Creatine Kinase MB (CK-MB) Assay Development Report

Theranos Inc.

Nov 18, 2011

Prepared by: Xiaoyan Du

TABLE OF CONTENTS

[TOC \o "1-3" \h \z \u]**LIST OF TABLES**

[TOC \h \z \c "Table"]

LIST OF FIGURES

1. ASSAY INFORMATION[TC "ASSAY INFORMATION" \F C \L "2"]

1.1 Assay Specifications

During a heart attack (or myocardial infarction), damaged heart tissue releases cardiac enzymes such as creatine kinase (CK). CK is found in the heart and skeletal muscles as well as in the liver and brain. CK-MB is a subtype of CK with a molecular mass of approximately 87,000 daltons that is found only in heart muscle. Low levels of the enzyme, < 5 ng/mL, are present in the blood stream of normal subjects. In acute myocardial infarction (AMI), plasma levels typically rise as early as 3-4 hours after the onset of symptoms, peaks within 9 to 30 hours, and remains elevated for approximately 65 hours post infarct. The pattern of serial determinations is more informative than a single determination.

This assay is designed to detect CK-MB in human whole blood, plasma and serum. The assay has a reportable range of 2.5 to 500 ng/mL, and is calibrated to the native CK-MB provided by Fitzgerald (Cat#30-AC65). This assay has no cross-reactivity with troponin T, CK-MM, CK-BB and myoglobin. Lipemic and rheumatoid factor (RF) positive serum, but not icteric and hemolyzed serum may dampen the measurement about 20-30% due to matrix effects.

1.1.1 Reference Assays [TC "Reference Assays and Standards" \f C \l "3"]

The following commercial ELISA kits have been used in house as predicate methods:

- DRG CK-MB ELISA (Cat # EIA-4361) range from 2-100 ng/mL
- Signosis CK-MB ELISA (Cat # EA-0304) range from 7.5-200 ng/mL

1.1.2 Materials and Methods for MTP Screening[TC "Materials and Methods" \f C \l "1"]

CK-MB assay is designed as a sandwich ELISA. A biotin-labeled anti-CK-MB antibody is coated on an avidin surface and serves as the capture surface. The sample (whole blood, plasma or serum) is diluted and then incubated on the capture surface for 10 minutes. An alkaline phosphatase-labeled anti-CK-MB antibody is incubated on the surface as detection antibody for 10 minutes. Then the surface is washed and the alkaline phosphatase substrate is incubated on the surface for 10 minutes. The resulting chemiluminescence is read in Relative Light Units (RLU).

Table [SEQ Table * ARABIC]: Materials

Name	Supplier	Catalog #
Human recombinant CK-MB	Fitzgerald	30R-AC074
Human native CK-MB	Fitzgerald	30-AC65
Mouse Anti-Human CK-MB Antibody (CAb)	Lifespan biosciences	LS-C14666
Mouse Anti-Human CK-MB Antibody (DAb)	Lifespan biosciences	LS-C27181
Alkaline Phosphatase Labeling Kit (SH)	Dojindo	LK13
Biotin Labeling Kit (SH)	Dojindo	LK10
Phospho Glo Substrate	KPL	55-60-04
Blocking Buffer (3% BSA in TBS, 0.05% Sodium Azide)	Sigma (BSA, Fraction V, 99% Pure)	A3059-500G

Carbonate-bicarbonate buffer	Sigma	C3041

2. ASSAY DEVELOPMENT
[TC "ASSAY OPTIMIZATION" \F C \L "2"]

2.1 Antibody Screening (MTP)[TC "Detection Antibody Conjugate Verification" \f C \l "1"]

To determine the optimal pair for the CK-MB ELISA, all combinations of 12 CK-MB antibodies were tested on a microtitre plate (MTP). The screening was performed with using Fitzgerald recombinant CK-MB (Cat#30R-AC074) diluted in assay buffer, 10 ug/ml of CAb and 100 ng/mL of detection antibody in blocking buffer.

Table [SEQ Table * ARABIC]: Antibody Information

	Vendor	Cat#	Clone#	Other
1	CalBiorcagents	M179	unknown	Mouse Monoclonal anti-human CK-MB
2	CalBioreagents	M180	unknown	Mouse monoclonal
4	CalBioreagents	M182	unknown	Mouse Monoclonal anti-human CK-MB
5	CalBioreagents	P195	poly	Goat poly anti-human CK-MB
6	Fitzgerald Industries	10-C40A	M9910207	Mouse monoclonal; No cross reactivity with CK-BB or CK-MM in ELISA
7	Fitzgerald Industries	10-C40B	M9910208	Mouse monoclonal; No cross reactivity with CK-BB or CK-MM in ELISA
8	Fitzgerald Industries	10R-C146B	CKMB59	Mouse monoclonal, purification method unknown
10	Fitzgerald Industries	10C-CR3013M	950430	Mouse monoclonal
11	LifeSpan BioScience,	LS-C14666	unknown	Mouse monoclonal; Protein G Column purification
12	LifeSpan BioScience,	LS-C27181	1302 (1F2/1)	Mouse monoclonal
14	BiosPacific	CK-MB 7502 (	7502-100086	Mouse monoclonal
15	Acris Antibodies	AM00686PU-N	BDI937	Mouse (IgG1) monoclonal

Table [SEQ Table * ARABIC]: Summary of Antibody Screen Results

MTP	Dab1	Dab2	Dab4	Dab5	Dab6	Dab7	Dab8	Dab10	Dab11	Dab12	Dab14	Dab15
Cab1												
Cab2												
Cab4												
Cab5												
Cab6												
Cab7												
Cab8												
Cab10												
Cab11												
Cab12												
Cab14												
Cab15												

Legend:

Great responses	Fair responses	No responses

Table [SEQ Table * ARABIC]: Summary of Best Pairs (MTP)

