Bone Marrow and Spleen Stain Index Visualizations

Billy Tomaszewski

2020-08-03

Contents

1 Overview

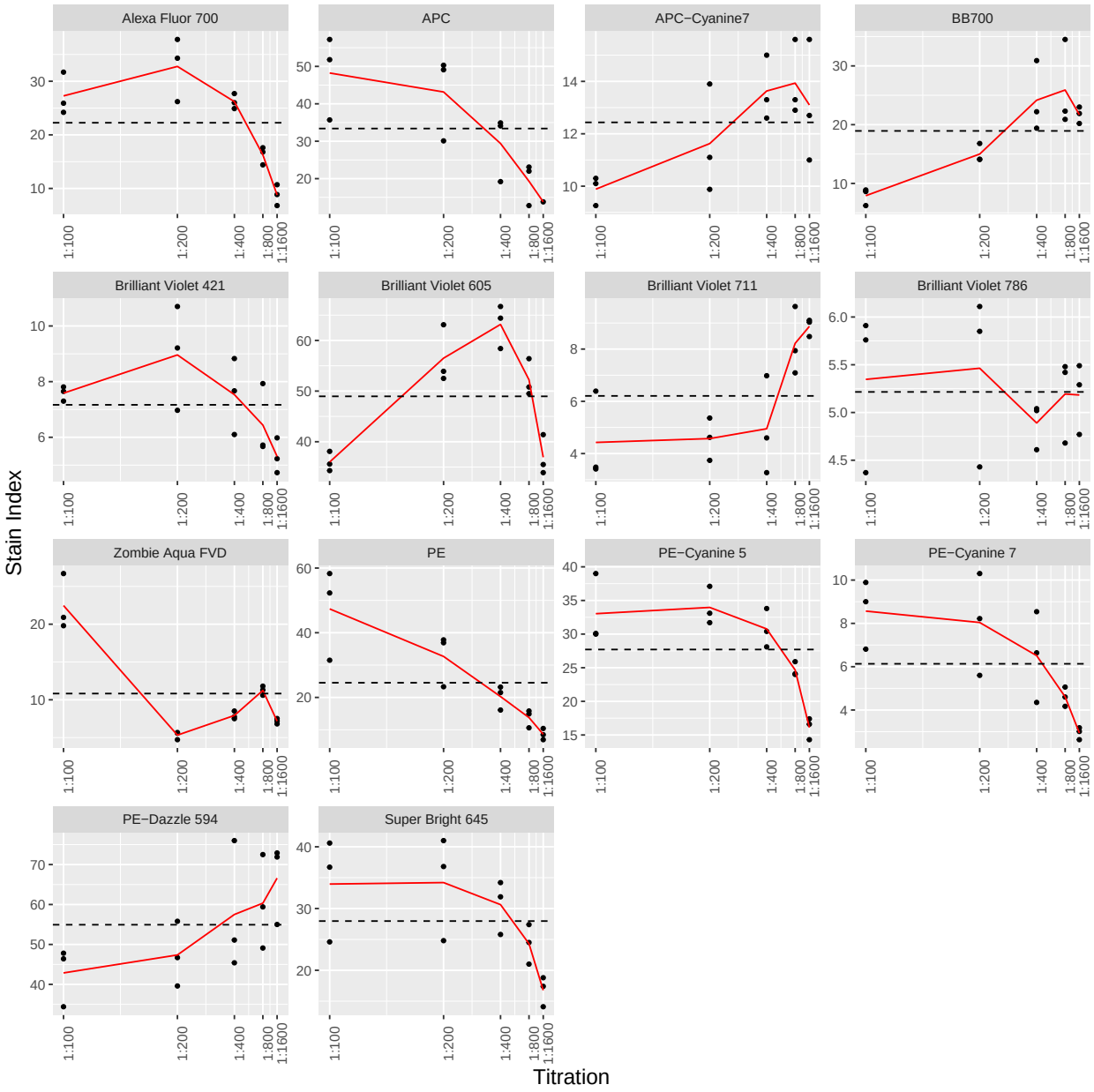
Titrations were performed on 3 biological samples from both Bone Marrow and Spleen. Bone Marrow was selected because of its relatvie enrichment for myeloid cells, and Spleen was used for its enrichment of lymphoid cells. Serial 2 fold dilutions were performed from 1:100-1:1600 (1:100,200,400,800,1600). This was perfomed to find the optimal titration for a 15 color panel that is being optimized. 14 colors x 3 samples x 5 dilutions resulted in 210 samples per organ. These samples were acquired on a BD LSRII, using a HTS so they could be acquired directly from a 96 well plate. In order to determine which titration was optimal, the FlowJo StainIndex plugin (v1.6) was used to automatically calculate stain index for each sample. Stain index is a metric which describes the quality of the signal produced, with higher Stain Indexs making it easier to resolve differences in cell types by flow cytometry. The equation used to for stain index is below:

