



Figure S1 Age and gender distribution (Straight lines represent reference intervals for different age groups: Upper limit P97.5 and lower limit P2.5). (A) Absolute count of CD3⁺CD16⁺CD56⁺ cells; (B) CD3⁺CD16⁺CD56⁺ cells (%); (C) absolute count of CD3⁺CD19⁺ cells; (D) CD3⁺CD19⁺ cells (%); (E) absolute count of CD3⁺ cells; (F) CD3⁺ cells (%); (G) absolute count of CD3⁺CD4⁺ cells; (H) CD3⁺CD4⁺ cells (%); (I) absolute count of CD3⁺CD8⁺ cells; (J) CD3⁺CD8⁺ cells (%); (K) CD4⁺/CD8⁺ ratio.

Table S1 Results comparison of quality control blood in 3 centers

Instrument	Type	Project	Center	QC Blood 1	QC Blood 2	QC Blood 3	QC Blood 4	QC Blood 5	Mean±2SD	Range	Number of out of control ^a
FACSCalibur [†]	High	CD3+ (%)	01	71	72	71	72	71	71.2±1.12	60.9-80.9	1
			02	71	71	71	71	71			
			03	72	71	70	71	72			
BD FACSCanto II	High	CD3+ (%)	01	71.2	71.01	71.26	71.72	71.91	71.763±1.3768	60.9-80.9	1
			02	71.99	70.37	71.11	71.87	72			
			03	72.93	71.97	71.92	72.89	72.3			
BD FACSLyric	High	CD3+ (%)	01	72.48	71.07	68.48	72.28	71.29	70.216±3.2361	60.9-80.9	0
			02	71.67	70.8	70.01	71.81	69.85			
			03	67.45	70.1	67.69	68.5	69.76			
FACSCalibur [†]	Low	CD3+ (%)	01	52	53	53	53	55	53.0±2.00	43.0-63.0	0
			02	53	52	53	53	54			
			03	54	52	51	54	53			
BD FACSCanto II	Low	CD3+ (%)	01	52.86	53.8	53.33	52.01	53.06	53.091±1.5255	43.0-63.0	0
			02	52.32	52.79	52.67	53.06	54.51			
			03	52.61	53.67	52.82	52.28	54.57			
BD FACSLyric	Low	CD3+ (%)	01	54.91	54.37	53.99	54.2	53.67	52.875±2.6101	43.0-63.0	0
			02	52.28	52.48	53.55	52.34	53.75			
			03	50.4	51.51	52.48	52.06	51.14			
FACSCalibur [†]	High	CD3+ (cells/ul)	01	1115	1271	1201	1191	1257	1237.2±115.74	1003.4-1505.1	1
			02	1240	1294	1274	1336	1256			
			03	1246	1145	1213	1292	1227			
BD FACSCanto II	High	CD3+ (cells/ul)	01	1158.22	1260.92	1271.86	1239.44	1349.97	1224.281±102.4005	1003.4-1505.1	1
			02	1196.53	1188.73	1157.8	1274.72	1237.35			
			03	1226.49	1190.55	1195.07	1185.63	1230.94			
BD FACSLyric	High	CD3+ (cells/ul)	01	1197	1252	1272	1161	1284	1205.3±114.44	1003.4-1505.1	0
			02	1147	1240	1195	1260	1262			
			03	1236	1122	1194	1117	1140			
FACSCalibur [†]	Low	CD3+ (cells/ul)	01	628	670	650	620	636	640.7±38.37	515.5-806.3	0
			02	665	636	655	634	648			
			03	636	609	609	651	664			
BD FACSCanto II	Low	CD3+ (cells/ul)	01	657.24	647.54	646.97	634.42	635.24	626.990±81.0412	515.5-806.3	1
			02	639.11	637.78	621.13	644.31	710.61			
			03	549.78	577.91	567.4	590.84	644.57			

Table S1 (continued)

Table S1 (continued)