CK-MB ng/mL	C1/D2					C1/D4					C2/D12				
	RLU1	RLU2	Mean	CV	Modulation	RLU1	RLU2	Mean	CV	Modulation	RLU1	RLU2	Mean	CV	Modulation
200	413041	401662	407351	2	503	241498	251394	246446	3	587	417037	422583	419810	1	233
20	41167	43479	42323	4	52	16023	17210	16616	5	40	53219	53391	53305	0	30
2	3682	3464	3573	4	4	1820	1492	1656	14	4	7963	6661	7312	13	4
0	1086	535	810	48	1	488	351	420	23	1	1979	1629	1804	14	1
	C2/D1					C1/D7					C10/D12				
	RLU1	RLU2	Mean	CV	Modulation	RLU1	RLU2	Mean	CV	Modulation	RLU1	RLU2	Mean	CV	Modulation
200	439459	447906	443682	1	1228	782476	770061	776269	1	602	503135	512117	507626	1	167
20	22899	23110	23005	1	64	68151	69819	68985	2	54	70061	72298	71180	2	23
2	1656	1453	1554	9	4	6845	6766	6806	1	5	9359	9443	9401	1	3
0	406	316	361	18	1	1484	1094	1289	21	1	3558	2506	3032	25	1
	C4/D1					C4/D12					C11/D12				
	RLU1	RLU2	Mean	CV	Modulation	RLU1	RLU2	Mean	CV	Modulation	RLU1	RLU2	Mean	CV	Modulation
200	373830	370940	372385	1	968	497452	533844	515648	5	205	562227	574622	568425	2	337
20	18334	17327	17831	4	46	59895	62841	61368	3	24	77026	77372	77199	0	46
2	1332	1011	1172	19	3	9341	8268	8805	9	4	9980	9926	9953	0	6
0	340	430	385	17	1	2653	2372	2513	8	1	1966	1411	1688	23	1
	C5/D2					C7/D12					C12/D4				
	RLU1	RLU2	Mean	CV	Modulation	RLU1	RLU2	Mean	CV	Modulation	RLU1	RLU2	Mean	CV	Modulation
200	1130495	1103655	1117075	2	420	417842	428462	423152	2	195	355064	359713	357388	1	490
20	472183	459914	466049	2	175	56463	55548	56005	1	26	36111	30874	33492	11	46
2	71022	69608	70315	1	26	8251	7788	8019	4	4	4326	3711	4019	11	6
0	2980	2335	2657	17	1	2155	2176	2165	1	1	836	622	729	21	1
	C6/D1					C8/D12					C12/D5				
	RLU1	RLU2	Mean	CV	Modulation	RLU1	RLU2	Mean	CV	Modulation	RLU1	RLU2	Mean	CV	Modulation
200	469606	478920	474263	1	1099	606470	644819	625645	4	260	1910379	1928641	1919510	1	107
20	25602	26972	26287	4	61	83339	85284	84312	2	35	658519	657877	658198	0	37
2	1675	1863	1769	7	4	9624	10661	10143	7	4	103983	106365	105174	2	6
0	531	332	432	33	1	2068	2744	2406	20	1	17750	18033	17891	1	1
	C7/D1					C7/D5					C5/D11				
	RLU1	RLU2	Mean	CV	Modulation	RLU1	RLU2	Mean	CV	Modulation	RLU1	RLU2	Mean	CV	Modulation
200	480064	484235	482149	1	885	1301430	1321740	1311585	1	124	1592823	1709073	1650948	5	323
20	23216	24680	23948	4	44	363968	385220	374594	4	35	634300	612578	623439	2	122
2	1644	1593	1619	2	3	54164	32244	43204	36	4	91293	92075	91684	1	18
0	656	433	545	29	1	10561	10561	10561	0	1	3858	6358	5108	35	1
	C8/D1					C5/D8					C12/D10				
	RLU1	RLU2	Mean	CV	Modulation	RLU1	RLU2	Mean	CV	Modulation	RLU1	RLU2	Mean	CV	Modulation
200	458330	450732	454531	1	1313	2421189	2532210	2476700	3	318	569871	596432	583151	3	1028
20	23163	21304	22234	6	64	1026341	1022191	1024266	0	131	89736	91599	90668	1	160
2	1583	1506	1544	4	4	172742	178146	175444	2	23	10179	10284	10232	1	18
0	335	358	346	5	1	7437	8147	7792	6	1	586	548	567	5	1
	C10/D1					C10/D5					C1/D15				
	RLU1	RLU2	Mean	CV	Modulation	RLU1	RLU2	Mean	CV	Modulation	RLU1	RLU2	Mean	CV	Modulation
200	419049	437598	428324	3	902	1910379	1928641	1919510	1	107	1081447	1152853	1117150	5	278
20	20996	20864	20930	0	44	658519	657877	658198	0	37	123996	137190	130593	7	33
2	1622	1681	1651	2	3	103983	106365	105174	2	6	14246	14718	14482	2	4
0	517	432	475	13	1	17750	18033	17891	1	1	3862	4173	4018	5	1
	C11/D1					C11/D5					C5/D14				
	RLU1	RLU2	Mean	CV	Modulation	RLU1	RLU2	Mean	CV	Modulation	RLU1	RLU2	Mean	CV	Modulation
200	422274	442881	432578	3	846	1894579	1941521	1918050	2	103	435253	430280	432766	1	219
20	21518	21230	21374	1	42	659228	667109	663169	1	35	65977	67569	66773	2	34
2	1774	1782	1778	0	3	110107	112471	111289	2	6	8339	9770	9054	11	5
0	556	467	512	12	1	18350	19055	18702	3	1	1926	2026	1976	4	1
	C12/D1					C12/D2					C15/D12				
	RLU1	RLU2	Mean	CV	Modulation	RLU1	RLU2	Mean	CV	Modulation	RLU1	RLU2	Mean	CV	Modulation
200	328804	342898	335851	3	267	649885	643641	646763	1	626	562227	574622	568425	2	337
20	12994	13239	13116	1	10	99244	93692	96468	4	93	77026	77372	77199	0	46
2	1828	2338	2083	17	2	14029	12200	13114	10	13	9980	9926	9953	0	6
0	938	1576	1257	36	1	1377	689	1033	47	1	1966	1411	1688	23	1
	C12/D8					C15/D5					C12/D11				
	RLU1	RLU2	Mean	CV	Modulation	RLU1	RLU2	Mean	CV	Modulation	RLU1	RLU2	Mean	CV	Modulation
200	657219	724356	690788	7	943	1894579	1941521	1918050	2	103	318748	352141	335445	7	518
20	120136	124807	122471	3	167	659228	667109	663169	1	35	55661	56629	56145	1	87
2	13215	13408	13312	1	18	110107	112471	111289	2	6	6654	7267	6961	6	11
0	791	675	733	11	1	18350	19055	18702	3	1	725	571	648	17	1
	C5/D15					C12/D5					C5/D10				
	RLU1	RLU2	Mean	CV	Modulation	RLU1	RLU2	Mean	CV	Modulation	RLU1	RLU2	Mean	CV	Modulation
200	2965148	3253164	3109156	7	358	2268333	2272732	2270532	0	130	2436503	2571193	2503848	4	58
20	1160421	1356224	1258323	11	145	759636	756219	757927	0	43	995595	1030109	1012852	2	23
2	195574	216870	206222	7	24	113586	113300	113443	0	6	197769	269609	233689	22	5
0	8532	8849	8691	3	1	17312	17602	17457	1	1	31314	55360	43337	39	1

2.2 Cross Reactivity and Interference (MTP)

Opiate family shares the closest structural. For example CK-MM and CK-BB. In addition, troponin and myoglobin are the antigens rising during heart attack. Therefore, the four above mentioned analytes were tested for cross reactivity and interference with CK-MB. Unacceptable interference was defined as greater than 120% or less than 80% of the controls. The candidate pairs with borderline or no cross reactivity/interference in the microtitre plate were chosen for the Theranos System screen.

The assay conditions were with DAb at 100 ng/mL in Blocking Buffer, CAb at 10 ug/mL. CK-MB calibrators were diluted in Blocking Buffer, with a 10-fold sample dilution.

In terms of interference test, CK-MB calibrators were diluted to assay buffer containing interfering substances at the doses indicated. If the recovery of CK-MB at each dose is within 80 -120%, it is considered as minimal interference. As to cross reactivity test, different doses of tested materials were directly detected by the antibody pairs. Each level of tested materials should be within 1% cross reactivity. Based on the interference and cross reactivity results (Table 5 and 6), antibody pairs C11/D12, C12/D2, C12/D10 and C7/D12 were advanced for the Theranos system screen.

Table 5: Interference (MTP) Results

Table 5A: Reagents for interference test

Related-analytes	Vendor	tested level; ng/ml
purified CK-MM	Biospacfic; Cat # 1-024	300 ng/ml
purified CK-BB	Lee; Cat # 191-25	100 ng/ml
Purified Troponin T	Abcam; Cat # ab9937	200 ng/ml
Purified Myoglobin	Abcam; Cat # ab77876	300 ng/ml

