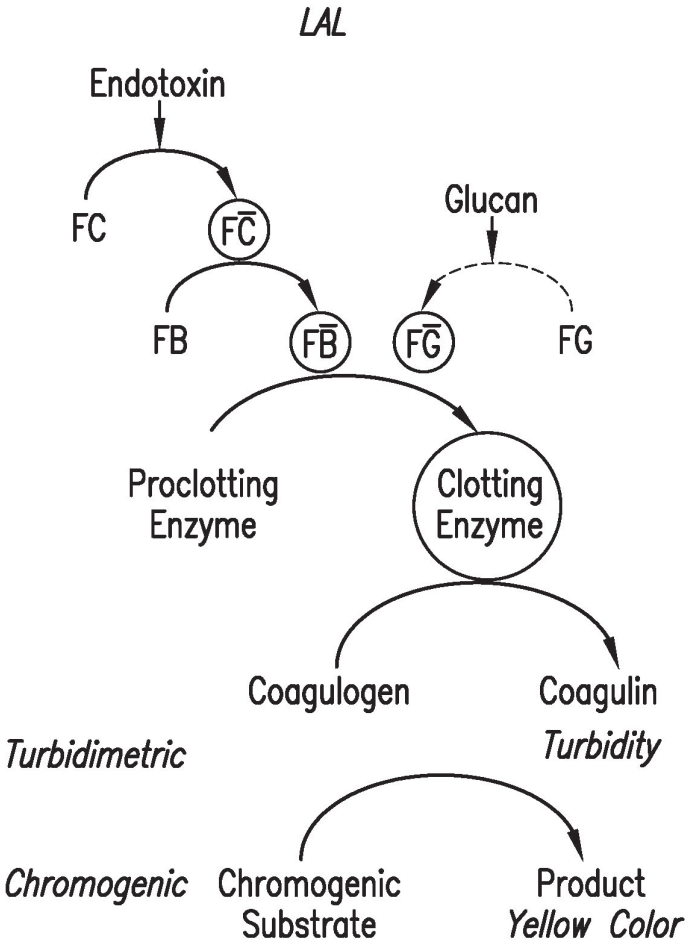

LAL
Endotoxin
FC
FC
Glucan
FB
FB
FG
FG
Proclotting Enzyme
Clotting Enzyme
Coagulogen
Coagulin
Turbidimetric
Turbidity
Chromogenic
Chromogenic Substrate
Product
Yellow Color

