

## Nonhuman Primate Immunology Quality Assessment Program SUMMARY

### Part I: GENERAL LABORATORY TECHNIQUES -

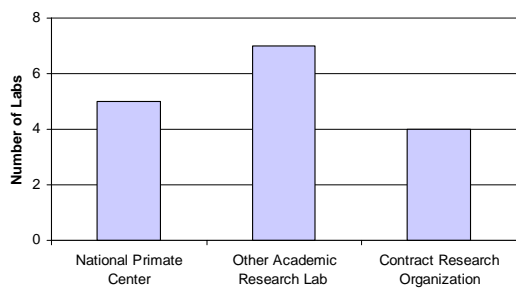
Data from participating labs provided a snap shot of current nonhuman primate immunophenotyping practices being employed in 15 participating laboratories. National Primate Centers, other academic labs and contract research organizations were all represented in this study.

**Method:** Each lab completed a questionnaire reporting their techniques for immunophenotyping nonhuman primate blood specimens.

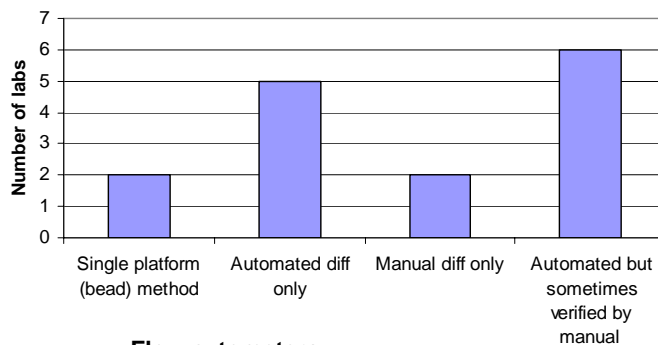
#### Key findings:

- Absolute counting:** Of 15 labs surveyed, 13 utilized a CBC to determine absolute lymphocyte counts; 2 used a single-platform bead technology.
- CBC instruments:** A wide variety of hematology instruments were in use. Most labs relied upon an automated differential count exclusively, or used an automated differential some of the time, to determine the absolute lymphocyte count. This is of particular interest because most of the between-lab error in lymphocyte subset counts was due to error in measuring absolute lymphocyte number.
- Whole blood lysis:** Fourteen of 15 labs used whole blood lysis technique. Four different lysis reagents were used.
- Flow cytometry:** 10 labs used 4-color analysis, 4 used 3-color analysis for this exercise. A wide variety of flow cytometers were used.
- Gating strategies:** No labs used CD45 to gate on lymphocytes. Instead, all relied on scatter gating. Only 1 or 14 labs used an automated method to determine the lymphocyte scatter gate. No labs calculated lymphocyte scatter gate purity or recovery.