Table 5B: Interference results

Ab pairs	CK-MB	CK-MB only			CK-MM 300 ng/mL			CK-BB 100 ng/mL			TnT 300 ng/mL			Myoglobin 300 ng/mL		
C12/2	ng/mL	Mean	Re-cal	Recovery	Mean	Re-cal	Recovery	Mean	Re-cal	**Recovery**	Mean	Re-cal	Recovery	Mean	Re-cal	Recovery
	200	62922	202	101	59158	189.8	94.9	49250	157.0	**78.5**	58661	188.2	94.1	60805	195.3	97.7
	20	6705	20	102	5482	16.5	82.5	5696	17.2	**85.9**	6463	19.6	97.9	6734	20.4	102.1
	2	804	2	97	827	2.0	100.7	810	2.0	**98.0**	725	1.7	84.8	593	1.3	64.2
	0	210	0		494	1.0		297	0.4		273	0.3		320	0.4	
C12/D4	CK-MB	Mean	Re-cal	Recovery	Mean	Re-cal	Recovery	Mean	Re-cal	**Recovery**	Mean	Re-cal	Recovery	Mean	Re-cal	Recovery
	200	20716	199	100	21119	203.5	101.8	18100	173.1	**86.5**	20939	201.7	100.9	22823	220.9	110.4
	20	2340	20	100	2221	18.9	94.7	2175	18.5	**92.6**	2550	22.0	110.2	2544	22.0	109.9
	2	430	2	110	432	2.2	110.8	390	1.8	**91.4**	377	1.7	85.2	403	2.0	97.5
	0	178	0		174	-0.2		259	0.6		278	0.8		244	0.5	
C12/D5	CK-MB	Mean	Re-cal	Recovery	Mean	Re-cal	Recovery	Mean	Re-cal	**Recovery**	Mean	Re-cal	Recovery	Mean	Re-cal	Recovery
	200	113297	203.7	101.9	117668	213.9	106.9	102580	179.4	**89.7**	119886	219.1	109.5	130982	245.7	122.9
	20	18075	20.4	101.8	16470	17.9	89.4	15197	17.5	**87.3**	19504	22.6	112.9	21854	26.2	131.2
	2	5643	1.8	88.6	6190	2.6	128.2	6268	2.7	**133.8**	7057	3.8	191.2	6478	3.0	149.1
	0	4670	42.1		4311	-0.1		5170	1.1		5345	1.3		5143	2.5	
C12/D8	CK-MB	Mean	Re-cal	Recovery	Mean	Re-cal	Recovery	Mean	Re-cal	**Recovery**	Mean	Re-cal	Recovery	Mean	Re-cal	Recovery
	200	77813	197.8	99	73941	187.3	94	65098	163.6	**82**	66450	167.2	84	65244	166.7	83
	20	8628	19.8	99	8045	18.3	92	7331	16.6	**83**	7499	17.0	85	7268	16.4	82
	2	1377	2.2	110	1188	1.7	87	1097	1.5	**77**	1044	1.4	70	806	0.8	42
	0	371	-0.2		513	0.1		405	-0.1		537	0.2		596	0.3	
C12/D10	CK-MB	Mean	Re-cal	Recovery	Mean	Re-cal	Recovery	Mean	Re-cal	**Recovery**	Mean	Re-cal	Recovery	Mean	Re-cal	Recovery
	200	58282	198.6	99	57501	195.7	98	54249	183.9	**92**	56440	191.8	96	60511	206.8	103
	20	6663	20.0	100	6655	20.0	100	6009	17.9	**89**	6421	19.2	95	6771	20.3	102
	2	972	1.6	80	1080	1.9	97	1091	2.0	**99**	1053	1.9	93	1196	2.3	116
	0	586	0.4		461	0.0		583	0.4		507	0.1		906	1.4	
C12/D11	CK-MB	Mean	Re-cal	Recovery	Mean	Re-cal	Recovery	Mean	Re-cal	**Recovery**	Mean	Re-cal	Recovery	Mean	Re-cal	Recovery
	200	33411	199.9	100	33954	203.6	102	31605	187.8	**94**	31319	199.3	100	34349	206.3	103
	20	4170	20.1	100	4061	19.4	97	4159	20.0	**100**	4028	19.3	96	4350	21.1	105
	2	895	2.0	102	825	1.6	82	898	2.1	**103**	788	1.5	73	906	2.1	105
	0	441	-0.4		668	0.8		817	1.6		488	-0.2		675	0.8	
C7/D12	CK-MB	Mean	Re-cal	Recovery	Mean	Re-cal	Recovery	Mean	Re-cal	**Recovery**	Mean	Re-cal	Recovery	Mean	Re-cal	Recovery
	200	35700	205.1	103	34099	194.7	97	34251	195.7	98	39572	230.7	115	35317	202.6	101
	20	4358	20.1	101	3803	17.2	86	3853	17.5	87	3969	18.1	90	4559	21.2	106
	2	794	1.6	80	730	1.3	63	810	1.7	84	904	2.2	108	1036	2.8	142
	0	561	0.4		418	-0.3		493	0.1		458	-0.1		572	0.5	
C8/D12	CK-MB	Mean	Re-cal	Recovery	Mean	Re-cal	Recovery	Mean	Re-cal	**Recovery**	Mean	Re-cal	Recovery	Mean	Re-cal	Recovery
	200	34768	195.9	98.0	36616	207.8	103.9	37106	211.0	**105.5**	37688	182.7	91.4	39769	228.4	114.2
	20	4313	19.9	99.3	4190	19.2	96.2	4212	19.3	**96.7**	4424	20.4	102.2	4795	22.4	111.8
	2	774	1.8	90.6	998	3.4	168.0	879	1.9	94.5	1134	**3.1**	**153.3**	1212	4.1	205.3
	0	442	0.1		619	1.0		661	1.7		568	0.9		628	1.1	
C2/D1	CK-MB	Mean	Re-cal	Recovery	Mean	Re-cal	Recovery	Mean	Re-cal	**Recovery**	Mean	Re-cal	Recovery	Mean	Re-cal	Recovery
	200	21974	201.6	101	21802	200.3	100.1	18760	176.7	**88.4**	15125	179.6	89.8	18442	174.2	87.1
	20	2281	20.1	100	2077	17.8	89.1	2059	17.6	**88.1**	2019	17.2	85.9	2252	19.7	98.7
	2	641	1.8	90	521	0.4	22.2	593	1.3	**62.6**	733	2.8	142.1	793	3.5	175.8
	0	500	0.2		386	-1.1		386	-1.1		596	1.3		760	3.1	
C4/D1	CK-MB	Mean	Re-cal	Recovery	Mean	Re-cal	Recovery	Mean	Re-cal	**Recovery**	Mean	Re-cal	Recovery	Mean	Re-cal	Recovery
	200	10147	224.8	112	10112	224.0	112	11036	246.1	123	10036	222.2	111	11546	258.4	129
	20	1234	20.4	102	1111	17.7	88	1204	19.7	99	1540	27.1	136	1728	31.3	156
	2	413			479			686			778			727		
	0	296	-0.2		452	3.2		483	3.9		396	2.0		748	9.7	
C11/D12	CK-MB	Mean	Re-cal	Recovery	Mean	Re-cal	Recovery	Mean	Re-cal	**Recovery**	Mean	Re-cal	Recovery	Mean	Re-cal	Recovery
	200	35322	200.2	100	31893	179.3	90	28744	160.4	80	29784	166.7	83	30135	168.8	84
	20	4237	20.0	100	4316	20.4	102	3695	17.0	85	3778	17.5	87	4172	19.7	98
	2	947	2.1	104	974	2.2	111	901	1.8	91	976	2.2	112	1023	2.5	124
	0	552	-0.1		567	0.0		590	0.1		637	0.4		783	1.2	
C15/D12	CK-MB	Mean	Re-cal	Recovery	Mean	Re-cal	Recovery	Mean	Re-cal	**Recovery**	Mean	Re-cal	Recovery	Mean	Re-cal	Recovery
	200	40252	199.3	100	37456	193.5	97	33047	160.2	80	34513	160.2	80	34595	174.1	87
	20	4903	20.0	100	4965	18.8	94	4779	20.4	102	4664	18.5	925	5082	21.7	109
	2	1079	1.5	76	1082	1.2	58	1304	3.4	169	1816	3.8	19	1982	6.4	319
	0	858	0.5		803	0.9		1669	8.6		1242	2.6		1646	4.8	

Table 6: Cross Reactivity (MTP) Results

Table 6A: Reagents for cross reactivity

Related-analytes	Vendor	tested range; ng/ml
purified CK-MM	Biospacfic; Cat # 1-024	0-300 ng/ml
purified CK-BB	Lee; Cat # 191-25	0-100 ng/ml
Purified Troponin T	Abcam; Cat # ab9937	0-200 ng/ml
Purified myoglobin	Abcam; Cat # ab77876	0-300 ng/ml

Table 6B: Cross-reactivity results

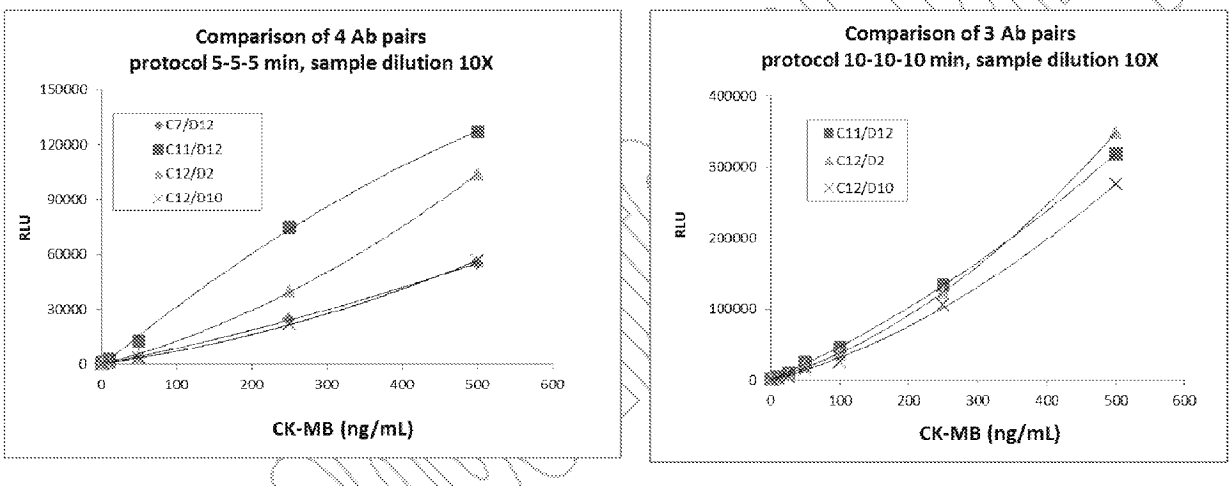
	ng/mL			ng/mL			ng/mL			ng/mL			ng/mL		
C12/D2	CK-MB	Mean	CV	CK-MM	Mean	CV	CK-BB	Mean	CV	TnT	Mean	CV	myoglobin	Mean	CV
	200	92700	4	300	782	8	100.0	908	25	200.0	762	10	300	706	9
	100	48410	1	100	782	6	50.0	760	21	100.0	737	4	100	935	7
	20	9841	6	30	786	27	25.0	892	47	50.0	850	6	30	628	19
	10	5516	3	10	661	7	12.5	741	6	25.0	737	17	10	684	3
	2	1766	17	3	618	4	6.3	696	25	12.5	611	21	3	714	20
	0	805	17	0	618	4	0.0	813	10	0.0	774	30	0	671	13
C12/D10	CK-MB	Mean	CV	CK-MM	Mean	CV	CK-BB	Mean	CV	TnT	Mean	CV	myoglobin	Mean	CV
	200	88810	1	300	632	9	100.0	782	11	200.0	793	21	300	879	11
	100	47467	0	100	712	1	50.0	719	15	100.0	883	16	100	812	15
	20	11017	4	30	747	16	25.0	634	16	50.0	717	6	30	778	21
	10	5946	5	10	908	3	12.5	821	28	25.0	865	28	10	837	17
	2	1937	6	3	939	5	6.3	1029	24	12.5	964	19	3	978	56
	0	795	17	0	962	4	0.0	786	19	0.0	972	16	0	820	2
C11/D12	CK-MB	Mean	CV	CK-MM	Mean	CV	CK-BB	Mean	CV	TnT	Mean	CV	myoglobin	Mean	CV
	200	61837	8	300	1751	15	100.0	895	3	200.0	885	10	300	1359	2
	100	31549	2	100	1412	62	50.0	1146	15	100.0	1037	16	100	1311	9
	20	6970	2	30	1057	49	25.0	893	14	50.0	837	6	30	1129	13
	10	4251	13	10	674	25	12.5	643	1	25.0	761	0	10	1098	17
	2	1427	16	3	1013	35	6.3	1001	5	12.5	1075	10	3	1061	27
	0	1020	5	0	711	24	0.0	1150	8	0.0	943	15	0	993	2
C7/D12	CK-MB	Mean	CV	CK-MM	Mean	CV	CK-BB	Mean	CV	TnT	Mean	CV	myoglobin	Mean	CV
	200	49972	2	300	444	7	100.0	560	17	200.0	525	1	300	607	1
	100	24577	0	100	434	10	50.0	463	5	100.0	469	15	100	494	8
	20	5382	2	30	489	25	25.0	397	12	50.0	330	7	30	502	40
	10	3265	5	10	440	34	12.5	304	10	25.0	318	9	10	442	12
	2	1022	3	3	446	9	6.3	335	4	12.5	471	13	3	531	20
	0	361	6	0	467	8	0.0	463	8	0.0	477	8	0	523	2
C8/D12	CK-MB	Mean	CV	CK-MM	Mean	CV	CK-BB	Mean	CV	TnT	Mean	CV	myoglobin	Mean	CV
	200	52097	1	300	523	6	100.0	603	4	200.0	644	2	300	634	15
	100	27892	0	100	527	5	50.0	760	1	100.0	739	22	100	597	15
	20	6576	10	30	520	13	25.0	514	41	50.0	712	27	30	578	6
	10	4112	3	10	712	37	12.5	636	8	25.0	717	10	10	535	9
	2	1262	1	3	673	14	6.3	690	19	12.5	669	2	3	648	5
	0	630	23	0	510	16	0.0	556	17	0.0	921	33	0	777	7
C2/D1	CK-MB	Mean	CV	CK-MM	Mean	CV	CK-BB	Mean	CV	TnT	Mean	CV	myoglobin	Mean	CV
	200	36776	3	300	1021	6	100.0	1110	49	200.0	970	8	300	1078	11
	100	15785	7	100	765	9	50.0	949	10	100.0	728	8	100	1098	10
	20	3253	2	30	848	3	25.0	871	18	50.0	1416	60	30	1079	8
	10	1725	3	10	550	8	12.5	670	34	25.0	829	13	10	1086	26
	2	740	2	3	538	16	6.3	639	17	12.5	763	14	3	1148	18
	0	525	6	0	521	6	0.0	773	13	0.0	777	5	0	804	17
C12/D11	CK-MB	Mean	CV	CK-MM	Mean	CV	CK-BB	Mean	CV	TnT	Mean	CV	myoglobin	Mean	CV
	200	33744	2	300	668	22	100.0	610	8	200.0	709	13	300	1195	37
	100	16677	2	100	532	0	50.0	616	15	100.0	799	2	100	1218	9
	20	4069	2	30	529	5	25.0	628	11	50.0	781	6	30	876	21
	10	2314	2	10	560	6	12.5	581	18	25.0	604	11	10	995	44
	2	863	8	3	529	5	6.3	734	18	12.5	635	19	3	820	23
	0	530	7	0	468	3	0.0	548	0	0.0	608	24	0	667	12