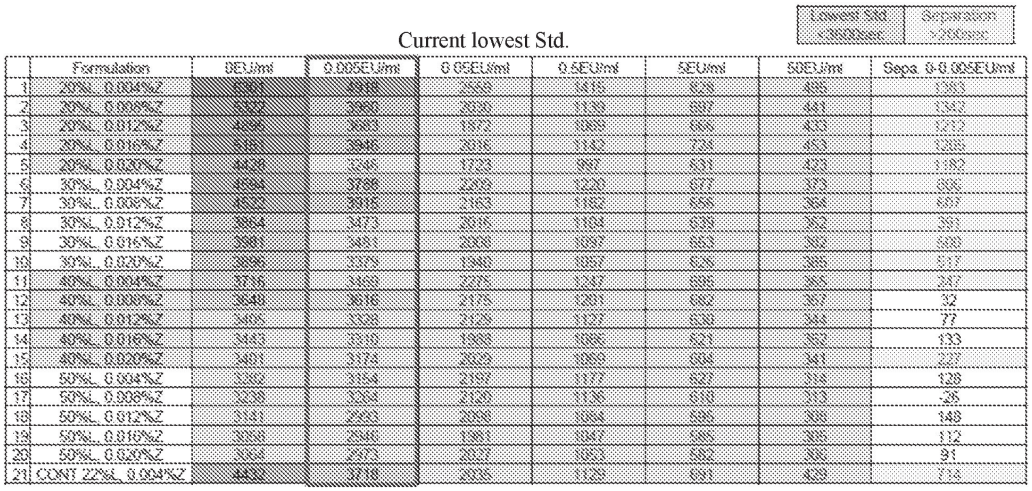
FIG. 1

FIG. 2

	200mOD		100mOD		50mOD	
	Lysate	Lysate-Coag.	Lysate	Lysate-Coag.	Lysate	Lysate-Coag.
Endo. Stds.	Rxn. Time	Rxn. Time	Rxn. Time	Rxn. Time	Rxn. Time	Rxn. Time
0 EU/ml	2557	2847	2272	2318	2010	1915
0.001EU/ml *	2644	2710	2295	2231	2044	1811
0.005 EU/ml	2552	2429	2198	1962	1943	1547
0.05 EU/ml	1972	1524	1743	1201	1525	943
0.5 EU/ml	1018	781	900	622	779	496
5 EU/ml	544	425	473	324	406	249
50 EU/ml	276	195	205	****	169	****
Corr. Coeff.	-0.991	-0.998	-0.985	-0.998	-0.984	-0.997
Slope	-0.249	-0.275	-0.265	-0.263	-0.270	-0.266
Blank to 0.005EU/ml Separation (Secs.)	5	418	74	356	67	368
0.005EU/ml Reaction Time (Mins.)	43	40	37	33	32	26
Blank to 0.001EU/ml Separation (Secs.)	-87	137	-23	87	-34	104
0.001EU/ml Reaction Time (Mins.)	44	45	38	37	34	30

*0.001EU/ml reaction time not included in calculation of line

FIG. 3

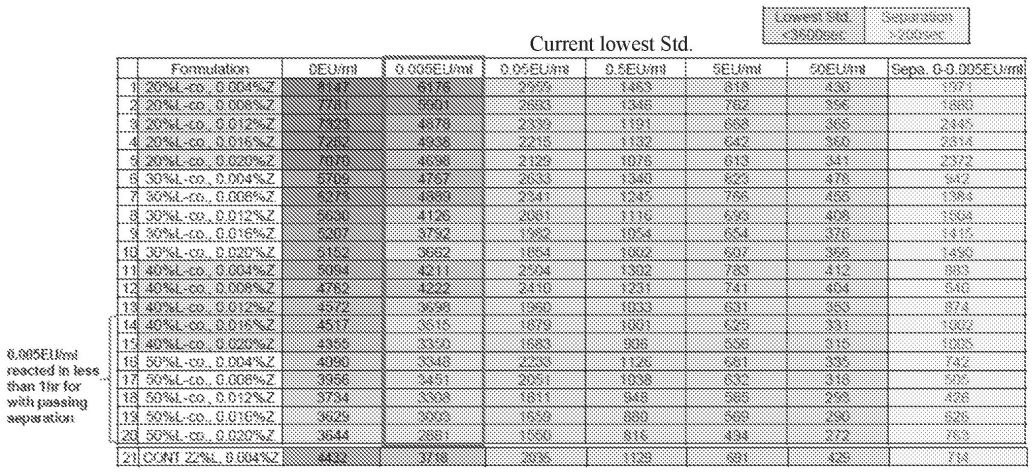


Lowest Std. <3600/ml | Separation >250/ml

Current lowest Std.

