Supplemental Table 2. Questionnaire Responses – Lab Gating SOP

Variable		N	%
Software Used for Data Analysis	FlowJo for Mac	48	43%
	FlowJo for PC	31	28%
	BD Diva	18	16%
	Beckman-Coulter Kaluza	4	4%
	Verity House	3	3%
	FCS Express	2	2%
	Weasel	2	2%
	Custom Software	2	2%
	Cyflogic	1	1%
Analysis Performed on	PC	60	54%
	Macintosh	50	45%
	Linux	1	1%
Biexponential scale adjustment	Sometimes	60	54%
	Never	28	25%
	Always	23	21%
Graphical Methods used to Visualize Data (Labs could select more than one option)	Dot plots	86	77%
	Histograms	33	30%
	Contour plots	31	28%
	Pseudo-color plots	14	13%
	Density plots	6	5%
	Zebra plots	5	5%
	Not Specified	4	4%
Cell Population used for First Gate	Lymphocytes	63	57%
	Singlets	17	15%
	Dump	13	12%
	Live Cells	6	5%
	CD3	5	5%
	Time	3	3%
	Custom Software	1	1%
	Unknown	3	3%
Doublet Exclusion	Always	58	52%
	Sometimes	35	32%
	Never	18	16%
Gating Style	Loose (Include Outliers)	73	66%
	Tight (Close to Central Population)	38	34%
Lymphocyte Gate Type	Freehand (polygon)	93	84%
	Elliptical	9	8%

	Freehand (polygon) or Elliptical	4	4%
	Rectangular	3	3%
	No Lymphocyte Gate Drawn	2	2%
CD3+ Gate Drawn Versus	Dump	25	23%
	SSC	20	18%
	CD4 and CD8	12	11%
	FSC	9	8%
	CD3 (histogram)	8	7%
	Cytokine	4	4%
	Dump or Live	3	3%
	Dump or SSC	3	3%
	SSC or FSC	3	3%
	Live Cells	2	2%
	Custom Software	2	2%
	CD4 and CD8 or Dump	1	1%
	CD4 or SSC	1	1%
	Dump or cytokine	1	1%
	Not specified	17	15%
CD3+ Gate Type	Rectangular	49	44%
	Freehand (Polygon)	41	37%
	Quadrant	8	7%
	Histogram	6	5%
	Elliptical	2	2%
	Other	5	5%
CD3 Dim cells included	Yes	82	74%
	No	29	26%
CD4 and CD8 cells gated in Same Plot	Yes, CD4 versus CD8	67	60%
	No, versus CD3	21	19%
	No, versus Cytokine	5	5%
	No, Histogram	3	3%
	No, Other (FCS, SSC, Software)	3	3%
	No, Not specified	12	11%
CD4 / CD8 Gate Type	Rectangular	48	43%
	Freehand (Polygon)	33	30%
	Quadrant	26	23%
	Elliptical	2	2%
	Other	2	2%
CD4/CD8 Dim cells included	Yes	84	76%
	No	27	24%
Reason for including Dim cells (n=84)	Down-regulation of co-receptor	46	55%
	Some Dim cells have Positive Response	19	23%