2.3 Theranos System Screen

The Theranos System test was completed with a regular serum standard curve to evaluate the antibody pair dose responses. CK-MB calibrators (Fitzgerald) were prepared in low CK-MB serum screened by DRG CK-MB ELISA kit. The assay conditions were DAb at 100 ng/mL in Stabilzyme, CAb at 10 ug/mL in Assay buffer and a 1:10 sample dilution.

Figure [SEQ Figure * ARABIC]: Theranos system screening for top 4 antibody pairs

A. **B.**



From the Theranos System screening, there were 2 pairs that both showed a good dose response and acceptable modulation at the lower assay range in the 5-5-5 min format. They are CAb 11 with DAb 12 (C11/D12) and CAb 12 with DAb 2 (C12/D2). Interestingly, antibody pair Cab12/Dab10 also showed nice responses in the 10-10-10 min format. Therefore, C11/D12, C12/D2 and C12/D10 were chosen for further tests.

2.4 Training Set

In order to evaluate if the three antibody pairs are able to track CK-MB from normal plasma and samples known to contain high level of CK-MBs, a total of 30 samples were measured by C11/D12, C12/D2, and C12/D10, as well as DRG CK-MB ELISA kit. The correlations of each comparison disclosed that C11/D12 showed the best correlation with DRG CK-MB ELISA kit and the reported CK-MB values by PromedDx using Roche Modular, Beckman Dxi and Roche Elecys analyzers. Thus, C11/D12 will be advanced but the other two pairs, C12/D2 and C12/D10 can be considered as backups as they also showed acceptable correlation to both DRG kit and predetermined CK-MB values by PromedDx. CK-MB ELISA kit from Signosis was also tested, but it turned out not to be able to measure CK-MB under 7.5 ng/mL. Therefore, the kit from Signosis is not used anymore in this assay.

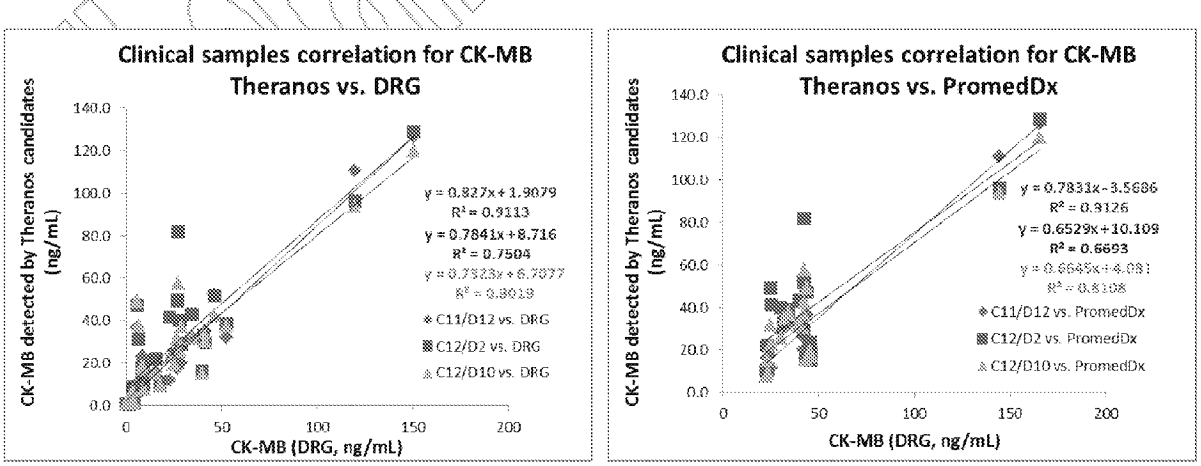
Table 7: Training Set 1—Comparison with DRG kit

Clinical samples IDs		C11/D12	C12/D2	C12/D10	DRG
		CK-MB ng/mL			CK-MB ng/mL
W070511101402	Plasma1	1.4	0.8	1.5	1.84
W070511101403	Plasma2	5.3	**31.1**	**37.8**	6.75
W070511002620	Plasma3	2.6	8.4	7.5	3.53
W070511002621	Plasma4	1.3	0.6	1.2	2.02
W070511002605	Plasma5	1.3	0.8	0.9	0.35
W070511002606	Plasma6	1.5	0.6	0.8	0.54
W070511002669	Plasma7	1.9	0.5	0.8	1.44
W070511002672	Plasma8	2.5	1.2	1.2	3.60
W070511002685	Plasma9	2.9	1.1	1.8	4.42
W070511002688	Plasma10	3.1	0.4	0.9	1.81
PromedDx #1		16.0	22.0	15.0	15.57
PromedDx #2		7.7	10.0	7.6	9.26
PromedDx #3		11.8	41.4	22.7	22.78
PromedDx #4		11.2	11.0	9.1	17.95
PromedDx #5		17.4	49.2	31.8	27.01
PromedDx #6		52.4	51.6	42.5	46.20
PromedDx #7		28.6	40.0	25.7	28.24
PromedDx #8		32.2	33.2	34.7	40.61
PromedDx #9		31.9	38.6	37.5	52.39
PromedDx #10		32.1	43.1	31.7	34.35
PromedDx #11		15.9	16.0	15.2	39.80
PromedDx #12		22.6	23.6	19.5	25.39
PromedDx #13		20.2	28.8	37.2	29.14
PromedDx #14		25.9	81.7	57.7	27.31
PromedDx #15		36.4	47.3	49.1	6.04
PromedDx #16		34.9	29.6	30.6	41.47
PromedDx #17		22.9	19.0	18.5	8.94
PromedDx #18		19.2	14.9	16.8	8.65
PromedDx #19		126.7	128.7	120.0	150.57
PromedDx #20		110.7	96.1	93.7	119.71

Table 8: Training Set 2—Comparison with PromedDx reported CK-MB values (the instruments used to measure each samples listed)

Clinical samples IDs	C11/D12	C12/D2	C12/D10	PromedDx	
	CK-MB ng/mL			CK-MB ng/mL	By analyzers
PromedDx #1	16.0	22.0	15.0	23	Roche Elecys
PromedDx #2	7.7	10.0	7.6	22.42	Roche Elecys
PromedDx #3	11.8	41.4	22.7	24.89	Roche Elecys
PromedDx #4	11.2	11.0	9.1	23.25	Roche Elecys
PromedDx #5	17.4	49.2	31.8	24.68	Roche Elecys
PromedDx #6	52.4	51.6	42.5	42.34	Roche Modular
PromedDx #7	28.6	40.0	25.7	30.21	Roche Modular
PromedDx #8	32.2	33.2	34.7	31.64	Roche Modular
PromedDx #9	31.9	38.6	37.5	34.2	Roche Modular
PromedDx #10	32.1	43.1	31.7	39.4	Roche Modular
PromedDx #11	15.9	16.0	15.2	43.1	Beckman Dxi
PromedDx #12	22.6	23.6	19.5	45.4	Beckman Dxi
PromedDx #13	20.2	28.8	37.2	41.9	Beckman Dxi
PromedDx #14	25.9	81.7	57.7	42.3	Dimension RXL
PromedDx #15	36.4	47.3	49.1	43.7	Beckman Dxi
PromedDx #16	34.9	29.6	30.6	41.77	Roche Modular
PromedDx #17	22.9	19.0	18.5	45.5	Beckman Dxi
PromedDx #18	19.2	14.9	16.8	45.5	Beckman Dxi
PromedDx #19	126.7	128.7	120.0	165.5	Beckman Dxi
PromedDx #20	110.7	96.1	93.7	144.4	Beckman Dxi

Figure 2: Correlations of Theranos CK-MB antibody pairs with DRG kit and the reported values by PromedDx



2.5 Effects of Coating Buffer

To find out the suitable buffer for coating capture antibody conjugates, Sea block, Super block and Starting block were tested. In comparison to 3% BSA TBS blocking buffer, all other buffers tested produced comparable results and all can be considered to use. BSA blocking buffer is chosen as the coating buffer.