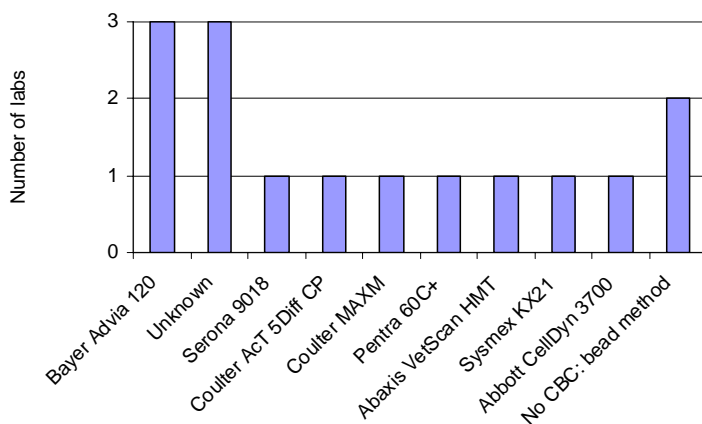
**Lab type:**



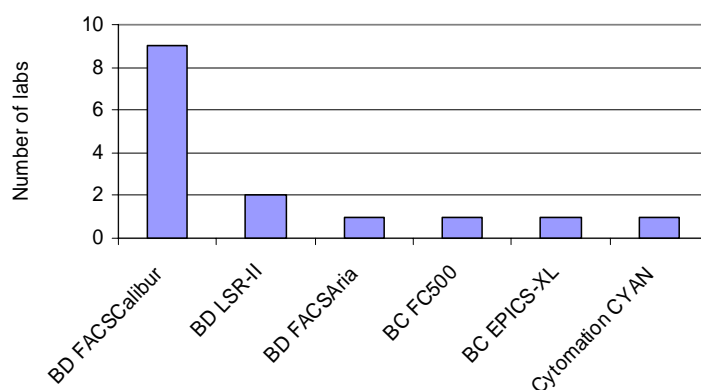
**Absolute counting methods**



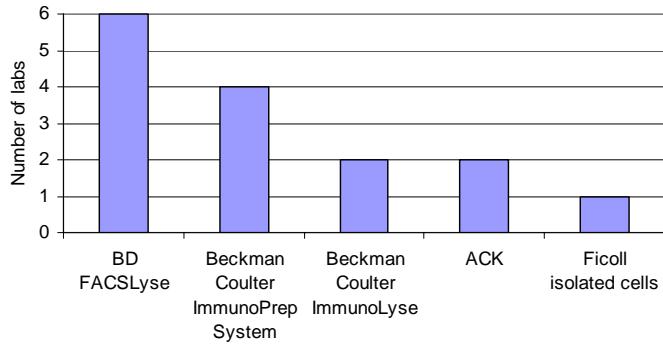
**CBC instruments**



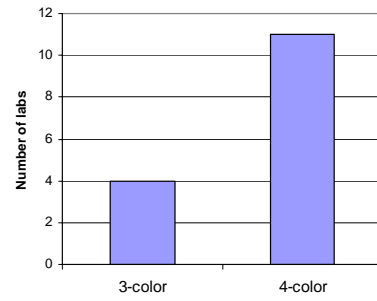
**Flow cytometers**



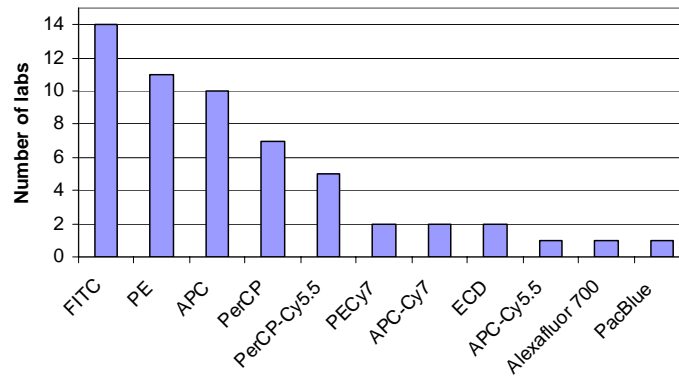
### RBC lysing method



### Number of colors used in flow cytometry



### Fluorochromes utilized



## Part II- PROFICIENCY TESTING

**Method:** Blood specimens were obtained from normal rhesus monkeys. Blood was drawn into 10ml EDTA Vacutainer tubes, then pooled, mixed and aliquoted into smaller tubes that contained no anticoagulant to ensure that each specimen was identical. Specimens were shipped by overnight courier in Styrofoam shippers. Of 15 shipments, 14 were delivered within 24hr. One lab received their specimens after 48 hrs. Thirteen labs performed CBCs and 14 labs completed the lymphocyte subset analysis portion.

**Hematology vs immunophenotyping:** Because hematology values (WBC, lymphocyte percent) were usually determined by a different assay method than subset values (%CD3, %CD3+4+, etc), these measurements are interpreted separately.

