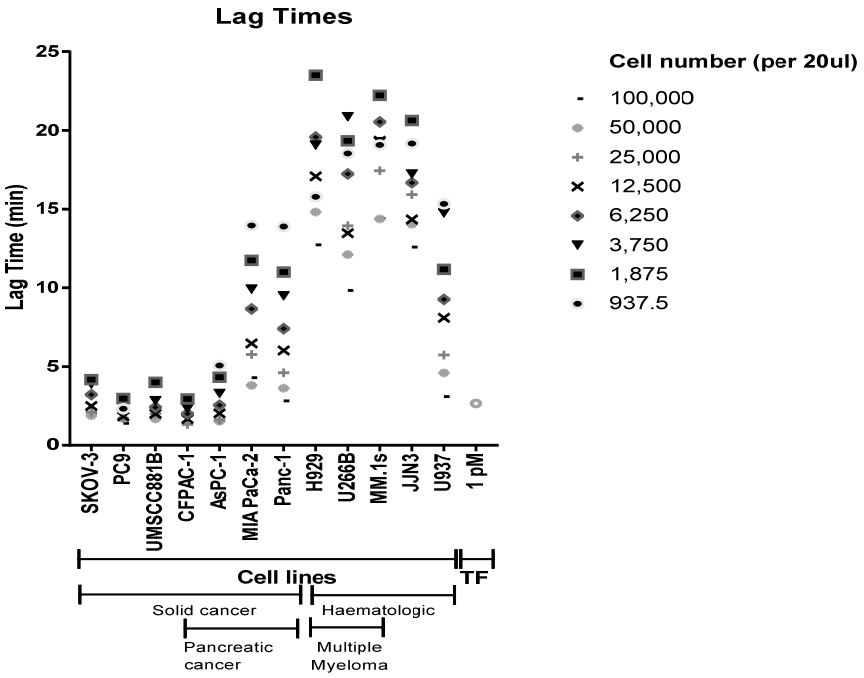
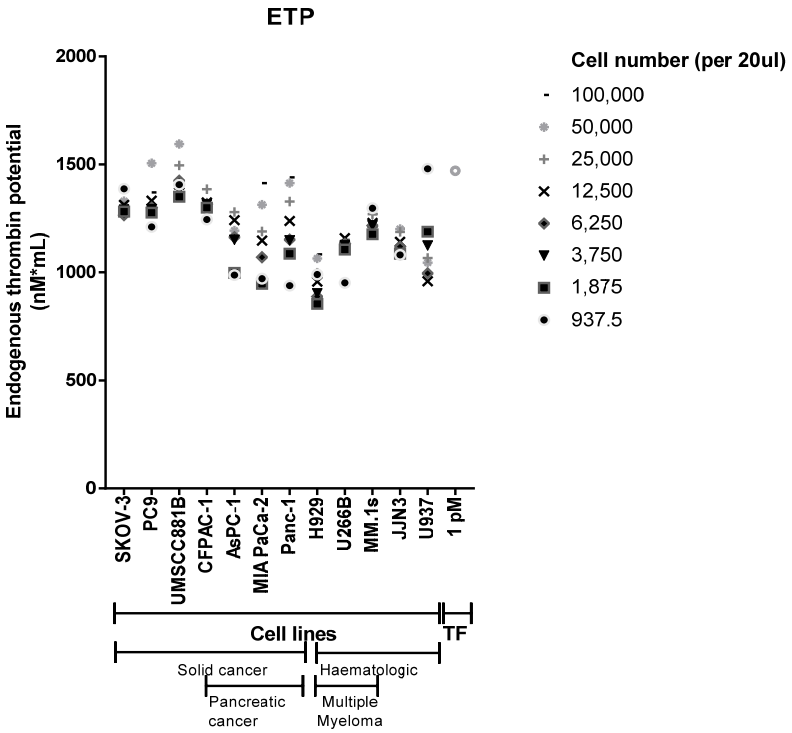
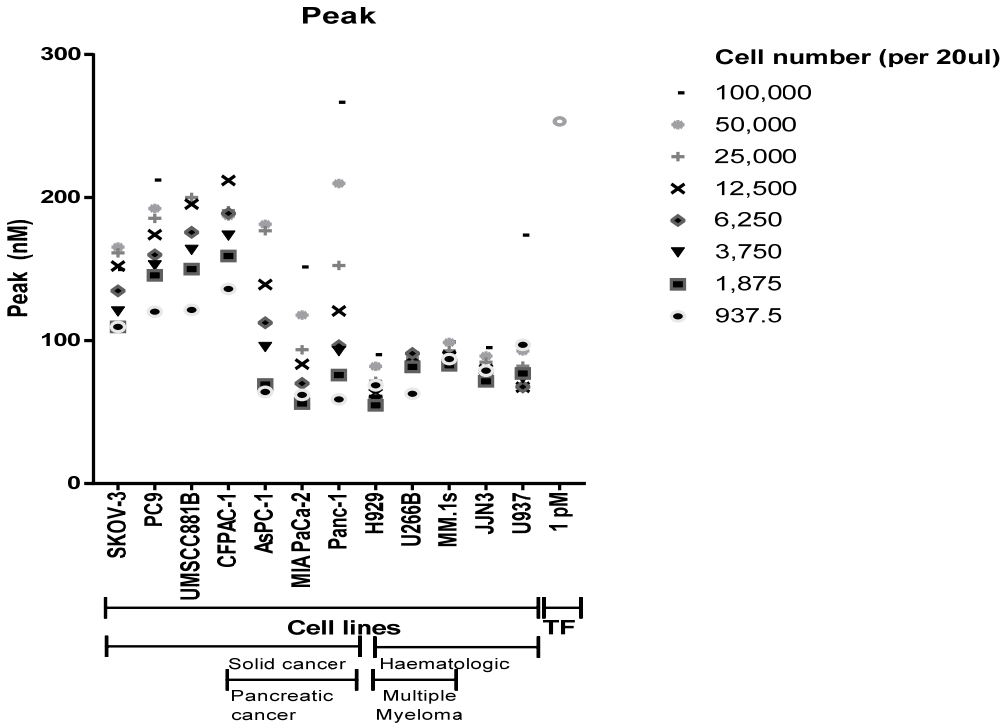
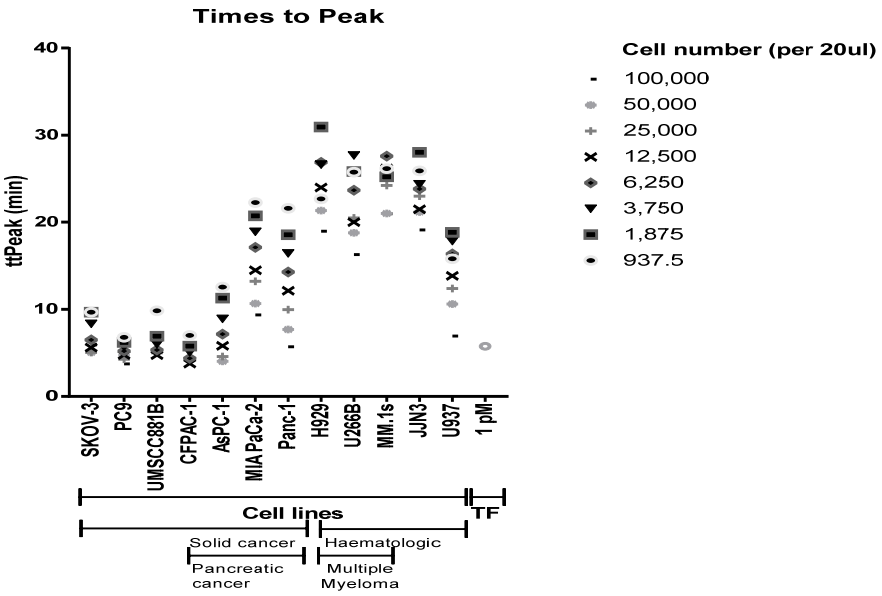