	Formulation	0EU/ml	0.005EU/ml	0.05EU/ml	0.5EU/ml	5EU/ml	50EU/ml	Sepa. 0-0.005EU/ml
1	20%_ 0.004%Z	5091	3818	2558	1419	828	485	1363
2	20%_ 0.008%Z	4757	3960	2638	1139	807	481	1547
3	20%_ 0.012%Z	4498	3663	1972	1069	666	433	1212
4	20%_ 0.016%Z	5161	3848	2018	1143	724	453	1288
5	20%_ 0.020%Z	4436	3545	1723	957	631	428	1182
6	30%_ 0.004%Z	4594	3788	2409	1220	677	373	806
7	30%_ 0.008%Z	4529	3818	2163	1162	668	384	687
8	30%_ 0.012%Z	3864	3473	2018	1104	639	362	391
9	30%_ 0.016%Z	3981	3481	2008	1097	653	382	499
10	30%_ 0.020%Z	3896	3378	1948	1057	626	385	417
11	40%_ 0.004%Z	3716	3493	2275	1247	695	365	247
12	40%_ 0.008%Z	3649	3616	2178	1281	662	367	32
13	40%_ 0.012%Z	3405	3328	2128	1127	630	343	77
14	40%_ 0.016%Z	3443	3310	1993	1086	621	362	133
15	40%_ 0.020%Z	3401	3178	2005	1060	608	341	223
16	50%_ 0.004%Z	3362	3154	2157	1177	627	314	128
17	50%_ 0.008%Z	3238	3264	2128	1136	618	313	-26
18	50%_ 0.012%Z	3151	2993	2058	1068	595	308	148
19	50%_ 0.016%Z	3058	2946	1981	1047	585	305	112
20	50%_ 0.020%Z	3064	2973	2027	1063	582	306	91
21	CONT 22%_ 0.004%Z	4492	3718	2035	1126	691	429	774

FIG. 4



	Formulation	0EU/ml	0.005EU/ml	0.05EU/ml	0.5EU/ml	5EU/ml	50EU/ml	Sepa. 0-0.005EU/ml
1	20%L-co., 0.004%Z	8147	6178	2979	1463	818	430	1771
2	20%L-co., 0.008%Z	7041	5601	2683	1348	782	396	1680
3	20%L-co., 0.012%Z	7078	4678	2099	1191	668	365	2445
4	20%L-co., 0.016%Z	7082	4936	2215	1172	642	360	2814
5	20%L-co., 0.020%Z	6010	4638	2129	1028	613	341	2372
6	30%L-co., 0.004%Z	5705	4787	2683	1388	855	478	942
7	30%L-co., 0.008%Z	6275	4680	2541	1245	766	455	1964
8	30%L-co., 0.012%Z	5636	4126	2081	1119	693	408	1558
9	30%L-co., 0.016%Z	5307	3792	1992	1054	654	376	1515
10	30%L-co., 0.020%Z	5152	3652	1854	1002	607	365	1450
11	40%L-co., 0.004%Z	5084	4211	2504	1302	783	412	873
12	40%L-co., 0.008%Z	4782	4222	2436	1231	741	404	546
13	40%L-co., 0.012%Z	4572	3696	1960	1033	631	353	874
14	40%L-co., 0.016%Z	4517	3516	1879	1001	625	331	1822
15	40%L-co., 0.020%Z	4355	3350	1683	908	556	315	1895
16	50%L-co., 0.004%Z	4086	3548	2258	1126	681	385	742
17	50%L-co., 0.008%Z	3956	3451	2051	1038	632	318	502
18	50%L-co., 0.012%Z	3794	3303	1811	948	593	293	536
19	50%L-co., 0.016%Z	3625	3009	1639	880	569	280	826
20	50%L-co., 0.020%Z	3644	2981	1566	816	534	272	753
21	CONT 22%L, 0.004%Z	4433	3758	2036	1158	691	426	716