Table 9: Coating Buffer Effects

Tested buffers	3% BSA blocking buffer			Sea-block buffer			Super-block buffer			Starting-block buffer		
CK-MB ng/mL	Mean	CV	Modulation	Mean	CV	Modulation	Mean	CV	Modulation	Mean	CV	Modulation
500	384907	3.4	352.8	409214	12.0	387.5	373185	20.2	343.3	382676	17.6	359.0
250	171095	23.1	156.8	191441	13.2	181.3	187449	8.8	172.4	201226	15.8	188.8
50	28472	24.9	26.1	26479	29.8	25.1	27535	16.2	25.3	29237	21.6	27.4
10	5809	8.4	5.3	5664	15.3	5.4	6246	18.6	5.7	6272	15.8	5.9
2.5	2556	6.2	2.3	2591	9.3	2.5	2853	18.7	2.6	2617	11.3	2.5
0	1091	12.9	1.0	1056	11.2	1.0	1087	24.8	1.0	1066	9.0	1.0

2.6 Capture (C11) and detection antibody (D12) titration

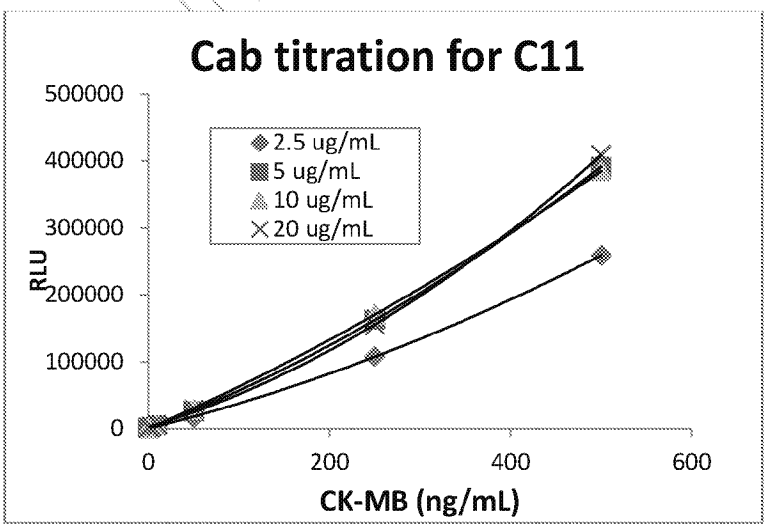
2.6.1 Capture Antibody Titration

The capture antibody C11 at 2.5, 5.0, 10, and 20 ug/ml were chosen for the titration. Dab 12 at 100 ng/mL in Stabilzyme buffer was used. The CK-MB calibrator was prepared in low CK-MB serum. Sample dilution is 1:10. Based on the results, capture antibody at 10 ug/ml yields strong dose responses and nice modulation in the low range, therefore 10 ug/ml of Cab 11 is chosen for CK-MB assay. However, the CK-MB standard curves almost overlapped to each other when capture antibodies were used at 5, 10 and 20 ug/ml, suggesting C11 at 5 ug/ml could be a good option as well.

Table 10: Capture antibody titration

Cab conc.	2.5 ug/mL		5 ug/mL		10 ug/mL		20 ug/mL	
CK-MB ng/mL	Mean	Modulation	Mean	Modulation	Mean	Modulation	Mean	Modulation
500	258875	336.2	391428	399.4	384907	352.8	409251	400.8
250	107785	140.0	162750	166.1	171095	156.8	154285	151.1
50	16394	21.3	25739	26.3	28472	26.1	28184	27.6
10	3423	4.4	5305	5.4	5809	5.3	5383	5.3
2.5	1593	2.1	1913	2.0	2556	2.3	2209	2.2
0	770	1.0	980	1.0	1091	1.0	1021	1.0

Figure 3: Capture antibody titration



2.6.2 Detection Antibody Titration

Next, Biostab and 3% BSA TBS blocking buffer were tested for detection antibody stabilizers in the Theranos system in order to know if they work better than Stabilzyme. In this test, capture antibody C11 at 10 ug/ml in BSA blocking buffer was used. Sample dilution is 1:10. It turned out that Stabilzyme displayed the best result (Figure 4). The detection antibody was titrated in Stabilzyme buffer at 3 levels at a 1:10 sample dilution of serum calibrators in order to determine the optimal working concentration (Figure 5). The DAb concentration of 100 ng/mL provided the best modulation across the standard curve, and in particular at the lower end of the assay where sensitivity is desired.

Figure 4: Stabilizers optimization for detection antibody

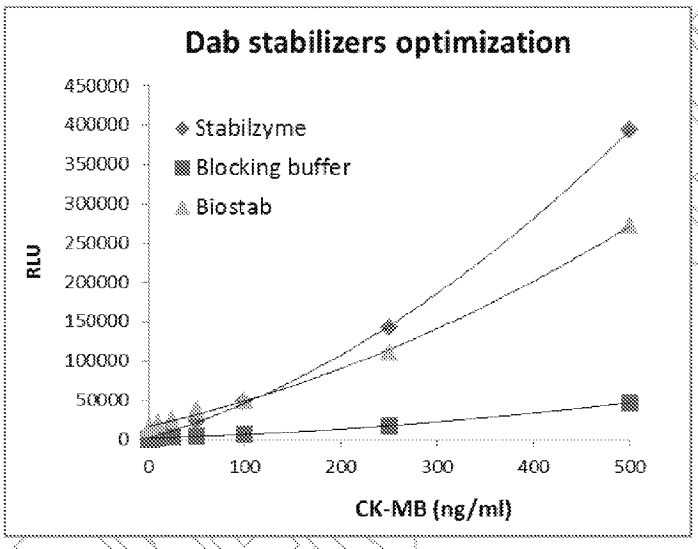
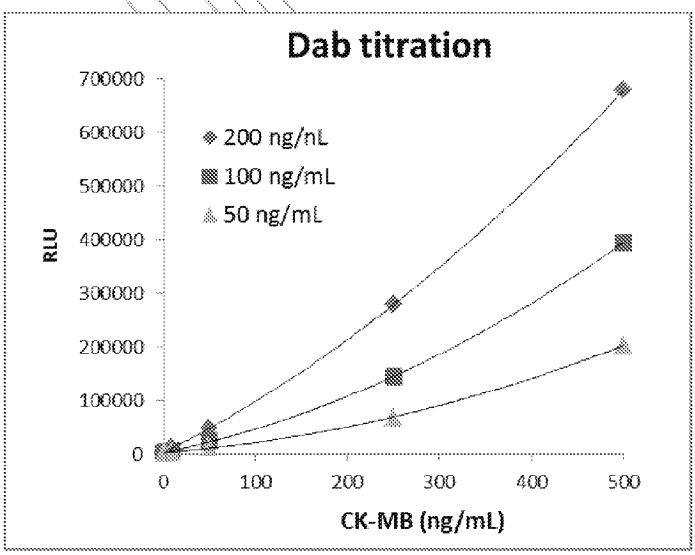


Figure 5: Detection antibody titration



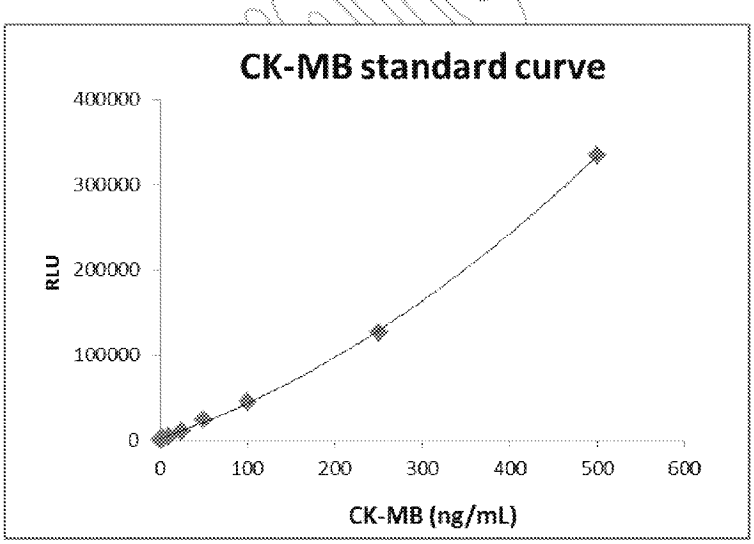
2.7 Theranos CK-MB standard curve

The final pair CAb 11 and Dab 12 was used for CK-MB assay in the Theranos system. Using the assay conditions described below, the current CK-MB ELISA displays a nice detection range from 2.5 to 500 ng/mL.
Capture antibody (C11) at 10 ug/ml in 3% BSA TBS blocking buffer
Detection antibody (D12) at 100 ng/ml in Stabilzyme buffer
Calibrator, native CK-MB from Fitzgerald spiked in low CK-MB serum
Sample dilution 1:10
Protocol 10-10-10 min