	Gate Placement less arbitrary	4	5%
	Dim cells are T cells	3	4%
	Not specified	12	14%
Reason for excluding Dim cells (n=27)	Dim cells can be Myeloid cells	11	41%
	Dim cells are Double Positive cells	4	15%
	No Dim Cells Present	3	11%
	Dim cells put in Separate gate	2	7%
	Background	1	4%
	No down-regulation	1	4%
	Not specified	5	19%
CD4+ CD8+ Double Positive Cells Included in a Gate	Double Positives Excluded	80	72%
	Double Positives in Separate Gate	10	9%
	Included in Both CD4 and CD8 Gate	11	10%
	Included in CD4 Gate	2	2%
	Included in CD8 Gate	3	3%
	Included, Gate Not Specified	5	5%
Cytokine Positive Gate Drawn	Rectangular	54	49%
	Quadrant	38	34%
	Freehand (polygon)	8	7%
	Histogram	7	6%
	Other	4	4%
Distance of Gate from Cytokine Negative Population	Somewhere in between Close and Far	47	42%
	Close to Negative Population	41	37%
	Far Away from Negative Population	5	5%
	Best Gate based on neg and pos controls	6	5%
	FMO	3	3%
	Isotype	3	3%
	Backgating	2	2%
	Compensation	1	1%
	Dependent on Cytokine	1	1%
	Staining Intensity	1	1%
	Software	1	1%
Backgating Used	Sometimes	49	44%
	Yes	47	42%
	No	15	14%
Same Gates Used for all Samples within a Donor	Yes, for consistency / comparability of results	73	66%
	Yes, reason not specified	8	7%
	No, due to changes based on stimulation	16	14%
	No, due to antibody variability	2	2%
	No, due to acquisition variability	1	1%
	No, due to intra-assay variation	1	1%

	No, reason not specified	3	3%
	No, Change gates *between* donors due to donor variability	7	6%

Phase II Questionnaire Responses – Comparison of SOPs

Greater confidence in results from	First time (Lab SOP)	62	57%
	Second time (Gating Strategy Instructions)	47	43%
Reasons for greater confidence in Your SOP (N=62) (Labs could state more than one reason)	Similar strategies	14	23%
	Cytokine Proximity Better in own SOP	8	13%
	First Gate on Lymphocytes	8	13%
	No inclusion of dims	8	13%
	Did not do Dump vs CD3 Gate	5	8%
	Include Double Positives	4	6%
	Don't use uniform gates	3	5%
	Time vs PE Gate	2	3%
	CD3 vs cytokines	2	3%
	All Steps of SOP	1	2%
	Backgating done in own SOP	1	2%
	CD4 not vs CD8	1	2%
	Shape of gate	1	2%
	Software	1	2%
	No FMO controls provided, in own SOP use FMO controls	4	6%
	Not Specified	8	13%
Reasons for greater confidence in Gating Strategy Instructions (N=47) (Labs could state more than one reason)	Dump vs CD3 Gate	21	45%
	Include Dim cells	9	19%
	Biexponential Scaling	8	17%
	CD4 vs CD8	5	11%
	Similar strategies	5	11%
	Cytokine Proximity Better in Gating Strategy Instructions	4	9%
	Lower background	1	2%
	Exclude Double Positives	1	2%
	No Histogram	1	2%
	Not Specified	1	2%
SOP established for general gating procedures	Yes	71	65%
	No	38	35%
Established training protocol to teach new lab members how to analyze data	No	62	57%
	Yes	47	43%
Support publication of general	Yes	98	90%

harmonization guidelines for gating	No	11	10%
Lab would adopt published guidelines	Yes	94	86%
even if different from lab own procedure	No	15	14%
Experience of Scientist	1 = Very Little Experience	1	1%
	2	0	0%
	3	19	17%
	4	42	39%
	5 = Very Experienced	47	43%
Number of years analyzing flow	1 – 5	28	26%
cytometry data	5.1 – 9.5	29	27%
	10 – 19	38	35%
	20+	14	13%
Ways personnel learned to gate and analyze complex flow cytometry data (Labs could select more than one option)	Training From Mentor	63	58%
	Learned On Own	60	55%
	Learned From Literature	59	54%
	Training From Others In Lab	45	41%
	Formal Training (Class)	40	37%
	Web-Based Training	11	10%
	Other: Learn from Conferences / Seminars / Experts in the field / Publications / Software Tutorials / Proficiency Panels	9	8%
Number of personnel in lab with gating experience	0	2	2%
	1	4	4%
	2	15	14%
	3	15	14%
	4	22	20%
	5	19	17%
	6 - 9	17	16%
	10 +	12	11%
	Unknown	3	3%
Person performing proficiency testing	Yes	75	69%
also routinely performs sample testing	Sometimes	24	22%
	No	10	9%