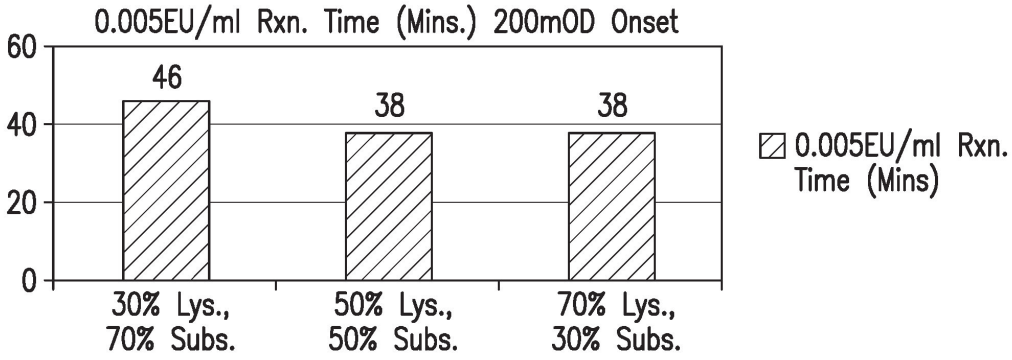


FIG.5A

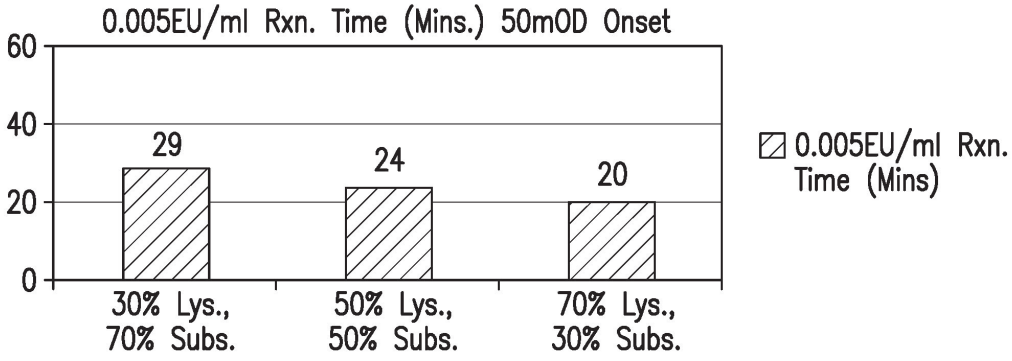


FIG.5B

	Current	New	Current	New
Sensitivity	0.005EU/ml	0.005EU/ml	0.001 EU/ml	0.001 EU/ml
Reaction Time	60 mins	20 mins	NA	27 to 45 mins
Separation	$\leq$300 secs	Sufficient	NA	Sufficient

FIG.5C

Lot	0.005EU/ml Rxn. (mins.)	0.001EU/ml Rxn. (mins.)
1	20	21
2	24	31
3	17	20

FIG.6A

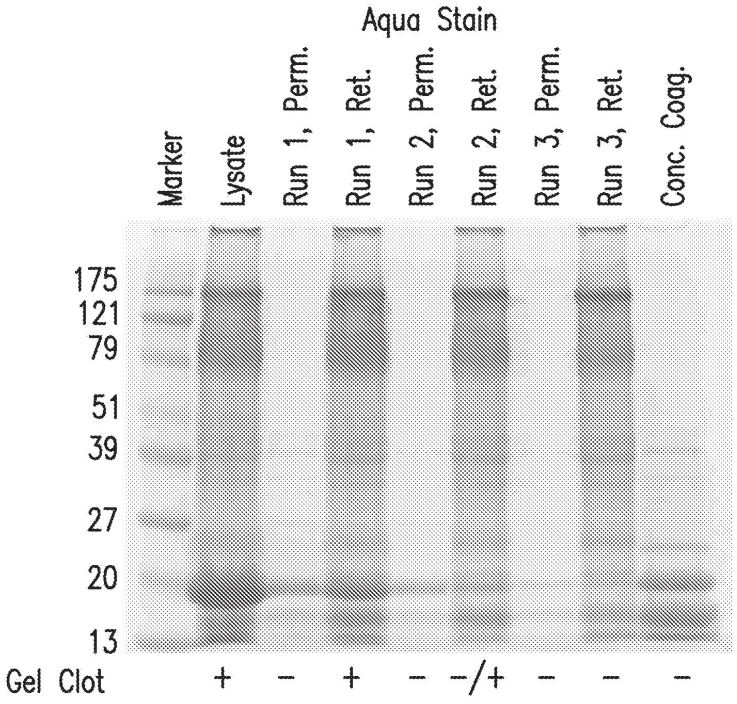

Aqua Stain
Marker
Lysate
Run 1, Perm.
Run 1, Ret.
Run 2, Perm.
Run 2, Ret.
Run 3, Perm.
Run 3, Ret.
Conc. Coag.
175
121
79
51
39
27
20
13
Gel Clot
+ − + − −/+ − − −