Table 11: CK-MB standard curve in serum

CK-MB ng/ml	Mean	CV	Modulation
500	334703	10	***330.08***
250	126758	7	***125.01***
100	45881	7	***45.25***
50	24534	12	***24.20***
25	10355	26	***10.21***
10	4818	11	***4.75***
2.5	1946	18	***1.92***
0	1014	18	***1.00***

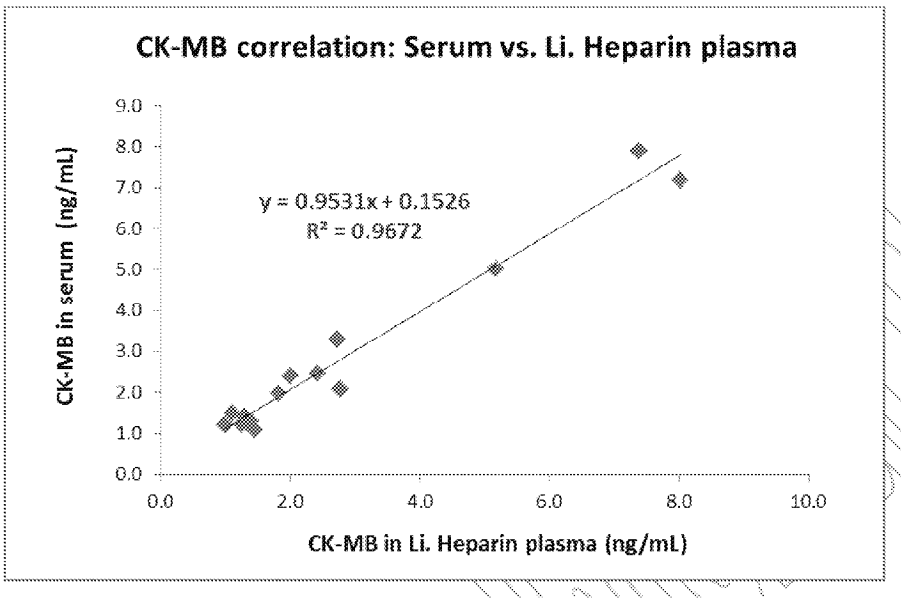
Figure 6: CK-MB standard curve in serum



2.8 Serum vs. Li. Heparin plasma

CK-MB levels in 14 matched serum and Li. Heparin plasma were evaluated by C11/D12 pair in the Theranos system. The two set samples showed a correlation about 95.3% and with R^2 of 0.9672. This result reveals that both serum and Li. Heparin plasma are compatible for the current CK-MB assay.

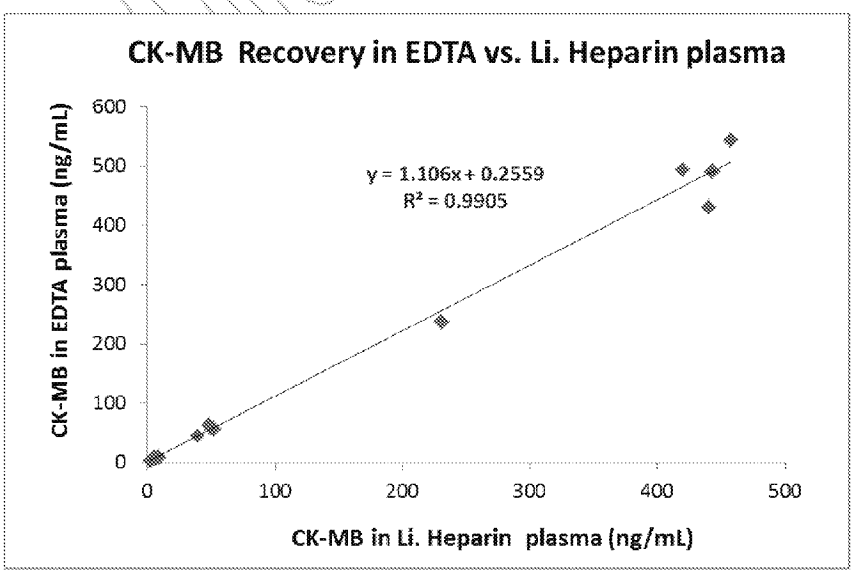
Figure 7: Detection of CK-MB in matched serum and Li. Heparin plasma



2.9 Anticoagulant effects: EDTA plasma vs. Li. Heparin plasma

Next, CK-MBs at different levels were spiked into matched EDTA and Li. Heparin plasma in order to evaluate anticoagulant effects. The back-calculated CK-MB concentrations were compared. Four donors were tested. Overall, CK-MB recovered from Li. Heparin plasma is about 90% of the recovery in EDTA plasma. Therefore, Li. Heparin plasma and serum is recommended for this assay, but EDTA plasma should also work considering about the 10% difference from Li. Heparin plasma.

Figure 8: Anticoagulant effects: EDAT plasma and Li. Heparin plasma



2.10 Whole Blood and Plasma Screen

To verify the normal ranges of CK-MB in whole blood, 10 male blood specimens were screened at a 1:10 sample dilution. CK-MB in the same plasma was also tested. In whole blood, CK-MB level ranges from 0.8 to 2.1 ng/mL. Meanwhile, CK-MB results in plasma correspond well with the expected normal range (< 5 ng/mL).

Table12: CK-MB level in whole blood and plasma

Stanford blood IDs	CK-MB (ng/mL)	
	Whole blood	Plasma
W070511101402	1.0	1.3
W070511101403	2.0	4.8
W070511002620	1.9	2.3
W070511002621	2.1	1.3
W070511002605	0.8	1.2
W070511002606	1.1	1.2
W070511002669	0.8	1.2
W070511002672	1.3	2.5
W070511002685	2.1	2.9
W070511002688	0.9	1.0
Ave. CK-MB	1.4	2.0

2.11 Interfering Matrixes

Hemolyzed, icteric, lipemic and Rheumatoid factor positive (RF+) serum samples were obtained from ProMedDx. The recovery of CK-MB spiked into these potentially interfering matrixes was evaluated in the Theranos System. The assay did not show any significant interference from icteric and hemolyzed serum, spike recovery was within 20% of nominal. **But lipemic and RF+ samples could reduce 20-30% of the CK-MB signal.**

Table 13: Matrixes effects

Calibrators	Lipemic #1		Lipemic #2		RF+ #1		RF+ #2	
Ck-MB ng/mL	Mean RLU	% Recovery	Mean RLU	% Recovery	Mean RLU	% Recovery	Mean RLU	% Recovery
500	288260	85.8	279417	84.4	247267	78.6	223127	73.9
250	111589	92.7	101019	86.1	80718	72.3	85990	76.1
50	15915	77.3	19356	95.4	16723	81.2	14219	68.6
10	4298	73.7	4275	73.7	5072	90.0	4195	72.9
2	1402	54.8	1833	71.8	1847	67.3	1995	85.4
0	1008		961		1133		848	
Calibrators	**Icteric #1**		**Icteric #2**		**Hemolyzed #1**		**Hemolyzed #2**	
Ck-MB ng/mL	Mean RLU	% Recovery	Mean RLU	% Recovery	Mean RLU	% Recovery	Mean RLU	% Recovery
500	356983	96.4	332488	92.9	362623	97.2	290561	86.1
250	123352	99.6	100497	85.8	150933	114.7	112048	92.6
50	19950	98.2	17539	86.1	23969	118.7	19665	95.2
10	4921	87.5	4160	71.9	6213	118.3	5160	86.6
2.5	1711	72.4	2110	91.8	2096	84.5	2643	95.0
0	1052		873		1040		1537	

2.12 Hematocrit effects

CK-MB was spiked into whole blood to measure recovery and it ranged from 79 -114% across the assay range. The same sample was also spun down to separate plasma, and to quantitate the CK-MB in plasma. Two blood samples were tested and the recovery ratio in plasma versus whole blood is 2.32 and 2.05, respectively (Figure 9). In addition, when we measured CK-MB directly from matched whole blood and plasma, the average CK-MB level was 1.4 ng/ml in whole blood vs. 2.0 ng/ml in plasma (Table 12), suggesting there is no red cells interference