**Between-lab variability** is used as an estimate of accuracy. The median value is considered the most accurate. The results for each lab are plotted for each specimen and your lab's deviation from the median is shown.

**Within-lab variability** is used as an estimate of precision. Specimens #1, #4 and #5 were aliquoted from the same animal's blood draw. The difference between the highest and lowest value obtained for these three replicate specimens was calculated and the percent variation was determined by dividing this difference by the median.

**Overall performance:** An overall performance of each laboratory, relative to the other 14 labs, is provided on page 4. This ranks your lab's between-lab agreement (accuracy) and within-lab variation (precision) for both hematology and immunophenotyping assays. Labs were divided into upper, middle and lower 1/3rds of each category based on a composite score for these measurements. This ranking is provided to identify your lab's strengths and potential weaknesses.

**Raw data files:** Raw data files are published as a technical note on the NHP Reagent Resource website: <http://nhpreagents.bidmc.harvard.edu/NHP/Protocols.aspx>

**Confidentiality:** Results from this study will be published but the identity of the participating labs will not be disclosed.

### Key findings:

#### **Between-lab variation-**

**Hematology-** WBC counts varied only ~30% between labs. However, % lymphocytes and absolute lymphocyte counts varied 2- to 3-fold between labs. These measurements were made in most labs using hematology instruments and utilized automated and/or manual differential counts).

**Immunophenotyping-** Overall, lymphocyte subset *percentages* varied 2-fold or less between labs. There was more between-lab variation in B cell and CD8+ T cell percentages than there was in CD4+ T cell percentages. However, between-lab variation in absolute lymphocyte subset counts varied 4-fold or greater for measurements on some specimens. This high degree of between-lab variation resulted from the additive effect of the high variation in absolute lymphocyte count and variation in lymphocyte subset percentages.

#### **Within-lab variation-**

Within-lab variation (variation in results on replicate specimens) differed widely between labs. Variation in hematology values (WBC, % lymphocytes) and in immunophenotyping subset percentages was near 0% in some labs but 30% or greater in other labs. No technical explanation for this within-lab variation was evident.

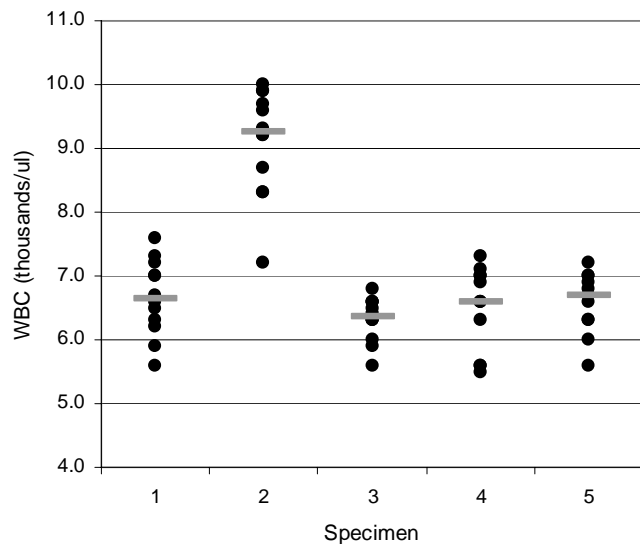


**PART II: ANALYSIS OF SPECIMENS – between-lab variation**

**X** Your lab's relative performance  
 Parameter: **WBC**

<i>Specimen ID</i>	<b>1*</b>	<b>2</b>	<b>3</b>	<b>4*</b>	<b>5*</b>
<b>MEDIAN OF ALL LABS</b> (range)	<b>6.7</b> (5.6-7.6)	<b>9.3</b> (7.2-10.0)	<b>6.4</b> (5.6-6.8)	<b>6.6</b> (5.5-7.3)	<b>6.7</b> (5.6-7.2)