FIG.7A

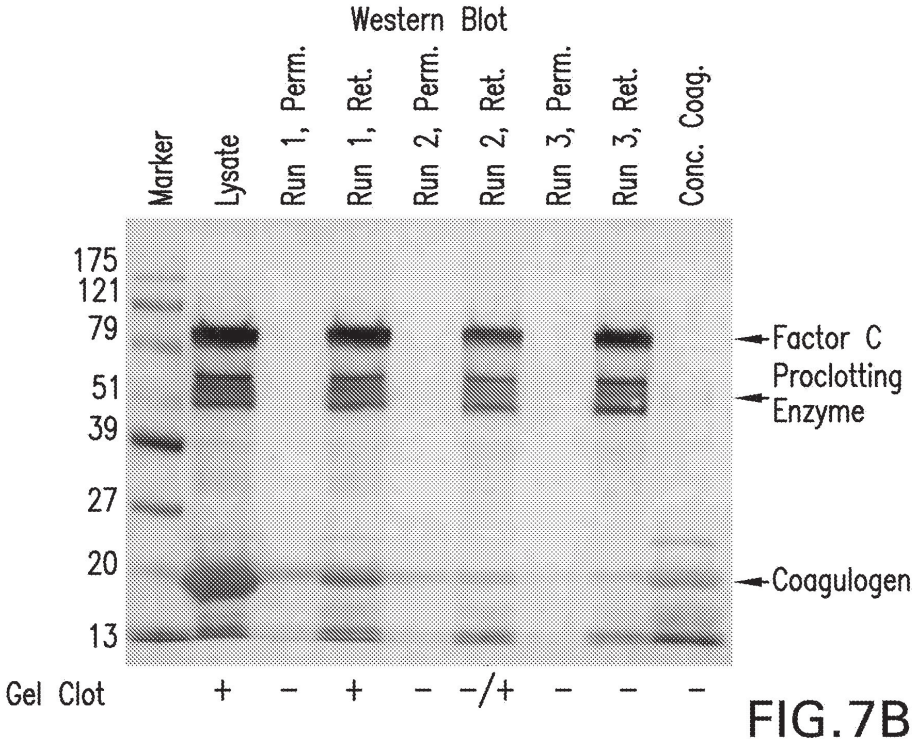

Western Blot
Marker
Lysate
Run 1, Perm.
Run 1, Ret.
Run 2, Perm.
Run 2, Ret.
Run 3, Perm.
Run 3, Ret.
Conc. Coag.
175
121
79
51
39
27
20
13
Factor C
Proclotting
Enzyme
Coagulogen
Gel Clot
+ − + − −/+ − − −