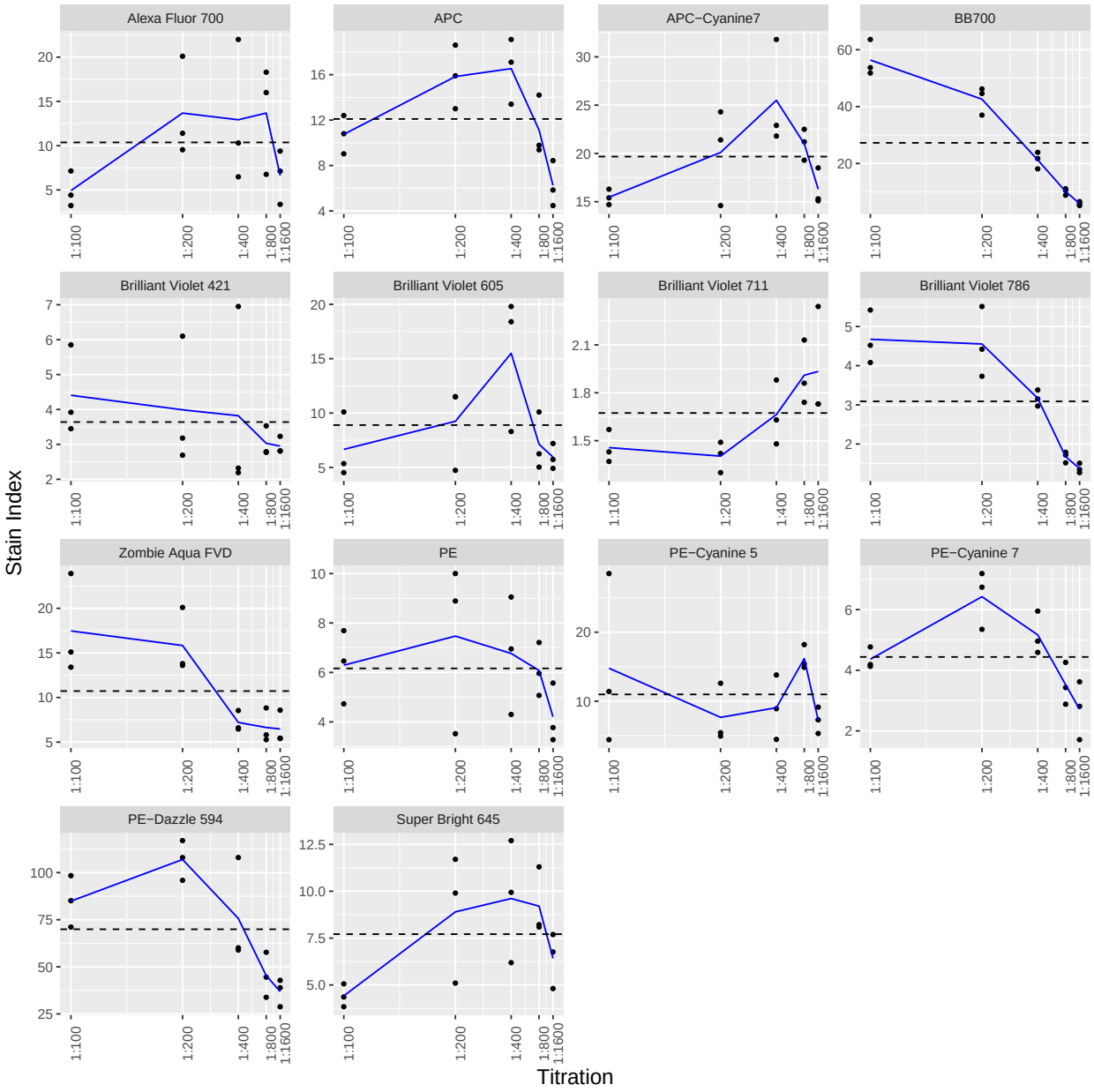
$$SI = \frac{MFI_{pos} - MFI_{neg}}{2 \times SD_{neg}}$$