Lag Times
Lag Time (min)
25
20
15
10
5
0
SKOV-3
PC9
UMSCC881B
CFPAC-1
AsPC-1
MIA PaCa-2
Panc-1
H929
U266B
MM.1s
JJN3
U937
1 pM
Cell lines
TF
Solid cancer
Haematologic
Pancreatic cancer
Multiple Myeloma
Cell number (per 20ul)
100,000
50,000
25,000
12,500
6,250
3,750
1,875
937.5


ETP
Endogenous thrombin potential (nM*mL)
2000
1500
1000
500
0
SKOV-3
PC9
UMSCC881B
CFPAC-1
AsPC-1
MIA PaCa-2
Panc-1
H929
U266B
MM.1s
JJN3
U937
1 pM
Cell lines
TF
Solid cancer
Haematologic
Pancreatic cancer
Multiple Myeloma
Cell number (per 20ul)
100,000
50,000
25,000
12,500
6,250
3,750
1,875
937.5


Peak
Peak (nM)
300
200
100
0
SKOV-3
PC9
UMSCC881B
CFPAC-1
AsPC-1
MIA PaCa-2
Panc-1
H929
U266B
MM.1s
JJN3
U937
1 pM
Cell lines
TF
Solid cancer
Haematologic
Pancreatic cancer
Multiple Myeloma
Cell number (per 20ul)
100,000
50,000
25,000
12,500
6,250
3,750
1,875
937.5


Times to Peak
ttPeak (min)
40
30
20
10
0
SKOV-3
PC9
UMSCC881B
CFPAC-1
AsPC-1
MIA PaCa-2
Panc-1
H929
U266B
MM.1s
JJN3
U937
1 pM
Cell lines
TF
Solid cancer
Haematologic
Pancreatic cancer
Multiple Myeloma
Cell number (per 20ul)
100,000
50,000
25,000
12,500
6,250
3,750
1,875
937.5