\* replicates



Median —



**PART II: ANALYSIS OF SPECIMENS – between-lab variation**

**Parameter: % lymphocytes**

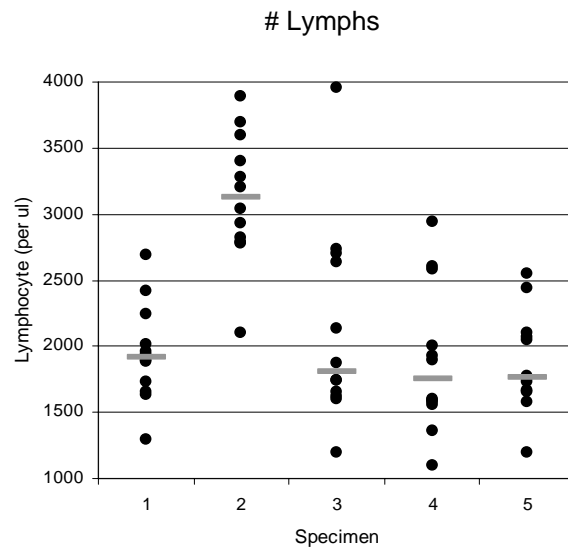
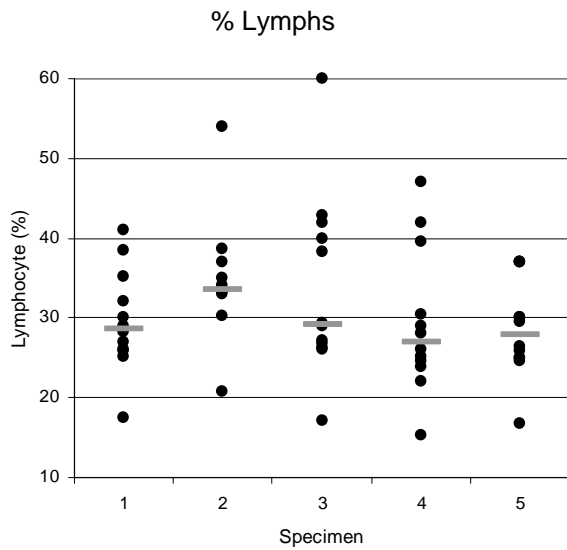
<i>Specimen ID</i>	<b>1*</b>	<b>2</b>	<b>3</b>	<b>4*</b>	<b>5*</b>
<b>MEDIAN OF ALL LABS</b> (range)	<b>28.6</b> (17.5-41.0)	<b>33.6</b> (20.8-54.0)	<b>29.2</b> (17.2-60-0)	<b>27.0</b> (15.3-47.0)	<b>28.0</b> (16.8-37-0)

\* replicates

**Parameter: Lymphocyte number**

<i>Specimen ID</i>	<b>1*</b>	<b>2</b>	<b>3</b>	<b>4*</b>	<b>5*</b>
<b>MEDIAN OF ALL LABS</b> (range)	<b>1919</b> (1300-2695)	<b>3126</b> (2100-3888)	<b>1808</b> (1200-3960)	<b>1749</b> (1100-2940)	<b>1765</b> (1200-2553)