Instrument	Type	Project	Center	QC Blood 1	QC Blood 2	QC Blood 3	QC Blood 4	QC Blood 5	Mean±2SD	Range	Number of out of control ^a
BD FACSLyric	Low	CD3+ (cells/ul)	01	660	668	676	643	625	636.8±94.13	515.5-806.3	0
			02	685	666	662	645	709			
			03	550	550	601	621	591			
FACSCalibur [†]	High	CD3-CD19+ (%)	01	16	15	16	16	16	15.9±0.92	12.1-20.1	1
			02	16	16	16	16	16			
			03	16	16	17	15	16			
BD FACSCanto II	High	CD3-CD19+ (%)	01	15.75	15.87	15.81	16.36	16.44	15.781±0.9743	12.1-20.1	1
			02	15.96	15.86	15.15	16.05	14.76			
			03	14.97	15.65	16.13	15.73	16.22			
BD FACSLyric	High	CD3-CD19+ (%)	01	14.53	16.45	15.35	16.09	16.29	15.713±1.7501	12.1-20.1	1
			02	16.19	16.17	17.54	16.08	16.43			
			03	14.89	14.7	14.57	15.55	14.87			
FACSCalibur [†]	Low	CD3-CD19+ (%)	01	24	24	23	24	22	23.7±1.77	17.9-29.9	1
			02	24	24	24	23	23			
			03	26	24	24	23	24			
BD FACSCanto II	Low	CD3-CD19+ (%)	01	24.33	25.14	24.19	24.19	25.08	24.463±1.3822	17.9-29.9	0
			02	23.95	23.84	23.33	24.71	23.47			
			03	24.95	25.08	23.9	25.28	25.51			
BD FACSLyric	Low	CD3-CD19+ (%)	01	24.45	24.26	24.42	24.04	23.98	23.702±1.4892	17.9-29.9	0
			02	22.28	24.83	24.17	22.88	22.53			
			03	23.5	24.06	23.37	23.64	23.12			
FACSCalibur [†]	High	CD3-CD19+ (cells/ul)	01	258	263	263	269	326	281.3±36.23	197.1-372.5	1
			02	286	288	288	295	282			
			03	278	264	300	291	268			
BD FACSCanto II	High	CD3-CD19+ (cells/ul)	01	260.2	287.35	297.37	288.31	332.14	272.231±45.4752	197.1-372.5	1
			02	267.19	263.02	251.29	286.84	245.62			
			03	254.1	259.73	256.23	259.3	274.77			
BD FACSLyric	High	CD3-CD19+ (cells/ul)	01	248	294	293	254	318	271.1±48.61	197.1-372.5	0
			02	264	274	305	273	293			
			03	260	240	249	260	242			

Table S1 (continued)

Table S1 (continued)

Instrument	Type	Project	Center	QC Blood 1	QC Blood 2	QC Blood 3	QC Blood 4	QC Blood 5	Mean±2SD	Range	Number of out of control ^a
FACSCalibur [†]	Low	CD3–CD19+ (cells/ul)	01	286	295	277	279	239	284.1±40.15	186.9-409.2	1
			02	304	305	286	278	263			
			03	260	302	310	273	304			
BD FACSCanto II	Low	CD3–CD19+ (cells/ul)	01	292.82	297.22	289.9	309.3	299.29	290.653±27.1495	186.9-409.2	0
			02	291.73	291.11	266.28	308.06	295.19			
			03	274.57	275.12	268.38	301.74	299.09			
BD FACSLyric	Low	CD3–CD19+ (cells/ul)	01	287	273	304	280	281	284.5±29.92	186.9-409.2	1
			02	295	317	289	288	291			
			03	267	271	270	294	261			
FACSCalibur [†]	High	CD3+CD4+ (%)	01	46	46	45	47	46	45.7±1.63	39.5-52.5	1
			02	45	45	47	46	46			
			03	46	45	45	44	46			
BD FACSCanto II	High	CD3+CD4+ (%)	01	45.23	44.58	47.15	48	47.93	46.275±2.1281	39.5-52.5	0
			02	45.73	44.56	46.26	46.72	46.13			
			03	47	46.26	45.7	47.16	45.72			
BD FACSLyric	High	CD3+CD4+ (%)	01	45.62	44.97	45.38	44.63	46.15	45.186±2.3069	39.5-52.5	1
			02	46.23	46.4	45.33	45.57	46.28			
			03	43.22	44.89	42.25	44.94	45.93			
FACSCalibur [†]	Low	CD3+CD4+ (%)	01	12	13	13	13	14	12.4±1.26	8.3-16.3	1
			02	12	12	12	12	12			
			03	12	12	12	12	13			
BD FACSCanto II	Low	CD3+CD4+ (%)	01	12.84	12.92	12.73	12.06	12.2	12.332±0.9839	8.3-16.3	0
			02	12.05	12.39	11.56	11.87	12.1			
			03	12.53	12.44	12.52	13.25	11.52			
BD FACSLyric	Low	CD3+CD4+ (%)	01	13.31	11.02	12.62	12.26	11.09	11.793±1.3233	8.3-16.3	1
			02	12.31	11.02	11.75	11.5	11.68			
			03	12.16	11.86	11.04	11.4	11.88			
FACSCalibur [†]	High	CD3+CD4+ (cells/ul)	01	712	844	808	756	718	784.7±100.09	651.0-976.5	0
			02	795	805	862	864	804			
			03	766	703	784	769	780			