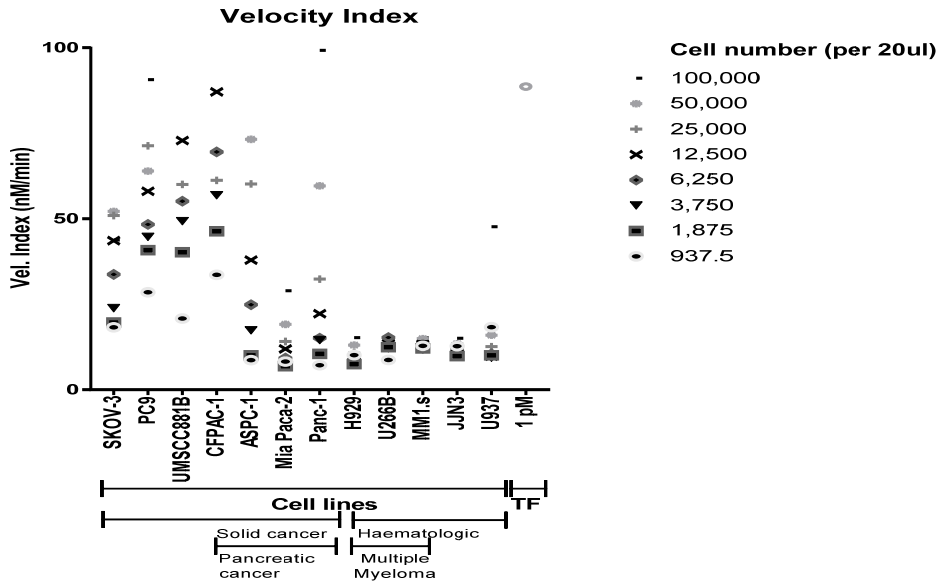
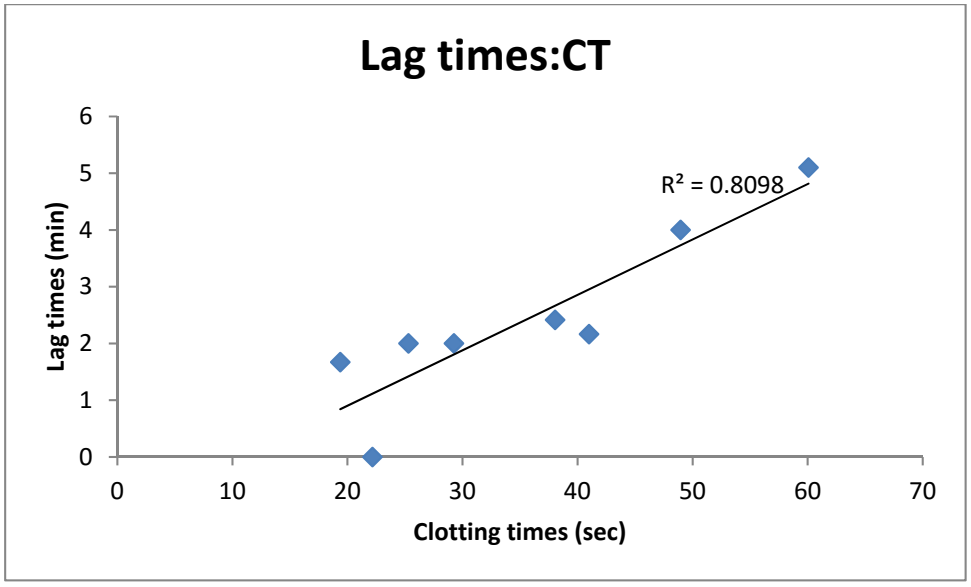


Figure 1. Thrombin generation with increasing cell numbers. TG was induced by 8 different concentrations of 12 cell lines (i.e 100,000 to 937.5 cells/20µl) in platelet-free plasma with low 1 pM TF standard preparations as controls. Results are mean of n=3 performed in triplicates (intra assay C of V of Lag times, ETP and the rest of the TG parameters were 4.78%, 3.53% and < 5% respectively, inter assay C of V of the TG parameters were < 10%). The 5 TG parameters measured by calibrated automated thrombography (CAT) assay are Lag times, Endogenous thrombin potential (ETP), Peak thrombin (Peak), Times to Peak (TTP or ttPeak), Velocity index (Vel.Index).

	Platelet free plasma	Lag time (min)	ETP (nM/min)	Peak (nM)	TTP (min)	Vel. Index (nM/min)
SKOV-3	NormTrol	1.6±0.2	1299±62.6	182.2±14.2	4.6±0.2	61.6±6.6
	F XII-deficient	1.9±0.3	1730.1±78.5*	173.3±24.2	5.3±0.1	50.8±3.4
	FVII-deficient	11.8±5.4**	991.7±171.5*	64.7±22.9**	20.3±6.8***	8.1±4.5***
PC9	NormTrol	1.8±0.1	1264.8±137	174±10.3	4.8±0.1	58±3.4
	F XII-deficient	1.5±0.1	1825.3±119*	233.4±61.5*	4.7±0.5	74.7±28.9
	FVII-deficient	9.2±0.1*	1609.7±131.5*	121.2±20.1*	15.2±4.2**	18.9±9.7***
UMSCC81B	NormTrol	1.5±0.2	1331.2±203	207.1±22.8	4.4±1.1	81.5±26.9
	F XII-deficient	1.7±0.1	1473.1±141.7	220.2±10	4±0.1	94.4±11
	FVII-deficient	10±4.4**	1510.1±120.1	103.8±64.2***	16.8±8**	18.9±15.8***
CFPAC-1	NormTrol	1.5±0.2	1294.1±46.6	217±12.4	3.7±0.2	95±16.4
	F XII-deficient	1.6±0.2	1722.3±107.2*	244.4±19.4**	4.2±0.3	93.7±16.6
	FVII-deficient	10.8±4.3***	1331.5±154.1	104.5±37.8***	17.6±6.1**	19±13.6***
AsPC-1	NormTrol	1.5±0.2	1263.9±78.8	162.1±12.4	4.8±0.8	50.8±13.4
	F XII-deficient	1.5±0.2	1750.7±123.7*	180.7±18.7**	5.7±6.8	41.3±12.9
	FVII-deficient	16.8±6.5**	1065.7±495.3	60.1±18.9***	26±6.8***	6.7±2.5***
MIA PaCa-2	NormTrol	7.6±1.4	1079.5±106.7	71±14.2	15.1±1.4	9.7±2.7
	F XII-deficient	5.1±2.6	1306.3±311.9	99.7±92.5	15.9±0.8	23.6±41.9
	FVII-deficient	19.7±6.3***	1260.6±304.3	84±32.1	28.1±6.8***	13.5±11.3
PANC-1	NormTrol	4.9±1.1	1166.3±95.6	91.7±26.5	11.6±2.2	14.4±6.2
	F XII-deficient	3.8±0.6	1474.4±330.9*	95.3±40.5*	13.5±2.7	11.5±7.4
	FVII-deficient	21.8±3.6***	1104.1±96	87.4±26.5	30.2±3.2**	10.6±3.6
H929	NormTrol	13.2±2.1	1122.2±70.7	89.5±23.2	19.7±2.8	14.6±5.9