\* replicates



Median —



**PART II: ANALYSIS OF SPECIMENS – between-lab variation**

**Parameter: % CD3+**

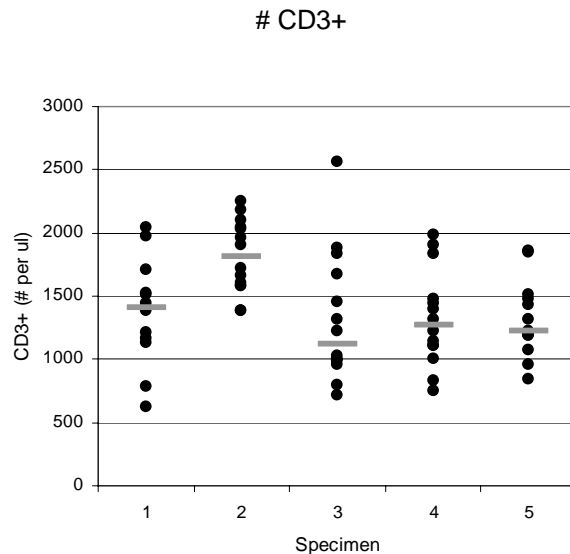
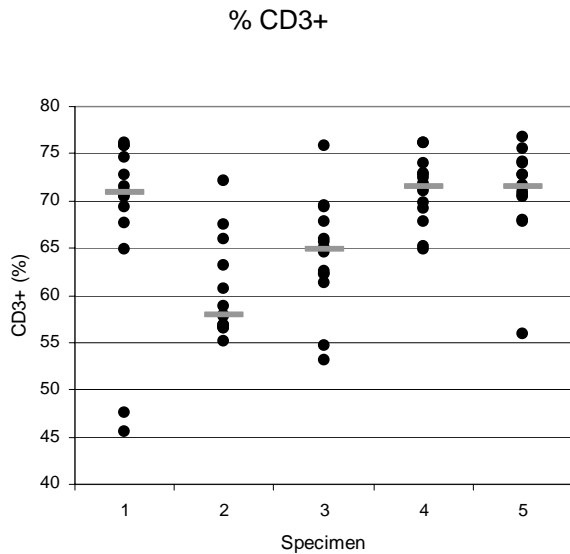
<i>Specimen ID</i>	<b>1*</b>	<b>2</b>	<b>3</b>	<b>4*</b>	<b>5*</b>
<b>MEDIAN OF ALL LABS</b> (range)	<b>70.9</b> (45.5-76.2)	<b>57.9</b> (55.1-72.2)	<b>64.8</b> (53.1-75.9)	<b>71.6</b> (64.9-76.2)	<b>71.5</b> (55.9-76.7)

\* replicates

**Parameter: CD3+ number**

<i>Specimen ID</i>	<b>1*</b>	<b>2</b>	<b>3</b>	<b>4*</b>	<b>5*</b>
<b>MEDIAN OF ALL LABS</b> (range)	<b>1407</b> (619-2043)	<b>1815</b> (1379-2251)	<b>1125</b> (718-2558)	<b>1266</b> (746-1984)	<b>1219</b> (847-1859)

\* replicates



Median —



**PART II: ANALYSIS OF SPECIMENS – between-lab variation**

Parameter: % CD3+4+

<i>Specimen ID</i>	<b>1*</b>	<b>2</b>	<b>3</b>	<b>4*</b>	<b>5*</b>
<b>MEDIAN OF ALL LABS (range)</b>	<b>52.6</b> (32.6-56.6)	<b>29.7</b> (26.2-33.8)	<b>49.2</b> (41.9-55.3)	<b>52.5</b> (45.6-56.5)	52.4 <b>(42.6-56.7)</b>

