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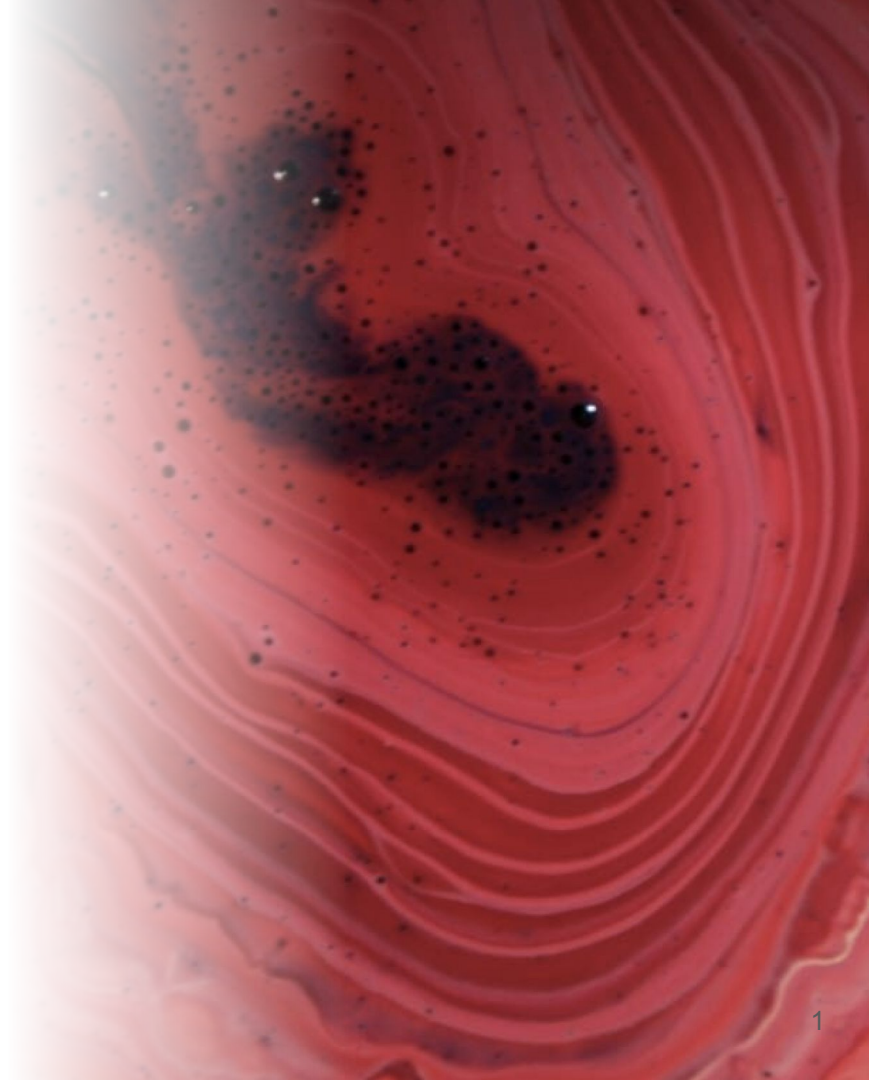
Minimum cell count for immune subset analysis

Teiko's carrier cell method for analysis of low cell count samples

April 2022

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Sample Dilution & Processing in Teiko Study

Teiko Study & Dataset
We processed and analyzed mass cytometry data for 5 dilutions of human PBMCs supplemented with mouse PBMCs as carrier cells. Total human PBMC counts per sample ranged from 5K to 500K and all samples were normalized to 500K with mouse PBMCs for barcoding, staining, and analysis as necessary.

Samples were stained with our Human pan-immune base panel. Anti-human CD45 was used to distinguish human PBMCs from mouse carrier cells for gating and analysis.

The following slides compare the immune cell population counts and frequencies across samples with varying numbers of human PBMCs.