Table S1 (continued)

Table S1 (continued)

Instrument	Type	Project	Center	QC Blood 1	QC Blood 2	QC Blood 3	QC Blood 4	QC Blood 5	Mean±2SD	Range	Number of out of control ^a
BD FACSCanto II	High	CD3+CD4+ (cells/ul)	01	724.44	775.38	796.97	813.21	832.15	781.367±63.0512	651.0-976.5	0
			02	754.39	766.68	739.23	822	818.02			
			03	782.74	762.34	793.51	757.37	782.08			
BD FACSLyric	High	CD3+CD4+ (cells/ul)	01	728	781	823	729	763	773.2±97.52	651.0-976.5	0
			02	728	839	760	826	846			
			03	832	704	770	715	754			
FACSCalibur [†]	Low	CD3+CD4+ (cells/ul)	01	152	169	154	147	162	149.9±21.45	92.0-214.7	0
			02	153	143	156	140	158			
			03	150	131	130	147	157			
BD FACSCanto II	Low	CD3+CD4+ (cells/ul)	01	164.64	158.19	156.37	139.94	146.46	144.512±24.4540	92.0-214.7	0
			02	147.61	148.04	140.81	140.3	163.24			
			03	123.78	131.48	128.15	141.6	137.07			
BD FACSLyric	Low	CD3+CD4+ (cells/ul)	01	163	147	159	148	129	142.3±27.94	92.0-214.7	0
			02	160	139	150	139	157			
			03	127	120	125	130	141			
FACSCalibur [†]	High	CD3+CD8+ (%)	01	22	24	22	24	23	22.3±1.63	16.4-30.4	2
			02	22	23	21	22	22			
			03	22	22	22	22	22			
BD FACSCanto II	High	CD3+CD8+ (%)	01	26.39	25.66	25.38	24.88	25.27	24.311±4.0389	16.4-30.4	0
			02	22.46	22.12	21.15	20.7	22.19			
			03	24.58	25.15	25.56	26.84	26.33			
BD FACSLyric	High	CD3+CD8+ (%)	01	21.1	22.59	21	23.64	22.66	21.221±2.5045	16.4-30.4	0
			02	22.51	21.97	21.32	21.27	20.75			
			03	19.97	20.62	19.29	19.83	19.79			
FACSCalibur [†]	Low	CD3+CD8+ (%)	01	33	33	36	32	37	34.5±2.91	30.1-44.1	0
			02	34	34	34	34	34			
			03	36	34	34	37	35			
BD FACSCanto II	Low	CD3+CD8+ (%)	01	40.59	41.7	39.42	39.65	40.94	38.451±5.8891	30.1-44.1	0
			02	33.62	34.39	35.02	35.2	36.18			
			03	38.53	40.31	38.69	38.92	43.61			

Table S1 (continued)

Table S1 (continued)

Instrument	Type	Project	Center	QC Blood 1	QC Blood 2	QC Blood 3	QC Blood 4	QC Blood 5	Mean±2SD	Range	Number of out of control ^a
BD FACSLyric	Low	CD3+CD8+ (%)	01	36.45	37.84	36.32	35.49	37.22	34.909±3.4045	30.1-44.1	0
			02	34.56	33.63	35.08	32.01	35.32			
			03	32.73	34.82	35.58	34.04	32.54			
FACSCalibur [†]	High	CD3+CD8+ (cells/ul)	01	344	432	404	396	365	385.1±51.97	298.5-529.4	0
			02	398	420	385	417	375			
			03	372	345	380	376	368			
BD FACSCanto II	High	CD3+CD8+ (cells/ul)	01	422.59	446.27	428.97	421.41	438.8	410.266±67.5728	298.5-529.4	1
			02	370.6	380.59	338.06	364.25	393.52			
			03	409.34	414.45	443.79	430.99	450.36			
BD FACSLyric	High	CD3+CD8+ (cells/ul)	01	337	392	381	386	375	362.9±54.72	298.5-529.4	0
			02	354	397	357	386	379			
			03	384	323	352	315	325			
FACSCalibur [†]	Low	CD3+CD8+ (cells/ul)	01	405	424	437	369	437	416.9±56.43	339.1-586.2	0
			02	429	398	426	399	435			
			03	463	369	383	443	436			
BD FACSCanto II	Low	CD3+CD8+ (cells/ul)	01	520.37	510.43	484.32	460.11	491.7	450.540±95.5245	339.1-586.2	0
			02	411.92	411.08	426.62	416.15	488.24			
			03	380.58	425.92	396.11	415.8	518.75			
BD FACSLyric	Low	CD3+CD8+ (cells/ul)	01	447	506	457	429	431	421.4±90.72	339.1-586.2	0
			02	448	424	447	386	476			
			03	342	351	403	389	385			
FACSCalibur [†]	High	CD3- CD16+CD56 (%)	01	11	12	11	11	12	11.5±1.49	4.7-18.7	2
			02	12	12	12	12	12			
			03	10	12	11	12	10			
BD FACSCanto II	High	CD3- CD16+CD56 (%)	01	12.13	11.83	12.44	11.49	11.4	11.387±1.4058	4.7-18.7	0
			02	10.97	12.02	12.14	10.38	12.03			
			03	10.18	10.93	10.67	11.35	10.84			