	F XII-deficient	16.5±2.9	1001.5±305.	48.8±16.1	28.5±4.2*	4.2±1.8
	FVII-deficient	21.3±4.7**	1318.6±384.9	76.9+36.1	30.3±6.4**	9.6±6.4
JJN3	NormTrol	15.4±1.6	1084.2±107.6	66.7±7.6	23.1±1.7	8.7±1.1
	F XII-deficient	15.3±3.4	824.9±150.5	37.5±5	30.3±6.4*	2.5±0.4
	FVII-deficient	23.6±0.9**	796.9±155.2	43.7±13.2	34±0.8**	4.3±1.3
U266B	NormTrol	15.2±1.3	1086.4±107.7	71±12.3	22.7±1.6	9.6±2
	F XII-deficient	19.3±3.4	681.8±39**	35±8.6	34±4***	2.4±0.4
	FVII-deficient	23.7±3.1**	861.3±20	46.8±12.2	34.8±4.7***	4.4±1.6
U937	NormTrol	9.8±1.6	999.9±114.9	60.4±20.4	17.8±1.1	7.6±1.7
	F XII-deficient	7.6±1.4	670.2±12	32.2±3.2	26.6±5.2*	1.8±0.3
	FVII-deficient	28.0±7***	967.9±243.5	46.4±5.1	39.2±6.4***	4.2±0.5
MM.1s	NormTrol	16.2±2.8	985.7±101.7	64.9±11.6	21.7±4.4	9.5±2.8
	F XII-deficient	20.4±9.0	664.9±94.5	33.8±7	35.8±9***	2.3±0.8
	FVII-deficient	29.4±2.4***	701.6±10	36.5±6.2	40.7±2.7***	3.2±0.5
TF standard	NormTrol	2.6±0.1	1398.7±174.8	254.2±6.1	5.4±0.1	93.5±3.7
	F XII-deficient	2.1±0.1	2010.8±163.8***	328.4±7.6**	5±0.1	111.6±0.6
	FVII-deficient	14.3±1.8***	1722.3±70.1*	218.5±23.6	18.5±2***	53.6±11.3***

Table 1. Results of Thrombin generation tests of 12 cancer cell lines and low 1pM TF standard preparation. Cells were collected and washed in PBS at concentration of 0.63×10^6/ml to induce TG in 3 different platelet-free plasma (NormTrol, Factor XII-deficient and Factor VII-deficient). TG parameters include Lag time, ETP, Peak, TTP and Velocity Index. Results are mean of n=4 ± S.D performed in duplicates. P values from two-way ANOVA multiple comparisons with NormTrol as baseline compared with Factor VII-deficient plasma and Factor XII-deficient plasma. Significance is at *p< 0.05, **p<0.01, ***p<0.001.