Figure 9: CK-MB recovery in plasma vs. whole blood

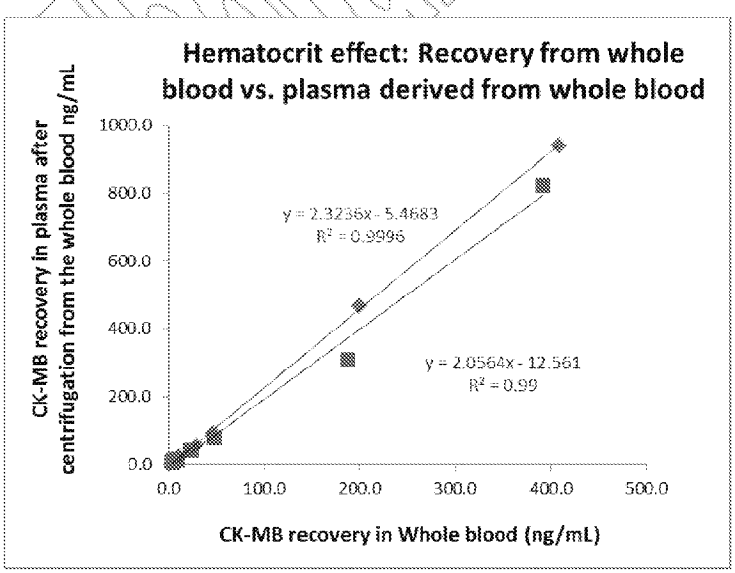


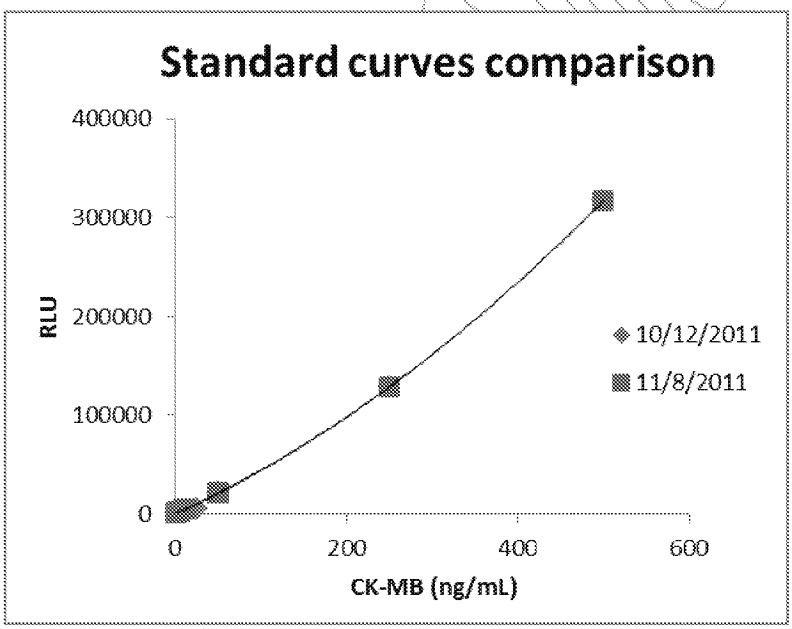
Table 14: CK-MB recovery in whole blood and plasma derived from the whole blood

	% of recovery			
CK-MB ng/mL	Blood #1	Blood #2	Plasma #1	Plasma #2
500	81.5	78.3	187.3	164.3
250	79.4	74.5	185.8	122.6
50	91.8	94.6	180.4	153.1
25	108.0	89.9	204.7	153.5
10	99.5	79.2	191.3	130.3
5	114.6	89.9	210.5	175.9
2.5	102.5	89.3	167.3	153.1

2.13 Comparision of different reagent lots

CK-MB analytes in serum were run twice in the Theranos system with the condition described in section 2.7. The capture surfaces (Tips) for the 2 tests were from different day of manufacturing lots (10/12/2011 vs. 11/08/2011). The standard curves were aligned to each other with nice overlay.

Figure 10: Comparison of 2 standard curves using 10-10-10 min protocol, 10X sample dilution



2.14 Protocol tests: sample dilution, incubation time, co-incubation format

In order to efficiently use the blood and reduce assay running time, sample dilution at 25 times and shorter assay incubation protocols such as 5-5-5 min and 2-2-1 min were tested. In addition, a thorough screen of co-incubation protocols was also performed. In general, co-incubation format is a better option for CK-MB assay. All the formats tested were satisfactory. At the condition when sample was diluted 25 times, reagents incubation 2-1 min, there is still

acceptable modulation at 2.5 ng/ml of CK-MB. Dab concentration was further tested for this short protocol and 50 ng/ml of Dab in Stabilzyme was finalized.

Table 15: Test different protocols

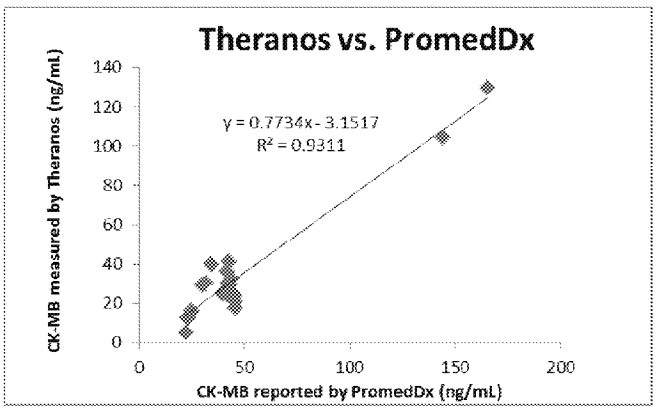
Sample dilution	10		10		10		10		25	
Protocol	10/10/10 min		5/5/5 min		Coincubation 5/5 min		Coincubation 2/1 min		10/10/10 min	
Dab conc. (ng/mL)	100		100		100		100		100	
CK-MB native	Ave. RLU	Modulation	Ave. RLU	Modulation	Mean	Modulation	Mean	Modulation	Ave. RLU	Modulation
500	317397	*309.66*	93522	*179.16*	754047	*870.72*	184156	*492.40*	94454	*122.35*
250	129057	*125.91*	39724	*76.10*	439945	*508.02*	80309	*214.73*	41347	*53.56*
50	21708	*21.18*	7473	*14.32*	65858	*76.05*	12557	*33.57*	7006	*9.08*
10	4886	*4.77*	1911	*3.66*	16108	*18.60*	3037	*8.12*	1737	*2.25*
5	3005	*2.93*	1422	*2.72*	7123	*8.23*	2060	*5.51*	1513	*1.96*
2.5	2006	*1.96*	797	*1.53*	4450	*5.14*	1244	*3.33*	1083	*1.40*
0	1025	*1.00*	522	*1.00*	866	*1.00*	374	*1.00*	772	*1.00*

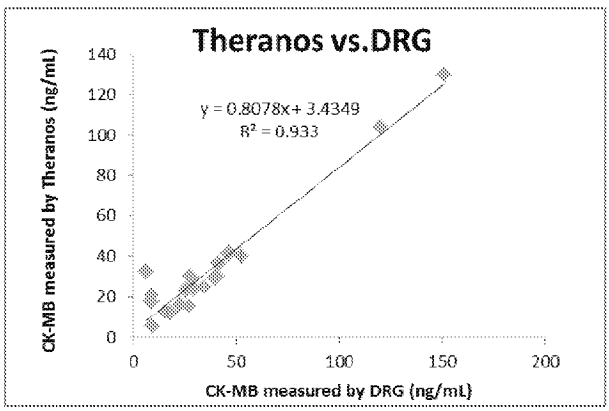
Sample dilution	25		25		25		25	
Protocol	Coincubation 5/5 min		Coincubation 2/1 min		Coincubation 2/1 min		Coincubation 2/1 min	
Dab conc. (ng/mL)	100		100		50		25	
CK-MB native	Ave. RLU	Modulation	Ave. RLU	Modulation	Ave. RLU	Modulation	Ave. RLU	Modulation
500	625615	*719.10*	146751	*403.16*	65108	*322.32*	27884	*149.91*
250	293511	*337.37*	61542	*169.07*	25608	*126.77*	10673	*57.38*
50	39277	*45.15*	13017	*35.76*	4557	*22.56*	2338	*12.57*
10	9803	*11.27*	2875	*7.90*	1175	*5.82*	621	*3.34*
5	5020	*5.77*	1414	*3.88*	668	*3.31*	424	*2.28*
2.5	3388	*3.89*	967	*2.66*	419	*2.07*	251	*1.35*
0	870	*1.00*	364	*1.00*	202	*1.00*	186	*1.00*

2.15 Clincal samples correlation using new protocol

The final protocol for CK-MB assay using C11/D12 has been determined. During capture antibody titration, 5 ug/ml of capture surface was found to be quite comparable to the antibody surface at 10 ug/ml (Figure 3 and Table 10). Therefore, capture antibody at 5 ug/ml in 3% BSA TBS blocking buffer and detection antibody at 50 ng/ml in Stabilzyme buffer are chosen for the final condition. Protocol is co-incubation time 2-1 min and sample dilution is 1:25. 20 clinical samples from PromedDx were re-tested and CK-MB values correlations were analyzed again. The results showed that the correlation relations maintain the same as previous (Figure 2).

Figure 11: Clinical samples correlation using co-incubation protocol





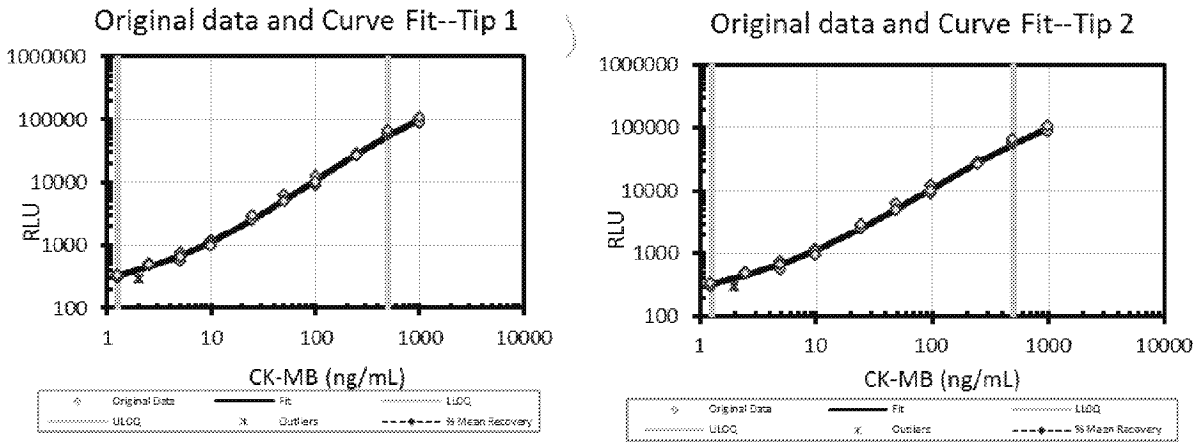
2.16 LLOQ and ULOQ

The assay range of CK-MB assay is 2.5 to 500 ng/mL. For LLOQ and ULOQ test, a broader range of CK-MB calibrators were run in the Theranos system using the final condition, 1 dose above 500 ng/ml and 1 dose below 2.5 ng/ml. It produced a nice curve with acceptable accuracy and precision as evaluated with Dexter1.0. Tip 1 and tip 2 were calibrated individually due to the variance between tips due to the shorter reagents incubation time.