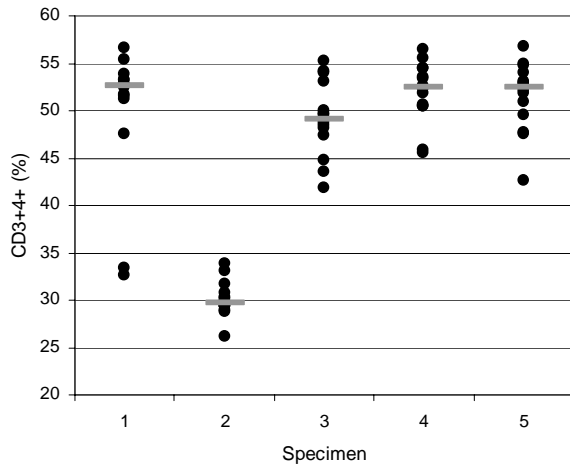
\* replicates

Parameter: CD3+4+ number

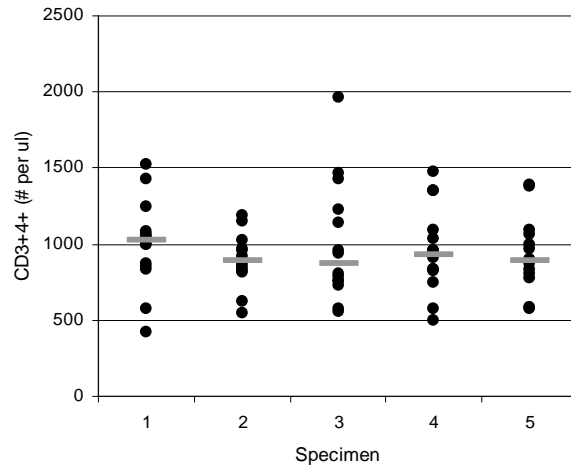
<i>Specimen ID</i>	<b>1*</b>	<b>2</b>	<b>3</b>	<b>4*</b>	<b>5*</b>
<b>MEDIAN OF ALL LABS (range)</b>	<b>1023</b> (424-1525)	<b>887</b> (549-1191)	<b>871</b> (553-1967)	<b>932</b> (502-1473)	892 <b>(572-1385)</b>

\* replicates

% CD3+4+



# CD3+4+



Median —



**PART II: ANALYSIS OF SPECIMENS – between-lab variation**

Parameter: % CD3+8+

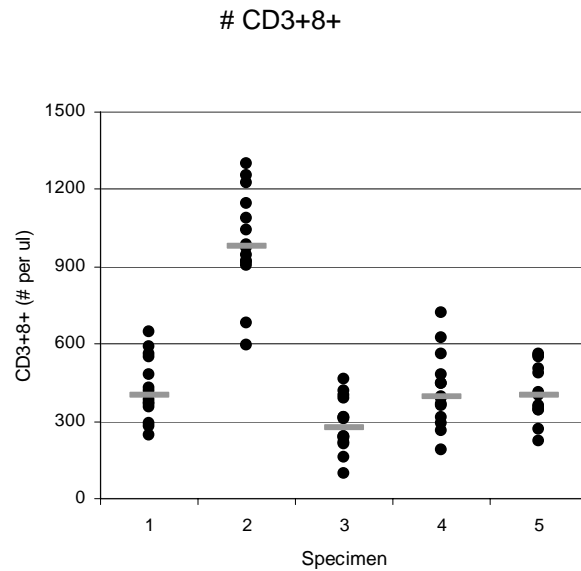
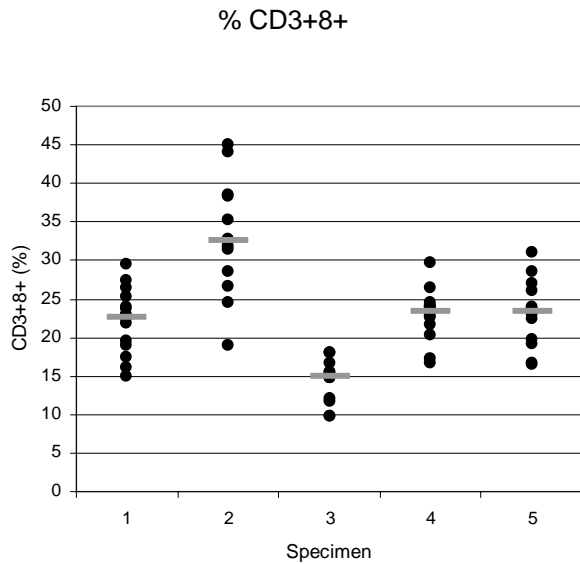
<i>Specimen ID</i>	<b>1*</b>	<b>2</b>	<b>3</b>	<b>4*</b>	<b>5*</b>
<b>MEDIAN OF ALL LABS (range)</b>	<b>22.7</b> (15.0-29.5)	<b>32.5</b> (18.9-45.0)	<b>14.9</b> (9.7-18.1)	<b>23.5</b> (16.7-29.7)	<b>23.3</b> (16.4-31.0)