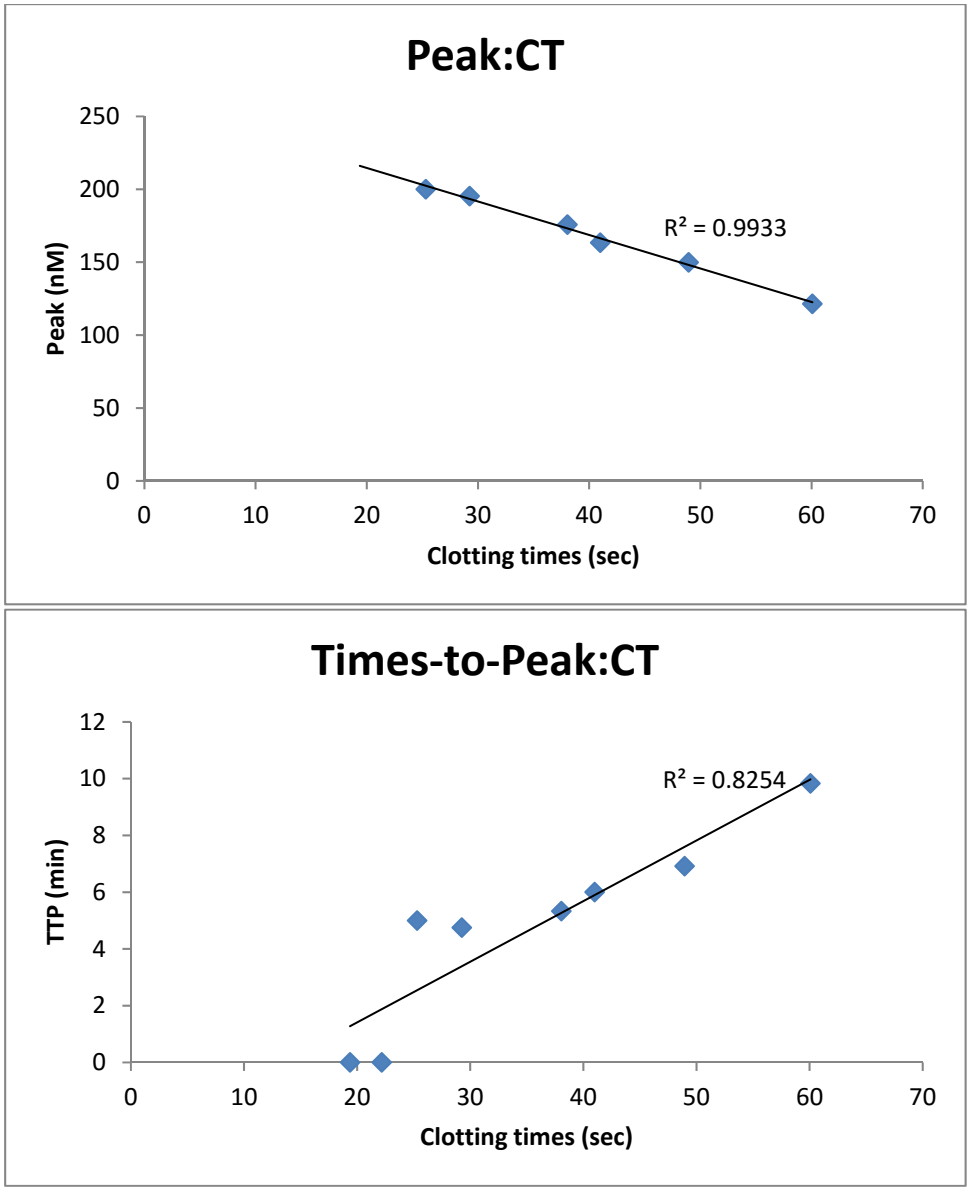


Figure 2. Clotting times versus Thrombin generation. TG parameters from the CAT assay were compared with clotting times from the one-step Thrombotrak Solo coagulometer. UMSCC81B cancer cell lines were used at decreasing concentrations (i.e 5, 2.5, 1.25, 0.63, 0.3, 0.15, 0.08, 0.04 x 10*6/ml) for both assays. CT for UMSCC81Bs had the highest correlation with the thrombin Peak (Pearson coefficient r^2=-0.997, p= 0.0007), followed by times-to-peak and lag times (r^2=0.908 and 0.899, p=0.0001 and 0.0002 respectively). Correlations with other TG parameters were slightly weaker (data not shown).