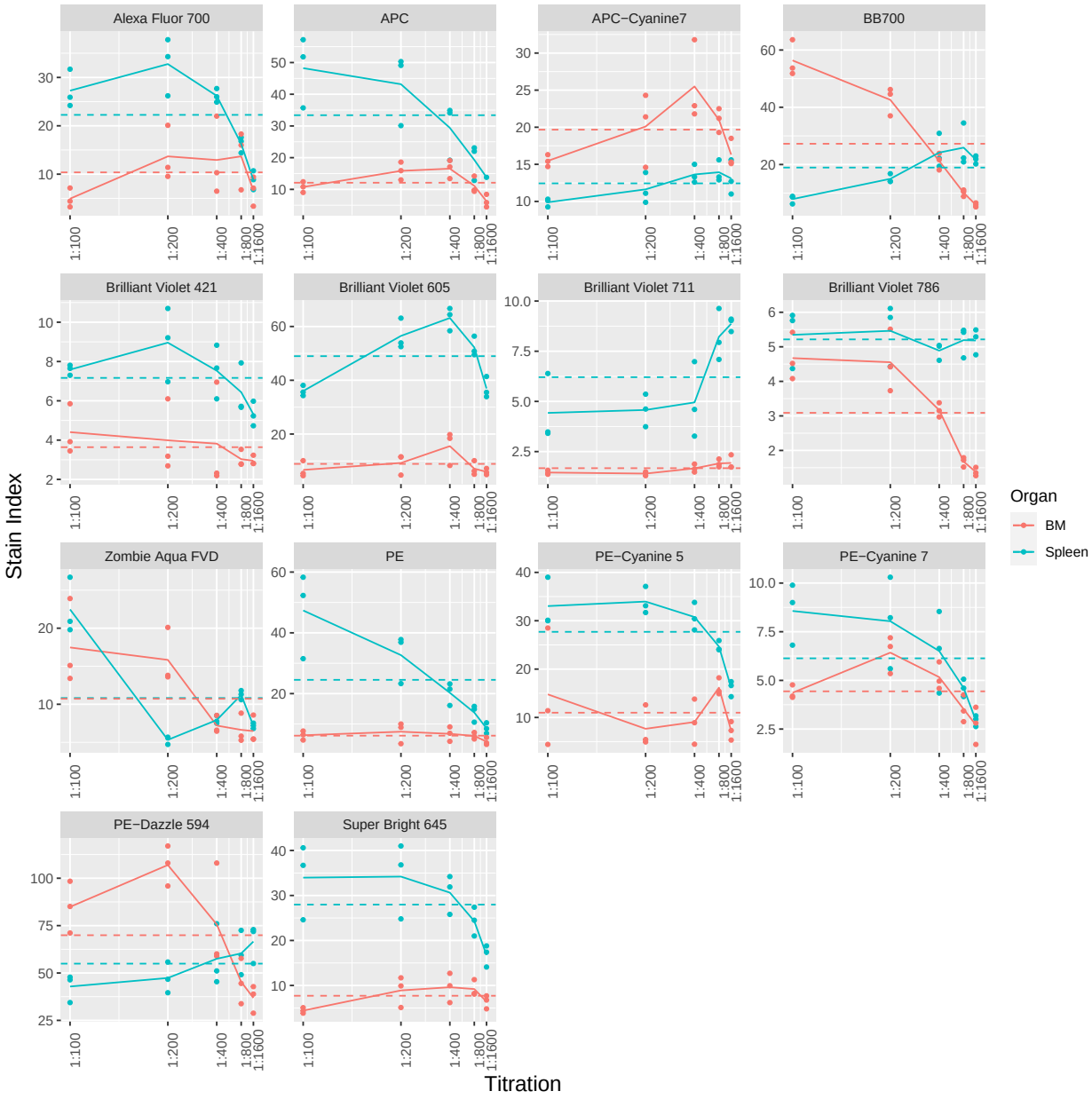
Where SI = Stain Index, MFI = Median Flourescent Intensity of a population, and SD = Standard Deviation, with *pos* and *neg* denoting whether the statistic was extracted from the positive or negative population in the sample.

2 Data Visualization

Below are the graphs of Titration vs. Stain Index for each organ, and then with them overlayed.


