
Highest value = 7.0700
Arithmetic mean = 1.1803
Median = 0.7850
Standard deviation = 1.2925
Standard error of the mean = 0.2044

SITE_3_SMART_CONC
Lowest value = 0.5200
Highest value = 6.7500
Arithmetic mean = 1.1702
Median = 0.7950
Standard deviation = 1.2469

Standard error of the mean = 0.1972

-- REGRESSION EQUATION ----------

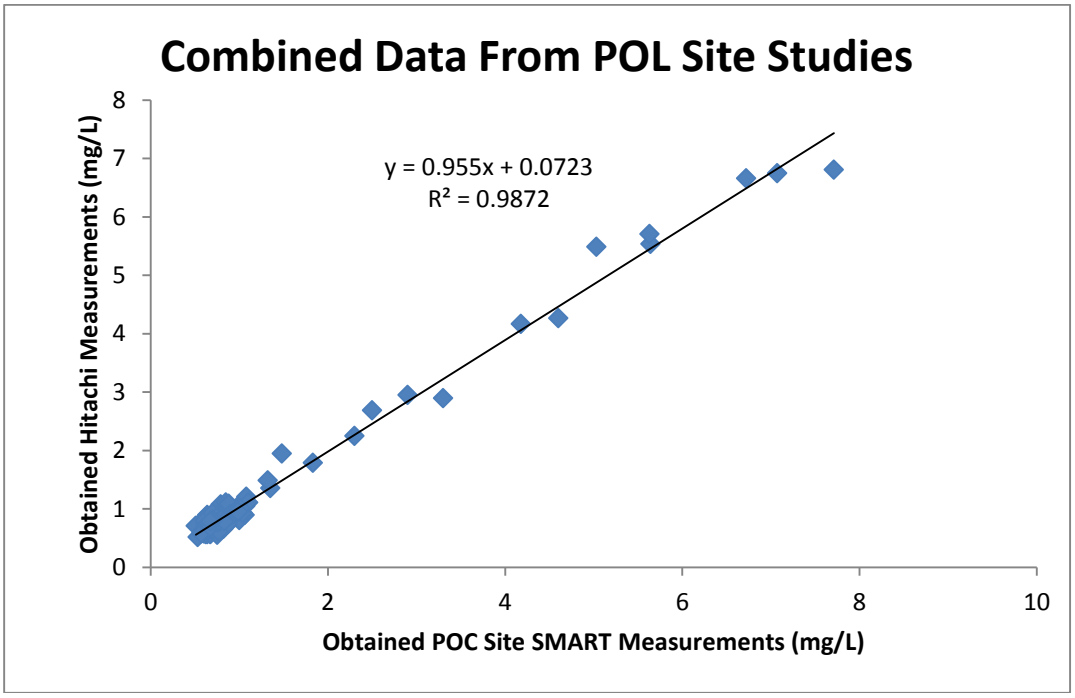
Y = 0.0000 + 1.0000 X

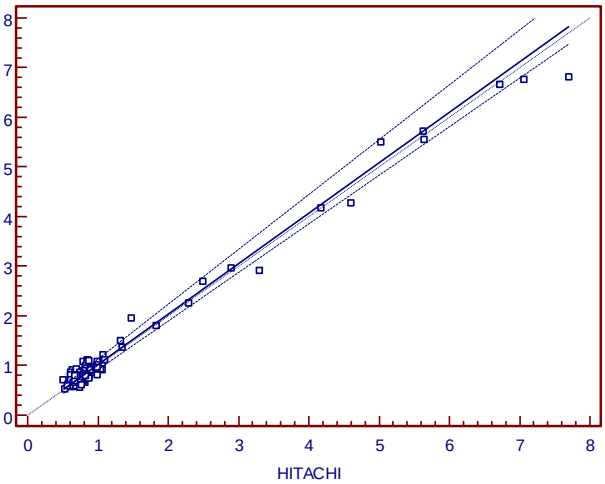
Intercept A : 0.0000
95% CI : -0.0708 to 0.0590