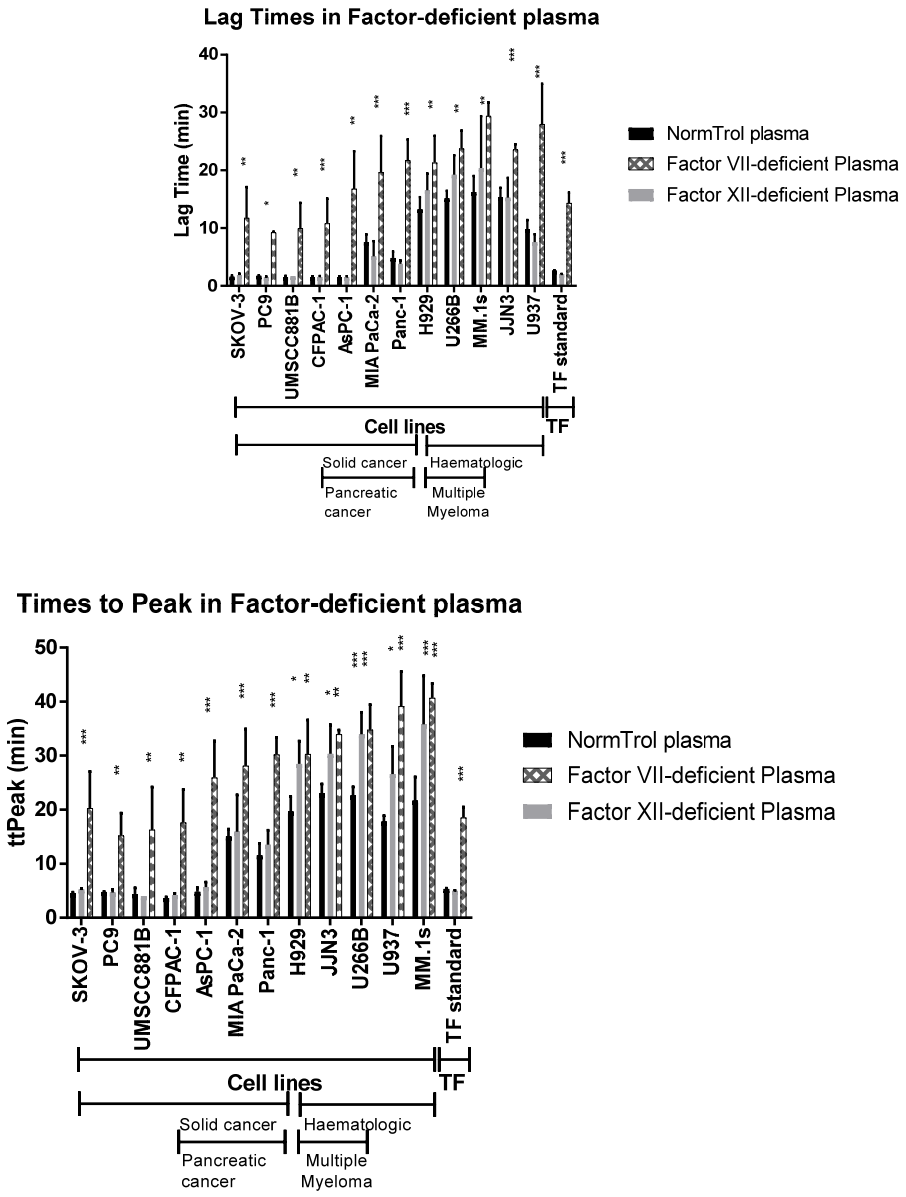
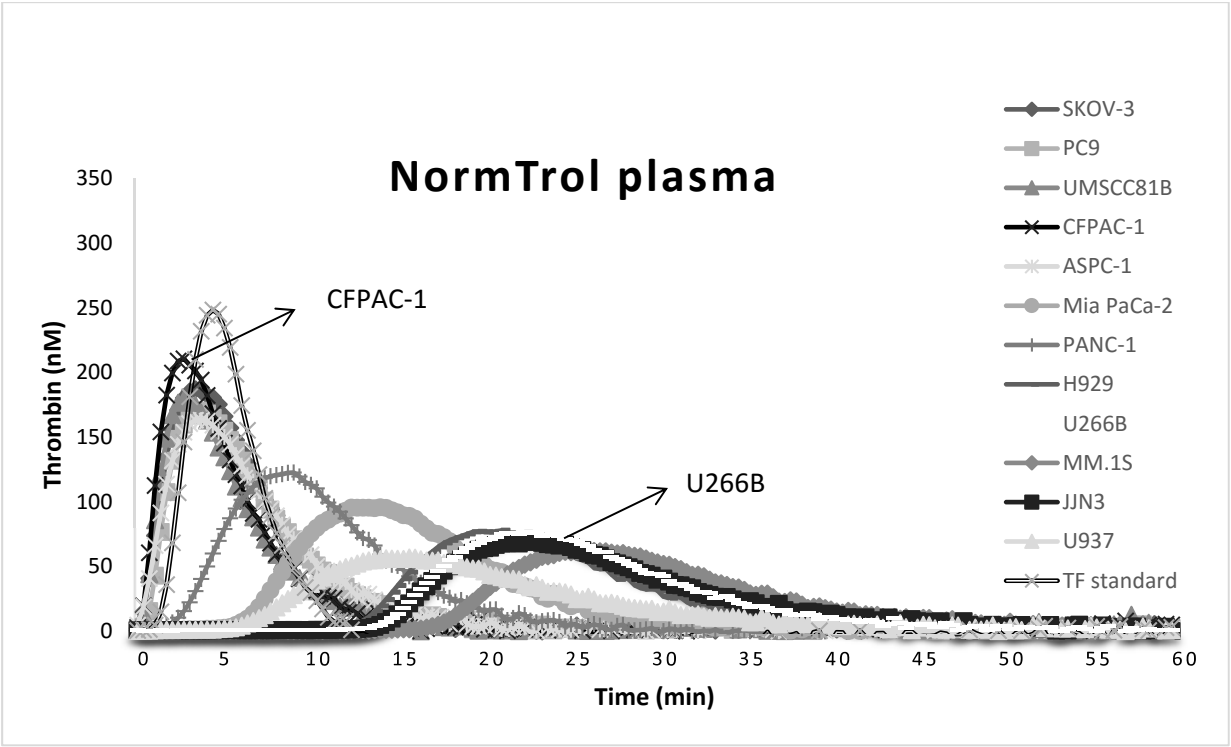
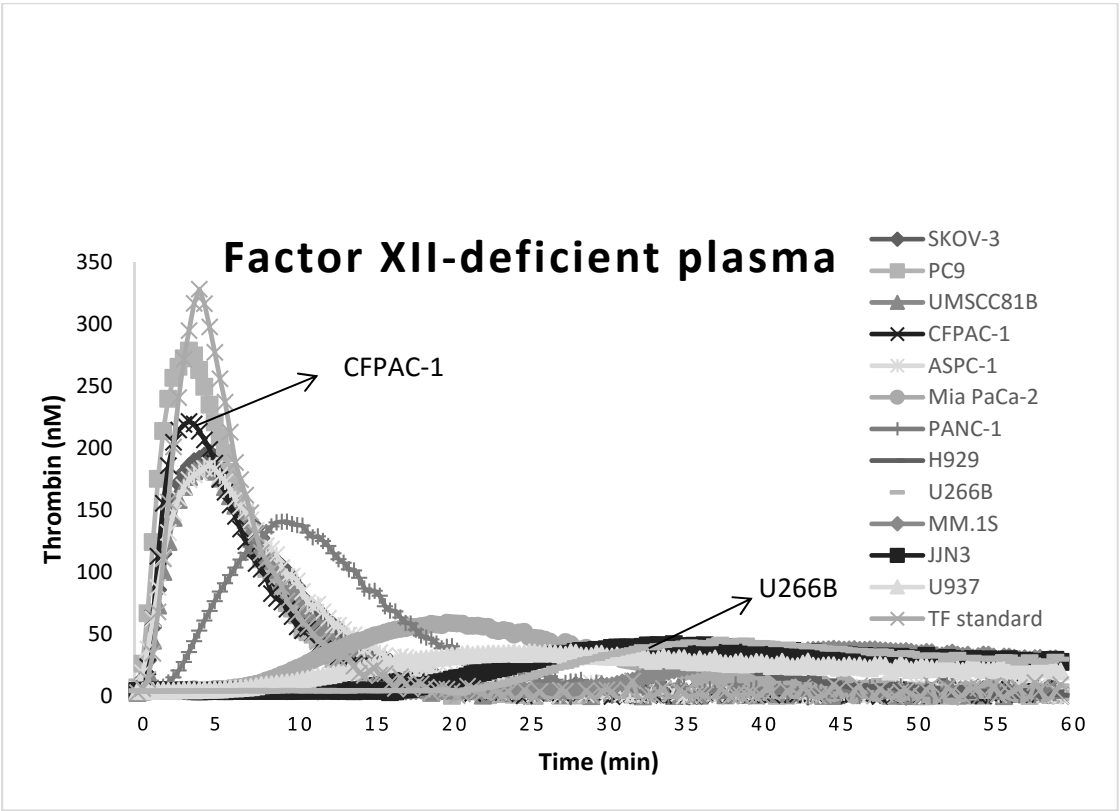