FIG.7B

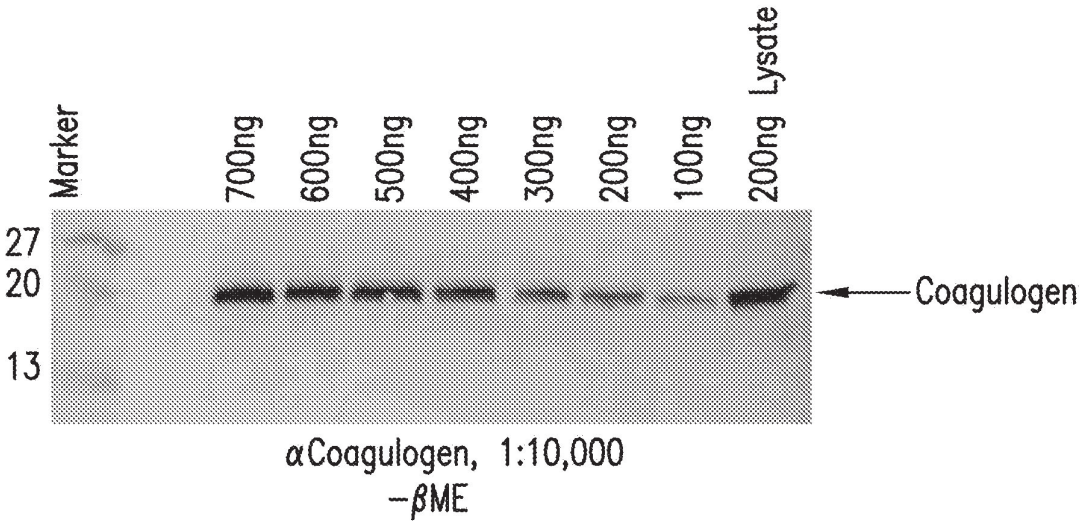

Marker
700ng
600ng
500ng
400ng
300ng
200ng
100ng
200ng Lysate
27
20
13
Coagulogen
αCoagulogen, 1:10,000
−βME

FIG.8A

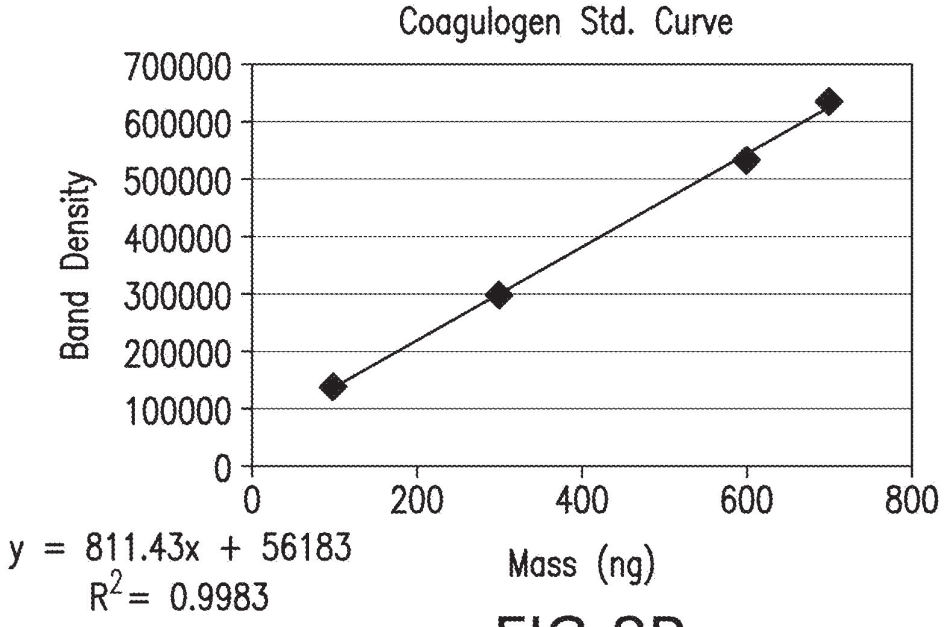

Coagulogen Std. Curve
Band Density
700000
600000
500000
400000
300000
200000
100000
0
0
200
400
600
800
Mass (ng)
y = 811.43x + 56183
R2 = 0.9983