Alexa Fluor 700
APC
APC−Cyanine7
BB700
Brilliant Violet 421
Brilliant Violet 605
Brilliant Violet 711
Brilliant Violet 786
Zombie Aqua FVD
PE
PE−Cyanine 5
PE−Cyanine 7
PE−Dazzle 594
Super Bright 645
Stain Index
Titration
1:100
1:200
1:400
1:800
1:1600


Alexa Fluor 700
APC
APC−Cyanine7
BB700
Brilliant Violet 421
Brilliant Violet 605
Brilliant Violet 711
Brilliant Violet 786
Zombie Aqua FVD
PE
PE−Cyanine 5
PE−Cyanine 7
PE−Dazzle 594
Super Bright 645
Stain Index
Titration
1:100
1:200
1:400
1:800
1:1600



Using this data we also wanted to make a table to display which titrations were the best for each organ, and how much above average they were. To decide which titration was best overall, we took each titration and divided it by the average SI for that flourophore for that organ. This gives a metric that calculates the fold change improvement for a given titration in a given organ. This value is called *FoldChange* in the below tables.

Organ	Fluorochrome	Titration	Average Stain Index	Weight	Fold Change
Spleen	BB700	1:100	7.91	-	0.42
Spleen	BB700	1:200	15.00	-	0.79
Spleen	BB700	1:400	24.17	++	1.28
Spleen	BB700	1:800	25.90	+++	1.37
Spleen	BB700	1:1600	21.70	+	1.15

Spleen	PE	1:100	47.37	+++	1.93
Spleen	PE	1:200	32.67	++	1.33
Spleen	PE	1:400	20.27	+	0.83
Spleen	PE	1:800	13.77	-	0.56
Spleen	PE	1:1600	8.60	-	0.35
Spleen	PEcy5	1:100	33.03	++	1.19
Spleen	PEcy5	1:200	33.97	+++	1.23
Spleen	PEcy5	1:400	30.77	+	1.11
Spleen	PEcy5	1:800	24.67	-	0.89
Spleen	PEcy5	1:1600	16.10	-	0.58
Spleen	PeD594	1:100	42.87	-	0.78
Spleen	PeD594	1:200	47.37	-	0.86
Spleen	PeD594	1:400	57.50	+	1.05
Spleen	PeD594	1:800	60.33	++	1.10
Spleen	PeD594	1:1600	66.60	+++	1.21
Spleen	PEcy7	1:100	8.57	+++	1.40
Spleen	PEcy7	1:200	8.04	++	1.31
Spleen	PEcy7	1:400	6.51	+	1.06
Spleen	PEcy7	1:800	4.61	-	0.75
Spleen	PEcy7	1:1600	2.94	-	0.48
Spleen	APC	1:100	48.23	+++	1.44
Spleen	APC	1:200	43.17	++	1.29
Spleen	APC	1:400	29.40	+	0.88
Spleen	APC	1:800	19.30	-	0.58
Spleen	APC	1:1600	13.80	-	0.41
Spleen	AF700	1:100	27.27	++	1.23
Spleen	AF700	1:200	32.77	+++	1.47
Spleen	AF700	1:400	26.20	+	1.18
Spleen	AF700	1:800	16.27	-	0.73
Spleen	AF700	1:1600	8.79	-	0.39
Spleen	APCcy7	1:100	9.89	-	0.80
Spleen	APCcy7	1:200	11.63	-	0.93
Spleen	APCcy7	1:400	13.63	++	1.10
Spleen	APCcy7	1:800	13.93	+++	1.12
Spleen	APCcy7	1:1600	13.10	+	1.05
Spleen	BV421	1:100	7.59	++	1.06
Spleen	BV421	1:200	8.96	+++	1.25
Spleen	BV421	1:400	7.53	+	1.05
Spleen	BV421	1:800	6.44	-	0.90

Spleen	BV421	1:1600	5.31	-	0.74
Spleen	LD_ZA	1:100	22.47	+++	2.08
Spleen	LD_ZA	1:200	5.32	-	0.49
Spleen	LD_ZA	1:400	7.92	+	0.73
Spleen	LD_ZA	1:800	11.23	++	1.04
Spleen	LD_ZA	1:1600	7.17	-	0.66
Spleen	BV605	1:100	36.00	-	0.74
Spleen	BV605	1:200	56.50	++	1.15
Spleen	BV605	1:400	63.17	+++	1.29
Spleen	BV605	1:800	52.23	+	1.07
Spleen	BV605	1:1600	36.93	-	0.75
Spleen	SB645	1:100	33.97	++	1.21
Spleen	SB645	1:200	34.20	+++	1.22
Spleen	SB645	1:400	30.63	+	1.10
Spleen	SB645	1:800	24.30	-	0.87
Spleen	SB645	1:1600	16.77	-	0.60
Spleen	BV711	1:100	4.43	-	0.71
Spleen	BV711	1:200	4.57	-	0.74
Spleen	BV711	1:400	4.95	+	0.80
Spleen	BV711	1:800	8.22	++	1.32
Spleen	BV711	1:1600	8.87	+++	1.43
Spleen	BV786	1:100	5.35	++	1.03
Spleen	BV786	1:200	5.46	+++	1.05
Spleen	BV786	1:400	4.89	-	0.94
Spleen	BV786	1:800	5.19	+	1.00
Spleen	BV786	1:1600	5.18	-	0.99