Figure 3: Influence of coagulation factors on thrombin generation in cell lines. TG in solid cancer cell lines such as PC was compared with Haematologic (e.g Multiple myeloma) cell lines *in vivo* on the CAT assay, each at constant cell number of 0.63×10^6/ml. Results are mean of n=4 ± S.D performed in duplicates. *P values are from two-way ANOVA multiple comparisons with NormTrol as baseline compared with Factor VII-deficient plasma and Factor XII-deficient plasma. Significance is at *p< 0.05, **p<0.01, ***p<0.001


NormTrol plasma
Thrombin (nM)
Time (min)
CFPAC-1
U266B
SKOV-3
PC9
UMSCC81B
CFPAC-1
ASPC-1
Mia PaCa-2
PANC-1
H929
U266B
MM.1S
JJN3
U937
TF standard


Factor XII-deficient plasma
Thrombin (nM)
Time (min)
CFPAC-1
U266B
SKOV-3
PC9
UMSCC81B
CFPAC-1
ASPC-1
Mia PaCa-2
PANC-1
H929
U266B
MM.1S
JJN3
U937
TF standard

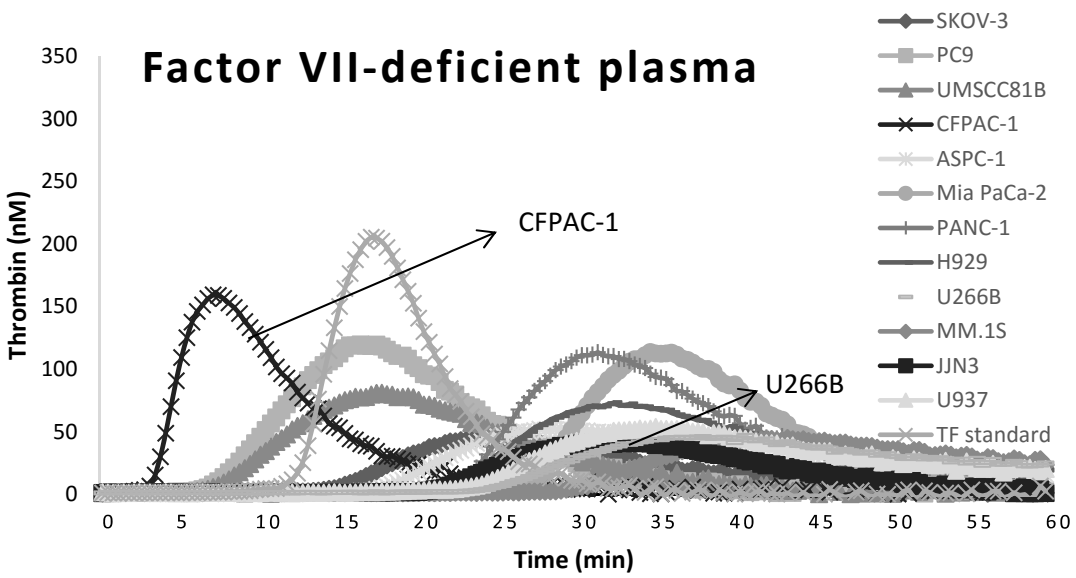
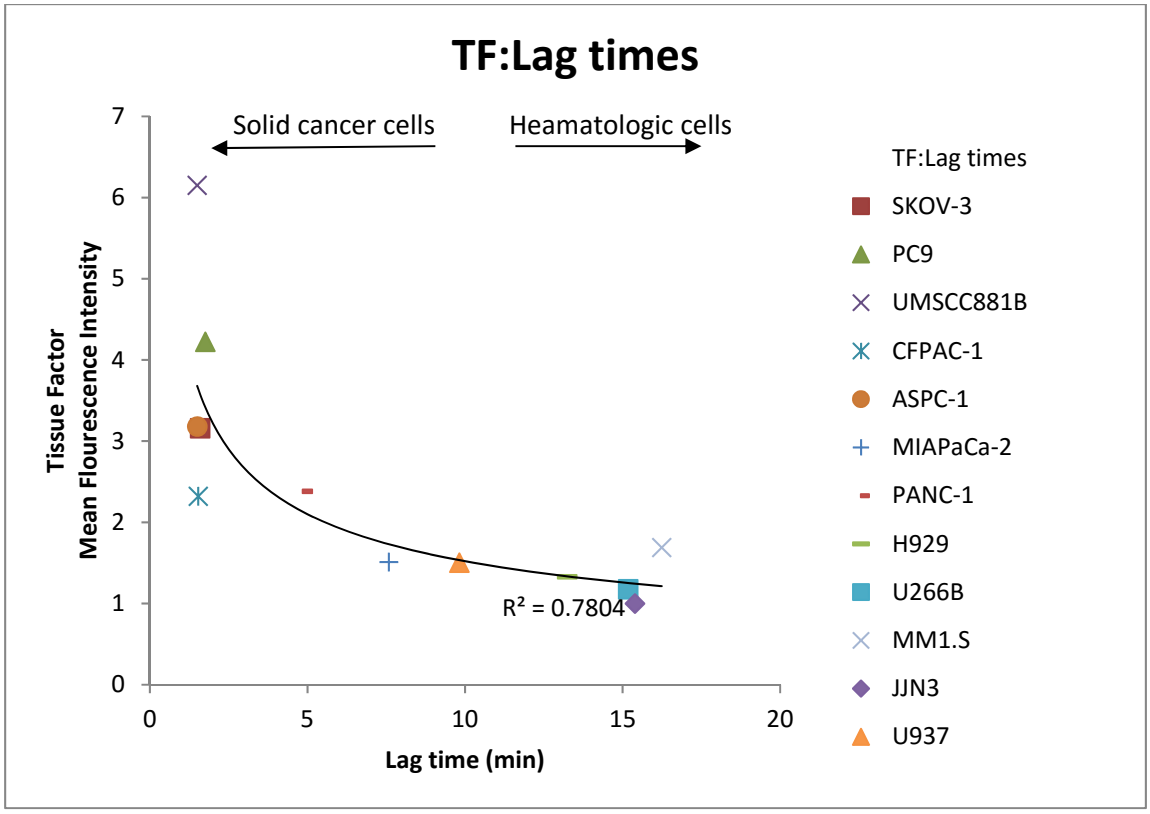