Table 16: Serum Standard Curve for Determination of LLOQ and ULOQ

CK-MB ng/mL	Ave.-Tip1	CV	Modulation	Ave.-Tip2	CV	Modulation	Log C	Log S1	Back-Recal	Recovery	Log S2	Back-Recal	Recovery
1000	129212	14	706	158529	10	817	3.000	5.111	987.7	98.8	5.200	1033.0	103.3
500	53670	16	293	58715	21	303	2.699	4.730	490.2	98.0	4.769	458.7	91.7
250	25988	8	142	32142	3	166	2.398	4.415	257.1	102.8	4.507	266.2	106.5
100	9699	22	53	10566	23	54	2.000	3.987	97.0	97.0	4.024	87.9	87.9
50	5615	16	31	7034	8	36	1.699	3.749	53.9	107.7	3.847	56.7	113.4
25	3005	8	16	3497	20	18	1.398	3.478	26.3	105.3	3.544	25.6	102.4
10	1310	18	7	1640	20	8	1.000	3.117	9.5	95.0	3.215	10.2	102.0
5	734	9	4	935	5	5	0.699	2.866	4.4	89.0	2.971	4.9	98.9
2.5	454	2	2	484	17	2	0.398	2.657	2.3	91.9	2.685	2.0	81.3
1.25	329	11	2	386	7	2	0.097	2.518	1.5	116.8	2.587	1.5	118.4
0	183	13	1	194	7	1		2.262	0.6		2.287	0.5	

Figure 12: LLOQ and ULOQ determination by Dexter1.0



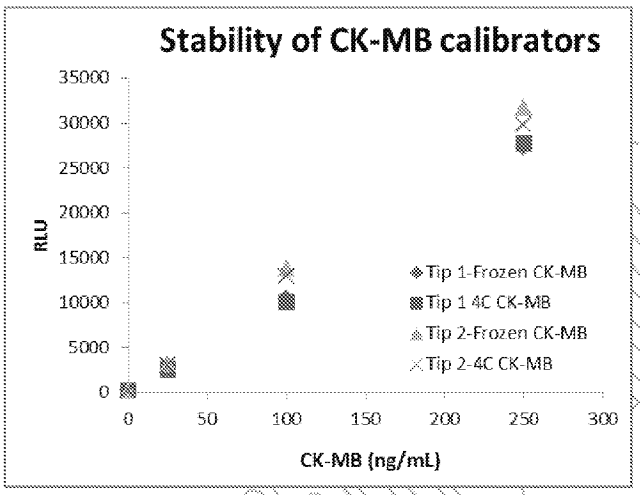
Tip 1			Tip 2		
LLOQ	**2.50**	**ng/ml**	LLOQ	**1.25**	**ng/ml**
ULOQ	**500.00**	**ng/ml**	ULOQ	**500.00**	**ng/ml**
desired LLOQ	**2.50**	**ng/ml**	desired LLOQ	**2.50**	**ng/ml**
desired ULOQ	**500.00**	**ng/ml**	desired ULOQ	**500.00**	**ng/ml**
LLOQ accuracy	**85**	**%**	LLOQ accuracy	**108**	**%**
LLOQ precision	**4.2**	**%**	LLOQ precision	**13.8**	**%**
ULOQ accuracy	**112**	**%**	ULOQ accuracy	**100**	**%**
ULOQ precision	**21.5**	**%**	ULOQ precision	**23.4**	**%**

2.17 Stability tests

2.17.1 Analyte stability at 4^0C

The stability of CK-MB when stored at 4^0C was tested. CK-MB calibrators in serum were stored at 4^0C over an 18-day period. Compared to calibrators stored at -80^0C, CK-MB at 4^0C maintained very good antigenic activity.

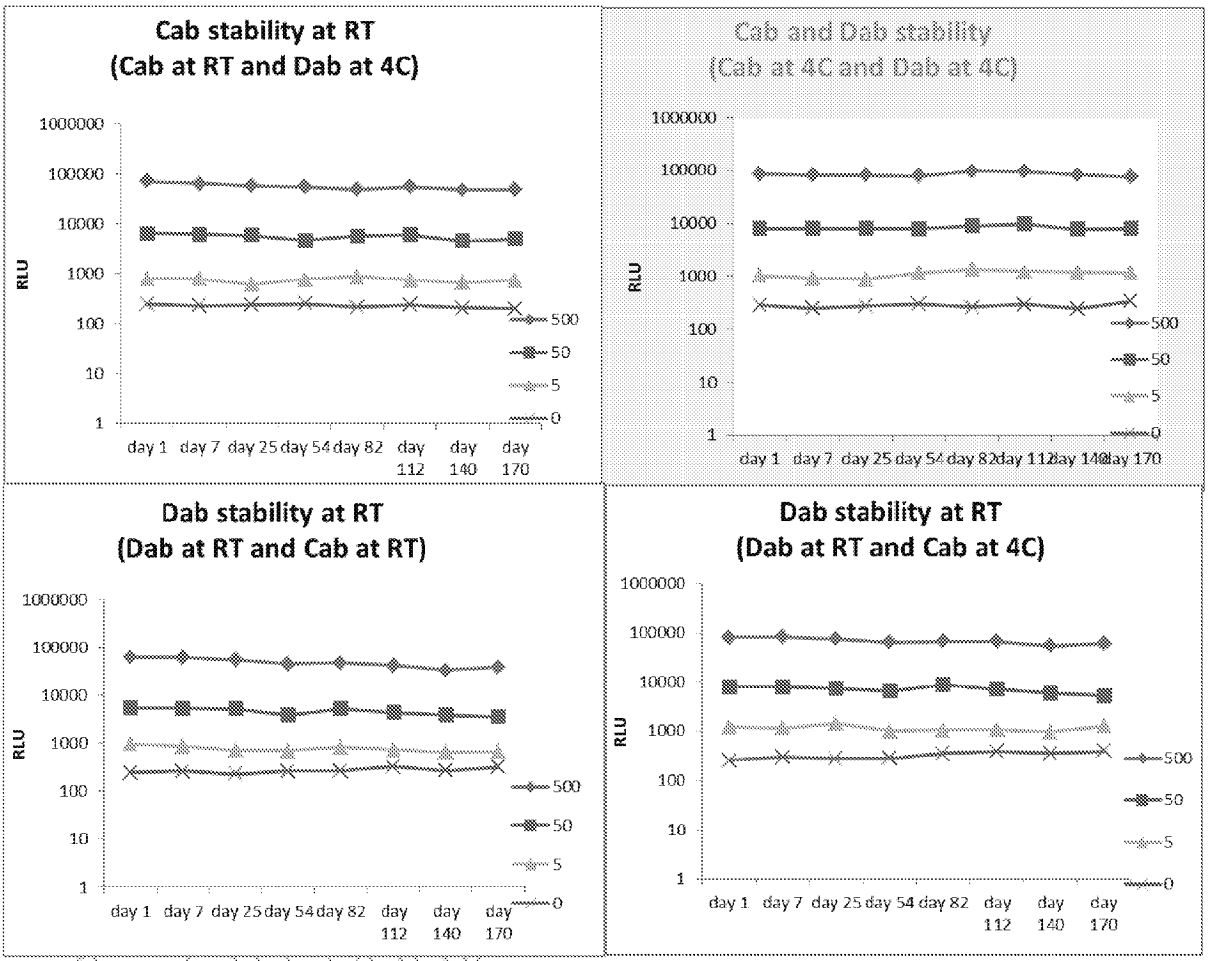
Figure 13: CK-MB analyte stability



2.17.2 Capture and detection antibody stability

Capture and detection antibodies were stored at room temperature (RT) and 4^0C, respectively. Four-point calibrators were run for each test periodically. Both capture and detection antibodies are stable for 170 days if stored at 4^0C. However, the signal dropped from 25 - 40 % if one of the antibodies or both antibodies were kept at RT. Therefore, shelf storage at 4^0C is recommended.

Figure 13: Stability of capture and detection antibodies


Cab stability at RT
(Cab at RT and Dab at 4C)
RLU
1000000
100000
10000
1000
100
10
1
day 1
day 7
day 25
day 54
day 82
day 112
day 140
day 170
500
50
5
0
Cab and Dab stability
(Cab at 4C and Dab at 4C)
Dab stability at RT
(Dab at RT and Cab at RT)
Dab stability at RT
(Dab at RT and Cab at 4C)