Table S1 (continued)

Table S1 (continued)

Instrument	Type	Project	Center	QC Blood 1	QC Blood 2	QC Blood 3	QC Blood 4	QC Blood 5	Mean±2SD	Range	Number of out of control ^a
BD FACSLyric	High	CD3- CD16+CD56 (%)	01	10.23	11.23	12.75	10.63	11.53	11.210±1.3494	4.7-18.7	1
			02	11.23	11.42	11.02	9.87	11.89			
			03	11.32	10.84	11.33	11.39	11.47			
FACSCalibur [†]	Low	CD3- CD16+CD56 (%)	01	22	21	21	20	21	21.0±1.69	16.5-26.5	1
			02	20	21	21	21	20			
			03	20	21	23	22	21			
BD FACSCanto II	Low	CD3- CD16+CD56 (%)	01	22.68	20.67	20.21	22.6	21.47	21.375±1.5324	16.5-26.5	0
			02	21.63	22.02	21.65	21.69	20.76			
			03	20.9	20.61	21.81	21.59	20.34			
BD FACSLyric	Low	CD3- CD16+CD56 (%)	01	20.05	19.32	19.97	19.77	20.63	20.537±1.8588	16.5-26.5	1
			02	22.53	20.41	19.88	21.23	20.51			
			03	22.25	20.76	20.83	19.32	20.6			
FACSCalibur [†]	High	CD3- CD16+CD56 (cells/ul)	01	167	199	176	188	245	199.9±41.52	109.7-304.3	1
			02	200	214	211	219	207			
			03	186	199	187	224	177			
BD FACSCanto II	High	CD3- CD16+CD56 (cells/ul)	01	200.43	214.26	233.98	202.52	230.41	196.408±37.8831	109.7-304.3	0
			02	183.61	199.37	201.32	185.6	200.19			
			03	172.79	181.42	169.53	187	183.69			
BD FACSLyric	High	CD3- CD16+CD56 (cells/ul)	01	174	201	243	168	225	193.6±41.31	109.7-304.3	1
			02	183	193	192	168	212			
			03	197	177	194	190	187			
FACSCalibur [†]	Low	CD3- CD16+CD56 (cells/ul)	01	253	258	259	230	235	251.9±37.17	198.7-337.5	1
			02	252	269	250	251	228			
			03	214	265	286	268	261			

Table S1 (continued)

Table S1 (continued)

Instrument	Type	Project	Center	QC Blood 1	QC Blood 2	QC Blood 3	QC Blood 4	QC Blood 5	Mean±2SD	Range	Number of out of control ^a
BD FACSCanto II	Low	CD3– CD16+CD56 (cells/ul)	01	273.02	244.39	242.17	288.94	256.24	254.198±34.5960	198.7-337.5	1
			02	263.41	268.93	247.09	270.46	261.13			
			03	230.05	226.06	244.98	257.6	238.5			
BD FACSLyric	Low	CD3– CD16+CD56 (cells/ul)	01	235	217	248	230	242	246.8±39.92	198.7-337.5	1
			02	299	261	238	267	265			
			03	253	234	241	240	232			

*, Center 01 used BD Calibur. ^a, the number of test results outside the range of mean ±2 SD. SD, standard deviation.