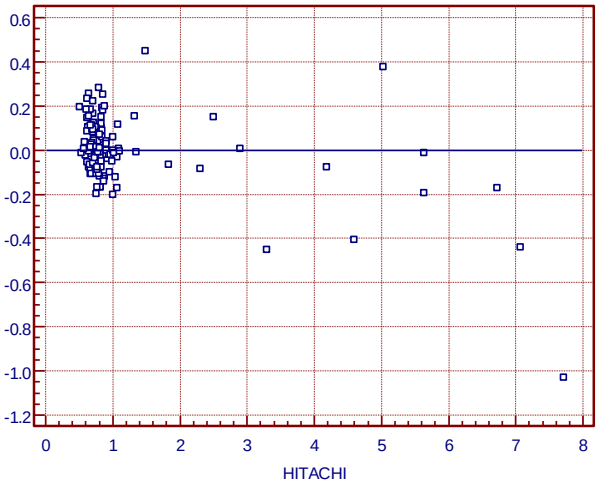
Slope B : 1.0000
95% CI : 0.9342 to 1.0769

Cusum test for linearity
No significant deviation from linearity (P>0.10)

All POL Sites Combined Method Comparison







Variable X : HITACHI
Variable Y : SMART

Sample size = 120

HITACHI
Lowest value = 0.5100
Highest value = 7.7100
Arithmetic mean = 1.2014
Median = 0.7900
Standard deviation = 1.3319
Standard error of the mean = 0.1211

SMART
Lowest value = 0.5200
Highest value = 6.8100

Arithmetic mean = 1.2196
Median = 0.8300
Standard deviation = 1.2801
Standard error of the mean = 0.1164

-- REGRESSION EQUATION ----------

Y = -0.0059 + 1.0176 X

Intercept A : -0.0059
95% CI : -0.0690 to 0.0338

Slope B : 1.0176
95% CI : 0.9794 to 1.1036

Cusum test for linearity
Significant deviation from linearity (P<0.05)

Regression results are summarized in the following table:

Regression analysis Summary

	Site 1	Site 2	Site 3	All 3 sites
N	40	40	40	120
Slope	0.9967	0.9049	0.9617	0.955
Intercept	0.1058	0.0731	0.0352	0.0723
R^2	0.9902	0.9902	0.9937	0.9872

Conclusion: Method Comparison data showed good correlation and met the method comparison acceptance criteria.

3. Expected values/ Reference range:

To verify the transferability of the reference interval from the predicate device, whole blood samples from 126 apparently healthy adults with age of 19-63 were tested using the Diazyme Cystatin C SMART assay according to CLSI C28-A3 guideline. The individual patient whole blood samples used for this study were from a certified commercial source, ProMedDx, LLC and came with an IRB certification.

The results are summarized below

Reagent Lot: SP00911-1

Sample ID	Gender	Age	Conc (mg/L)
11708690	M	59	0.91
11708691	F	25	1.06

11708692	M	24	1.11
11708693	M	23	0.96
11708694	M	26	0.95
11708696	M	29	0.69
11708697	M	53	0.76
11708699	M	54	0.56
11708700	M	23	0.89
11708701	M	44	0.66
11708702	M	57	0.82
11708703	F	26	0.75
11708704	M	32	0.98
11708705	M	55	1.05
11708706	M	22	0.92
11708707	M	23	0.57
11708708	M	43	0.82
11708709	F	31	0.87
11708710	M	22	0.87
11708711	M	45	0.99
11708712	M	24	0.89
11708713	M	41	0.68
11708714	M	43	0.88
11708715	M	21	0.85
11708716	M	63	0.58
11708718	M	42	1.13
11708719	M	36	0.92
11708720	M	27	0.65
11708721	M	26	0.79
11708722	M	22	0.48
11708723	M	22	0.68
11708725	M	33	0.62
11708726	M	47	0.96
11708727	M	45	0.67
11708728	M	41	0.78
11708729	M	26	0.97
11708730	F	53	0.88
11708731	M	28	0.63
11708732	M	29	0.61
11708733	M	34	0.58
11708735	M	48	0.58
11708736	M	48	0.87
11708737	F	32	0.98

11708738	M	46	0.61
11708739	M	48	0.83
11708741	M	24	0.80
11708742	M	39	0.60
11708743	M	32	0.66
11708744	M	28	0.52
11708745	M	60	1.03
11708746	M	25	1.03
11708747	M	54	0.94
11708748	M	58	0.68
11708749	M	26	0.64
11708750	M	35	0.63
11708751	M	41	0.91
11708752	M	36	0.58
11708753	F	54	0.93
11708754	M	52	0.70
11708755	M	30	0.90
11708756	F	34	1.06
11708757	M	25	0.78
11708758	M	26	0.59
11708759	M	43	1.20
11708760	M	22	0.55
11708761	M	49	0.94
11708762	M	31	0.97
11708763	M	56	0.87
11708764	M	44	0.82
11708765	M	47	0.49
11708766	M	27	0.78
11708767	M	43	0.68
11708768	M	36	0.64
11708769	M	27	0.63
11714649	F	35	0.53
11714650	M	39	0.65
11714651	M	40	0.83
11714652	F	34	0.63
11714653	M	40	0.64
11714654	F	27	0.76
11714655	M	35	0.77
11714656	M	49	0.60
11714657	M	56	0.69
11714658	M	50	0.67