Organ	Fluorochrome	Titration	Average Stain Index	Weight	Fold Change
BM	AF700	1:100	4.93	-	0.48
BM	AF700	1:200	13.68	++	1.32
BM	AF700	1:400	12.93	+	1.25
BM	AF700	1:800	13.69	+++	1.32
BM	AF700	1:1600	6.63	-	0.64
BM	APC	1:100	10.74	-	0.89
BM	APC	1:200	15.83	++	1.31
BM	APC	1:400	16.53	+++	1.37
BM	APC	1:800	11.12	+	0.92
BM	APC	1:1600	6.25	-	0.52
BM	APCcy7	1:100	15.47	-	0.79

BM	APCcy7	1:200	20.10	+	1.02
BM	APCcy7	1:400	25.50	+++	1.30
BM	APCcy7	1:800	21.00	++	1.07
BM	APCcy7	1:1600	16.30	-	0.83
BM	BB700	1:100	56.37	+++	2.07
BM	BB700	1:200	42.60	++	1.56
BM	BB700	1:400	21.23	+	0.78
BM	BB700	1:800	10.15	-	0.37
BM	BB700	1:1600	5.90	-	0.22
BM	BV421	1:100	4.41	+++	1.21
BM	BV421	1:200	3.99	++	1.10
BM	BV421	1:400	3.82	+	1.05
BM	BV421	1:800	3.03	-	0.83
BM	BV421	1:1600	2.95	-	0.81
BM	BV605	1:100	6.66	-	0.75
BM	BV605	1:200	9.24	++	1.04
BM	BV605	1:400	15.50	+++	1.74
BM	BV605	1:800	7.13	+	0.80
BM	BV605	1:1600	5.95	-	0.67
BM	BV711	1:100	1.46	-	0.87
BM	BV711	1:200	1.40	-	0.84
BM	BV711	1:400	1.66	+	0.99
BM	BV711	1:800	1.91	++	1.14
BM	BV711	1:1600	1.93	+++	1.16
BM	BV786	1:100	4.67	+++	1.51
BM	BV786	1:200	4.55	++	1.47
BM	BV786	1:400	3.17	+	1.03
BM	BV786	1:800	1.68	-	0.54
BM	BV786	1:1600	1.38	-	0.45
BM	LD_ZA	1:100	17.47	+++	1.63
BM	LD_ZA	1:200	15.83	++	1.48
BM	LD_ZA	1:400	7.20	+	0.67
BM	LD_ZA	1:800	6.64	-	0.62
BM	LD_ZA	1:1600	6.47	-	0.60
BM	PE	1:100	6.29	+	1.02
BM	PE	1:200	7.47	+++	1.21
BM	PE	1:400	6.77	++	1.10
BM	PE	1:800	6.08	-	0.99
BM	PE	1:1600	4.21	-	0.68

BM	PEcy5	1:100	14.77	++	1.35
BM	PEcy5	1:200	7.66	-	0.70
BM	PEcy5	1:400	9.06	+	0.82
BM	PEcy5	1:800	16.17	+++	1.47
BM	PEcy5	1:1600	7.25	-	0.66
BM	PEcy7	1:100	4.36	+	0.98
BM	PEcy7	1:200	6.43	+++	1.45
BM	PEcy7	1:400	5.17	++	1.16
BM	PEcy7	1:800	3.52	-	0.79
BM	PEcy7	1:1600	2.71	-	0.61
BM	PeD594	1:100	84.87	++	1.21
BM	PeD594	1:200	106.97	+++	1.53
BM	PeD594	1:400	75.67	+	1.08
BM	PeD594	1:800	45.33	-	0.65
BM	PeD594	1:1600	36.83	-	0.53
BM	SB645	1:100	4.42	-	0.57
BM	SB645	1:200	8.90	+	1.15
BM	SB645	1:400	9.61	+++	1.25
BM	SB645	1:800	9.20	++	1.19
BM	SB645	1:1600	6.42	-	0.83

From the above tables we can conclude which titrations work the best across organs.