FIG.8B

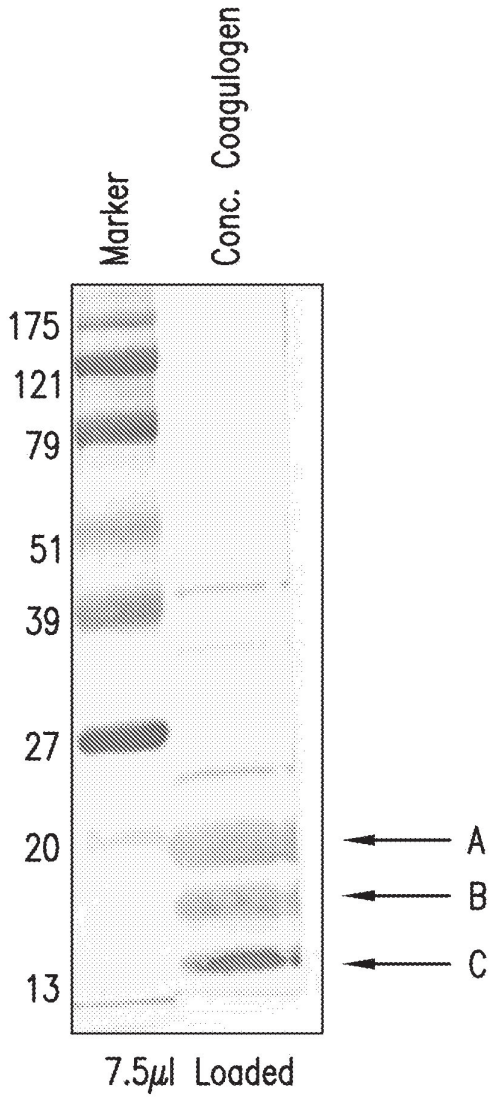

Marker
Conc. Coagulogen
175
121
79
51
39
27
20
13
A
B
C
7.5μl Loaded

FIG.9

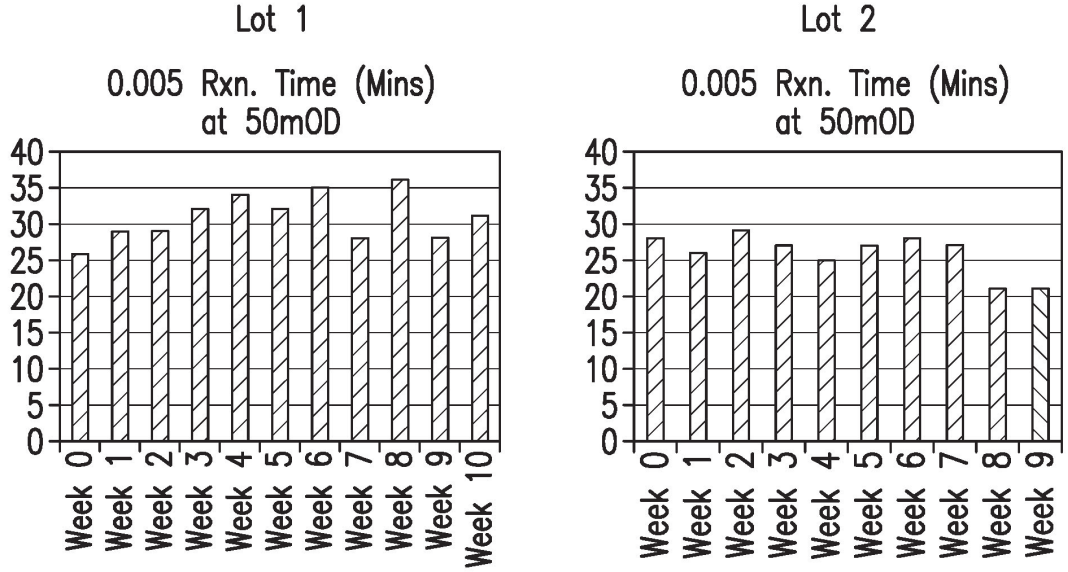


FIG.10A