11714659	M	22	0.62
11714660	M	20	0.77
11714661	F	21	0.58
11714662	M	45	0.51
11714663	M	61	0.57
11714664	F	27	0.91
11714665	F	22	0.38
11714666	M	41	0.50
11714667	M	45	0.65
11714668	M	32	0.67
11714669	M	34	0.60
11714670	M	56	0.85
11714671	M	22	0.86
11714672	M	41	0.68
11714673	M	29	0.67
11714674	M	19	0.53
11714675	M	52	0.62
11714676	M	25	0.58
11714677	M	22	0.53
11714678	F	22	0.87
11714679	M	35	0.54
11714680	M	24	0.58
11714681	M	22	0.32
11714682	M	24	0.98
11714683	F	46	0.64
11714684	M	28	0.91
11714685	M	52	0.73
11714686	M	25	0.73
11714687	M	30	0.57
11714688	M	39	0.46
11714689	M	49	0.63
11714690	M	36	0.56
11714692	M	29	0.76
11714693	M	54	0.95
11714695	M	53	0.46
11714696	M	26	0.70
11714697	M	58	0.58
11714698	M	40	0.55
11708698	M	36	0.65
11708717	F	34	0.62
11708724	M	27	0.8

11708734	M	61	0.96

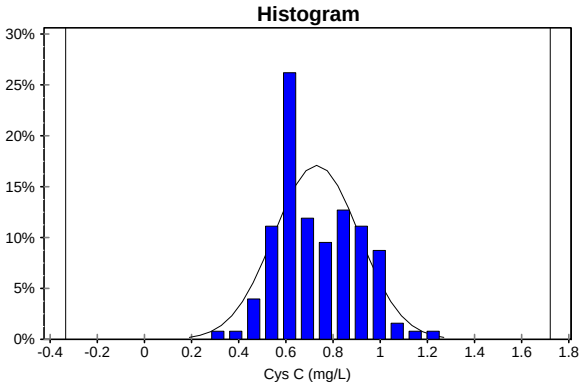
EP Evaluator 8 Software was used to establish the reference interval. The analysis is shown below.

Reference Interval Estimation: Combined

Central 95% Interval
(N = 126)

	Lower		Upper		Confidence
	Value	*90% CI*	*Value*	*90% CI*	*Ratio*
Nonparametric (CLSI C28-A)	**0.46**	**0.32 to 0.50**	**1.06**	**0.99 to 1.20**	**0.33**
Alternatives:					
Transformed Parametric	0.43	0.41 to 0.46	1.11	1.06 to 1.18	0.13
Parametric	0.39	0.35 to 0.44	1.07	1.03 to 1.11	0.13

Confidence Limits for Nonparametric CLSI C-28A method computed from C28-A Table 8.

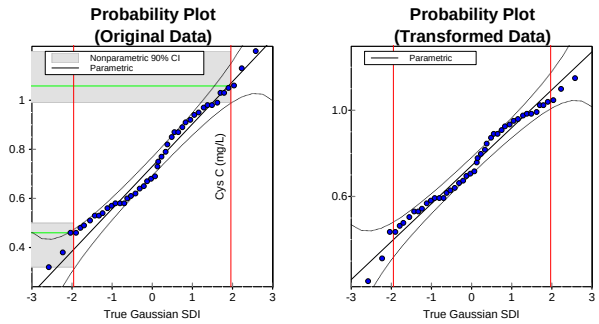


Selection Criteria:

Bounds	None
Filter	None

Statistics:

Mean	0.730 mg/L
SD	0.173
Median	0.680
Range	0.32 to 1.20
N	126 of 128
Distinct values	55
Zeroes	0
Central 95% Index	3.2 to 123.8
Analyst	Tech
Expt. Date	01 Mar 2012



Normalizing Transformation

Exponent	0.25
Constant	0.00

The expected normal range is 0.46 to 1.06 mg/L in 95% of the population tested.