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# Sample Information

Teiko Sample ID	Description	Total cell count (millions)	Viability (%)	Cell count prior acquisition	Estimated event in acquisition	Debarcoding with 0.3 separation
Hu500K_Ms0	0.5 million human cells mixed with 0 mouse cells	0.5	pre-fixed	0.28	0.24	0.241
Hu250K_Ms250K	250K human cells mixed with 250K mouse cells	0.5	pre-fixed	0.28	0.24	0.238
Hu100K_Ms400K	100K human cells mixed with 400K mouse cells	0.5	pre-fixed	0.28	0.24	0.236
Hu25K_Ms500K	25K human cells mixed with 0.5 million mouse cells	0.5	pre-fixed	0.28	0.24	0.253
Hu5K_Ms500K	5K human cells mixed with 0.5 million mouse cells	0.5	pre-fixed	0.28	0.24	0.206

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Immune subset counts and frequencies

Row Labels	Hu500K	Hu250K	Hu100K	Hu25K	Hu5K
Hu CD45+	200972	99578	42706	11932	3653
leukocytes	86428	41154	17725	4774	1433
iNKT	59	27	13	3	0
gd T cells	508	244	100	30	7
NKT cells	447	224	109	22	6
Tregs	2999	1446	657	182	50
CD4 T central memory	11532	5365	2220	620	187
CD4 T effector memory	4049	2025	892	240	76
CD4 T naive	15067	6938	2958	810	227
CD4 TEMRA	291	128	59	19	1
CD8 T central memory	899	377	170	40	8
CD8 T effector memory	2119	974	432	112	29
CD8 T naive	6322	2931	1261	342	95
CD8 TEMRA	425	199	78	30	13
CD4- CD8- T cells	944	480	197	43	14
CD4+ CD8+ T cells	120	66	20	13	1
B memory	4955	2393	1054	279	120
B naive	10216	4822	2070	554	169
Plasma cells / Plasmablasts	92	51	29	8	2
NK (CD16-)	380	193	97	23	10
NK (CD16+)	9565	4478	1994	567	155
Classical Mono (CD14+ CD16-)	8996	4648	1984	465	145
Intermediate Mono (CD14+ CD16+)	489	241	113	25	8
Non-classical Mono (CD14- CD16+)	803	407	157	53	16
conventional DCs	798	371	160	27	14
plasmacytoid DCs	148	66	38	9	2
Basophils	958	468	218	72	15

Row Labels	Hu500K	Hu250K	Hu100K	Hu25K	Hu5K
Hu CD45+					
leukocytes					
iNKT	0.07%	0.07%	0.07%	0.06%	0.00%
gd T cells	0.59%	0.59%	0.56%	0.63%	0.49%
NKT cells	0.52%	0.54%	0.61%	0.46%	0.42%
Tregs	3.47%	3.51%	3.71%	3.81%	3.49%
CD4 T central memory	13.34%	13.04%	12.52%	12.99%	13.05%
CD4 T effector memory	4.68%	4.92%	5.03%	5.03%	5.30%
CD4 T naive	17.43%	16.86%	16.69%	16.97%	15.84%
CD4 TEMRA	0.34%	0.31%	0.33%	0.40%	0.07%
CD8 T central memory	1.04%	0.92%	0.96%	0.84%	0.56%
CD8 T effector memory	2.45%	2.37%	2.44%	2.35%	2.02%
CD8 T naive	7.31%	7.12%	7.11%	7.16%	6.63%
CD8 TEMRA	0.49%	0.48%	0.44%	0.63%	0.91%
CD4- CD8- T cells	1.09%	1.17%	1.11%	0.90%	0.98%
CD4+ CD8+ T cells	0.14%	0.16%	0.11%	0.27%	0.07%
B memory	5.73%	5.81%	5.95%	5.84%	8.37%
B naive	11.82%	11.72%	11.68%	11.60%	11.79%
Plasma cells / Plasmablasts	0.11%	0.12%	0.16%	0.17%	0.14%
NK (CD16-)	0.44%	0.47%	0.55%	0.48%	0.70%
NK (CD16+)	11.07%	10.88%	11.25%	11.88%	10.82%
Classical Mono (CD14+ CD16-)	10.41%	11.29%	11.19%	9.74%	10.12%
Intermediate Mono (CD14+ CD16+)	0.57%	0.59%	0.64%	0.52%	0.56%
Non-classical Mono (CD14- CD16+)	0.93%	0.99%	0.89%	1.11%	1.12%
conventional DCs	0.92%	0.90%	0.90%	0.57%	0.98%
plasmacytoid DCs	0.17%	0.16%	0.21%	0.19%	0.14%
Basophils	1.11%	1.14%	1.23%	1.51%	1.05%