Table S2 Nested ANOVA for the influence of gender, age, and region on the value of lymphocyte subsets

		Df	Sum. Sq	Mean. Sq	F-value	P-value (>F)
CD-CD16+CD56+ (cells/ul)	Gender	1	721376.559	721376.559	20.454	0.000*
	Gender: Age	6	290145.333	48357.556	1.371	0.224
	Gender: Age: Region	16	549740.340	34358.771	0.974	0.484
	Residual	472	16646594.856	35268.209		
CD3–CD16+CD56+ (%)	Gender	1	1367.064	1367.064	24.847	0.000*
	Gender: Age	6	634.815	105.803	1.923	0.075
	Gender: Age: Region	16	877.862	54.866	0.997	0.458
	Residual	472	25969.309	55.020		
CD3–CD19+ (cells/ul)	Gender	1	66117.489	66117.489	7.068	0.008*
	Gender: Age	6	51359.039	8559.840	0.915	0.484
	Gender: Age: Region	16	338722.392	21170.150	2.263	0.004*
	Residual	472	4415329.103	9354.511		
CD3–CD19+ (%)	Gender	1	217.203	217.203	16.642	0.000*
	Gender: Age	6	105.397	17.566	1.346	0.235
	Gender: Age: Region	16	332.624	20.789	1.593	0.067
	Residual	472	6160.338	13.052		
CD3+ (cells/ul)	Gender	1	34097.462	34097.462	0.297	0.586
	Gender: Age	6	3340684.402	556780.734	4.858	0.000*
	Gender: Age: Region	16	2049239.693	128077.481	1.117	0.335
	Residual	472	54098967.663	114616.457		

Table S2 (continued)

Table S2 (continued)

		Df	Sum. Sq	Mean. Sq	F-value	P-value (>F)
CD3+ (%)	Gender	1	478.127	478.127	8.212	0.004*
	Gender: Age	6	582.306	97.051	1.667	0.127
	Gender: Age: Region	16	851.807	53.238	0.914	0.553
	Residual	472	27482.167	58.225		
CD3+CD4+ (cells/ul)	Gender	1	387191.943	387191.943	8.371	0.004*
	Gender: Age	6	142770.685	23795.114	0.514	0.798
	Gender: Age: Region	16	888584.074	55536.505	1.201	0.263
	Residual	472	21832178.677	46254.616		
CD3+CD4+ (%)	Gender	1	1394.010	1394.010	33.066	0.000*
	Gender: Age	6	957.132	159.522	3.784	0.001*
	Gender: Age: Region	16	924.095	57.756	1.370	0.152
	Residual	472	19898.991	42.159		
CD3+CD8+ (cells/ul)	Gender	1	143317.143	143317.143	4.020	0.046*
	Gender: Age	6	1722187.283	287031.214	8.052	0.000*
	Gender: Age: Region	16	574811.822	35925.739	1.008	0.447
	Residual	472	16826332.856	35649.010		
CD3+CD8 (%)	Gender	1	167.851	167.851	3.473	0.063
	Gender: Age	6	1447.980	241.330	4.993	0.000*
	Gender: Age: Region	16	770.949	48.184	0.997	0.459
	Residual	472	22811.758	48.330		
CD4+/CD8+	Gender	1	3.283	3.283	11.220	0.001*
	Gender: Age	6	14.394	2.399	8.200	0.000*
	Gender: Age: Region	16	5.956	0.372	1.272	0.210
	Residual	472	138.088	0.293		

* The difference is statistically significant. ANOVA, analysis of variance; Df, degrees of freedom.

Table S3 Z-test and difference analysis grouped by Gender-1

	Male		Female		Z	Z standard (Z*)	Z difference (Z-Z*)
	Mean	SD	Mean	SD			
N	233	233	263	263			
CD3-CD16+CD56+ (cells/ul)	440.181	203.701	363.768	173.203	4.47	4.31	0.16 [†]
CD3-CD16+CD56+ (%)	20.591	7.935	17.264	7.010	4.92	4.31	0.61 [†]
CD3-CD19+ (cells/ul)	222.636	105.487	245.769	92.129	-2.59	4.31	-1.72
CD3-CD19+ (%)	10.375	3.902	11.701	3.420	-4	4.31	-0.31
CD3+ (cells/ul)	1439.807	345.823	1456.420	348.076	-0.53	4.31	-3.78
CD3+ (%)	67.723	7.711	69.690	7.598	-2.86	4.31	-1.45
CD3+CD4+ (cells/ul)	715.491	213.929	771.473	216.195	-2.89	4.31	-1.42
CD3+CD4+ (%)	33.518	6.574	36.877	6.698	-5.63	4.31	1.32 [†]
CD3+CD8+ (cells/ul)	608.724	199.705	574.665	194.099	1.92	4.31	-2.39
CD3+CD8+ (%)	28.616	7.429	27.450	6.831	1.81	4.31	-2.5
CD4+/CD8+	1.297	0.572	1.460	0.562	-3.2	4.31	-1.11

* Statistic Z with a “critical” value purposed by “Harris and Boyd”. [†] The difference is statistically significant. SD, standard deviation.