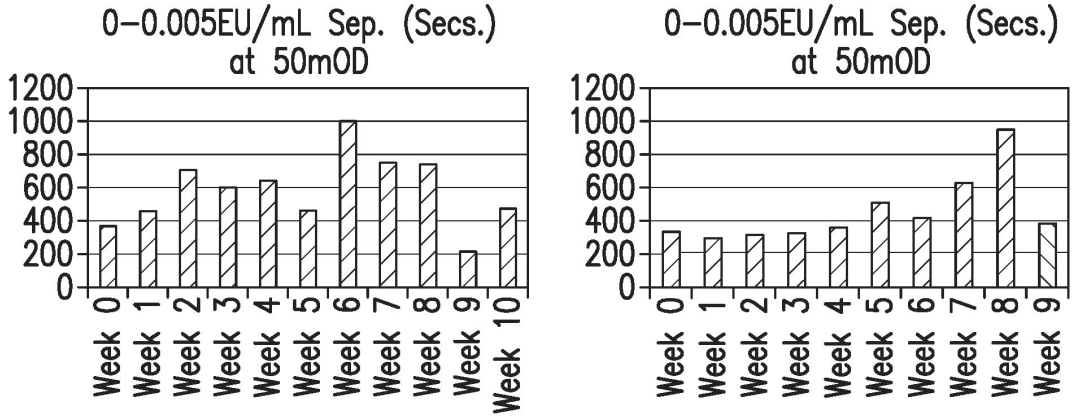


FIG.10B

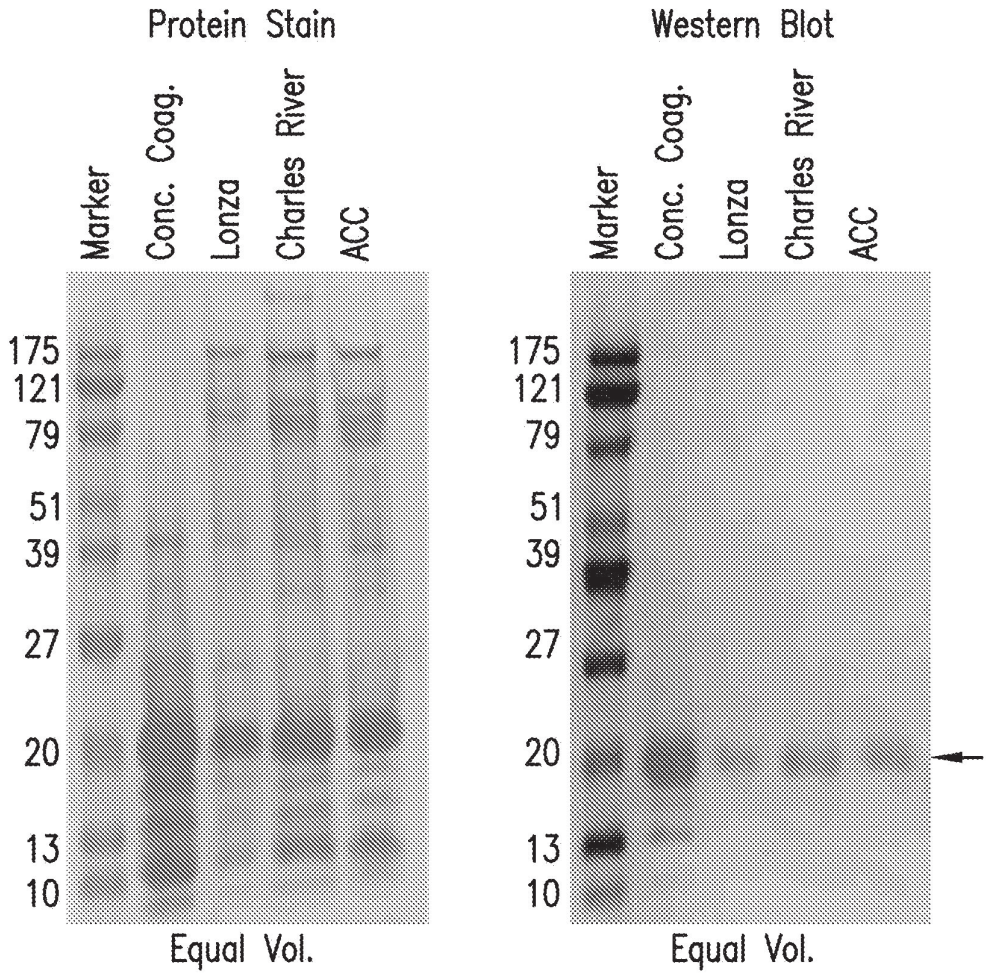


FIG.11A

Manufacturer	Protein Conc. (mg/ml)
Lonza	2.9
Charles River	5.8
ACC	8.7

FIG.11B