Figure 4. Thrombin generation curves. The graphs show the differences in TG in platelet-free plasma (NormTrol, Factor VII-deficient and Factor XII-deficient) induced by 12 cancer cell lines with low 1pM TF as control. Pancreatic CFPAC-1 and Multiple myeloma U266B are highlighted here as examples to compare the differences in TG.


TF:Lag times
Solid cancer cells
Heamatologic cells
Tissue Factor
Mean Flourescence Intensity
Lag time (min)
R² = 0.7804
TF:Lag times
SKOV-3
PC9
UMSCC881B
CFPAC-1
ASPC-1
MIAPaCa-2
PANC-1
H929
U266B
MM1.S
JJN3
U937

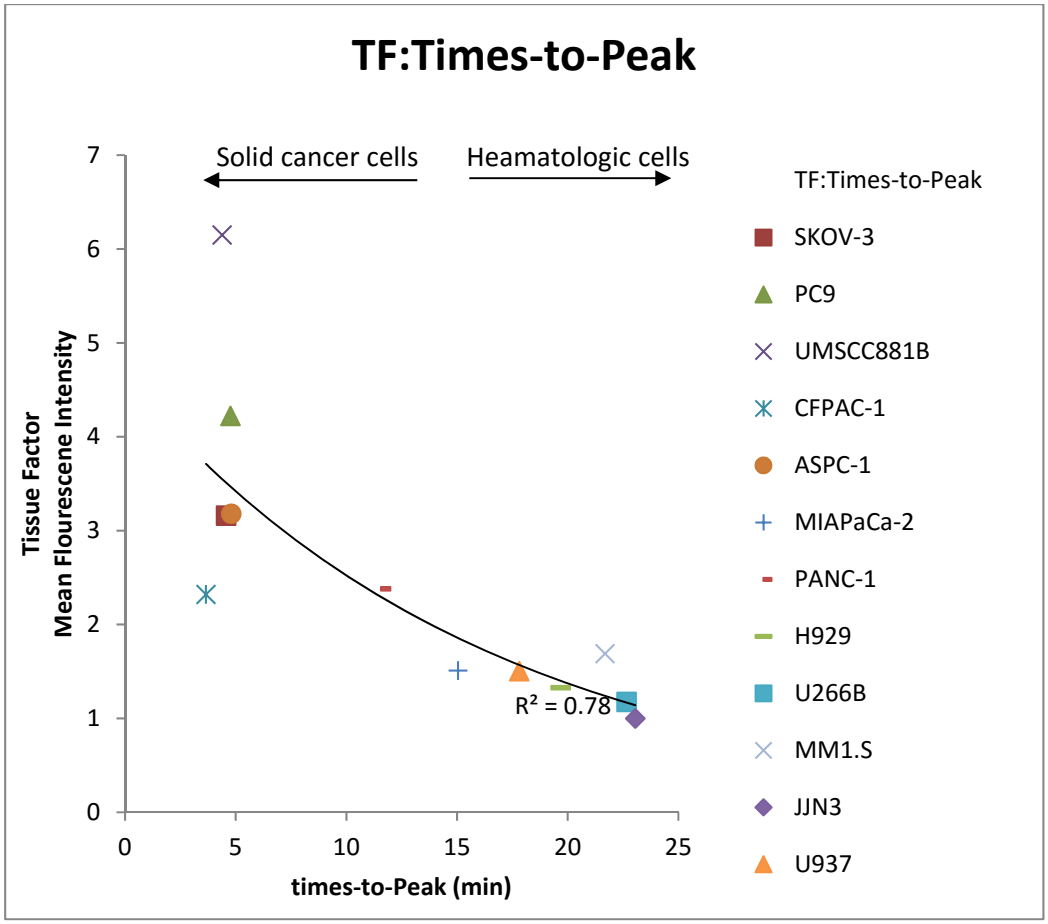


Figure 5: Influence of Tissue Factor (TF) on Thrombin generation in cell lines. Following 24 hour cell culture, flow cytometry was used to assess mean TF surface expression of cell lines at 0.63 x 10*6/ml cell concentration. There was an inverse correlation of TF expression with averaged TG parameters i.e as TF expression increases, Lag times and times-to-peak decreases. There existed a near linear relationship of TG parameter in NormTrol with TF expressed per 10^5 cells on Log_{10}/Log_{10} transformed data using regression and r^2 values (not shown). Results are mean of n=3 performed in triplicates.