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Immune subset composition

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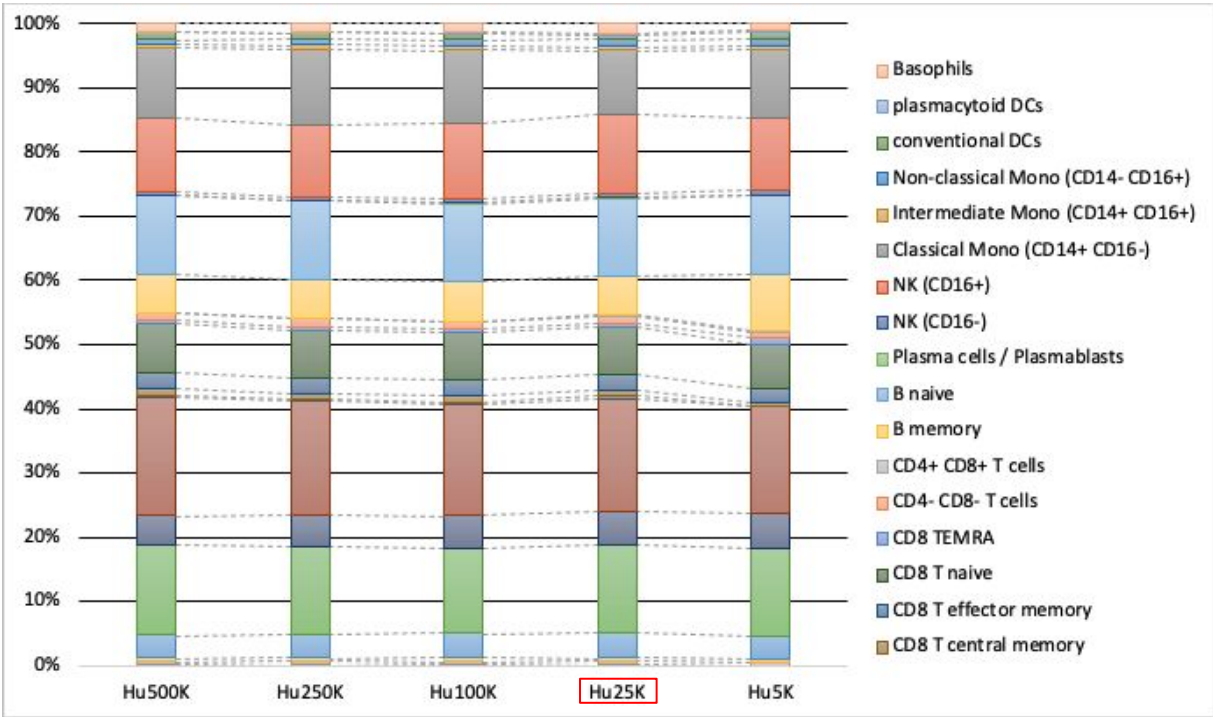
Summary of Teiko's assessment of minimum cell counts for immune profiling

- The relative frequencies of immune subsets are stable across samples with an input of 500K to 25K human PBMCs as starting material.
- While still detectable, the event counts of rare populations, like pDCs, plasma cells, and CD4 TEMRA, falls below 20 with an input of 25K cells or less.

Recommend a minimum of 50K cells per sample for accurate detection of all major immune subsets. For studies focused on rare subsets, higher cell counts are recommended.