\* replicates

Parameter: CD3+8+ number

<i>Specimen ID</i>	<b>1*</b>	<b>2</b>	<b>3</b>	<b>4*</b>	<b>5*</b>
<b>MEDIAN OF ALL LABS (range)</b>	<b>401</b> (248-647)	<b>979</b> (293-1302)	<b>276</b> (97-463)	<b>419</b> (191-720)	<b>403</b> (222-562)

\* replicates



Median



**PART II: ANALYSIS OF SPECIMENS – between-lab variation**

Parameter: % CD3-20+

<i>Specimen ID</i>	<b>1*</b>	<b>2</b>	<b>3</b>	<b>4*</b>	<b>5*</b>
<b>MEDIAN OF ALL LABS (range)</b>	<b>17.6 (8.1-19.7)</b>	<b>32.0 (14.1-36.1)</b>	<b>19.9 (10.2-21.4)</b>	<b>18.1 (0.7-19.7)</b>	<b>18.3 (8.2-19.2)</b>

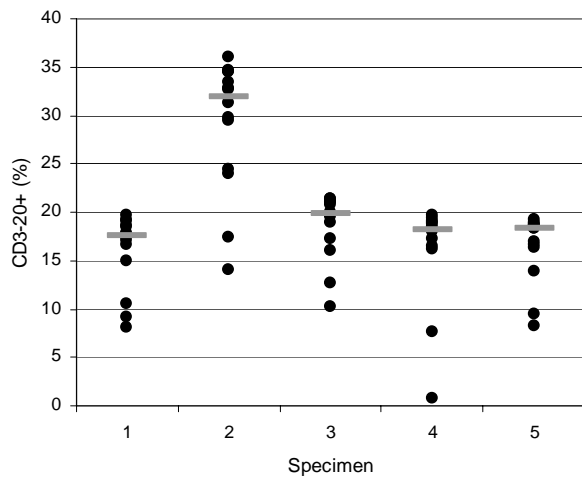
\* replicates

Parameter: CD3-20+ number

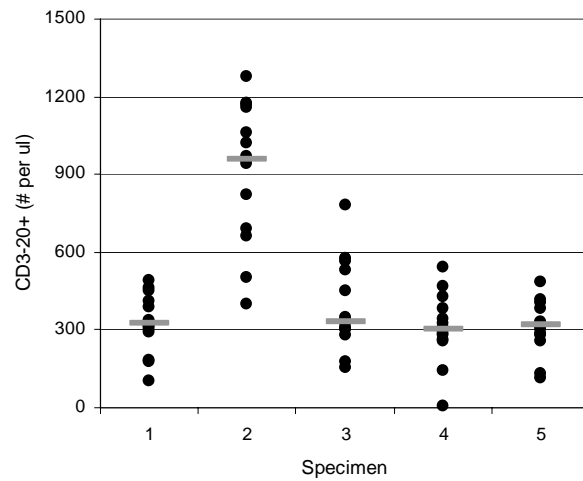
<i>Specimen ID</i>	<b>1*</b>	<b>2</b>	<b>3</b>	<b>4*</b>	<b>5*</b>
<b>MEDIAN OF ALL LABS (range)</b>	<b>323 (105-489)</b>	<b>960 (398-1277)</b>	<b>333 (152-783)</b>	<b>303 (8-541)</b>	<b>317 (113-484)</b>

\* replicates

% CD3-20+



# CD3-20+



Median



**PART II: ANALYSIS OF SPECIMENS – within-lab variation**

**Parameter: Hematology values  
Variation between replicates**

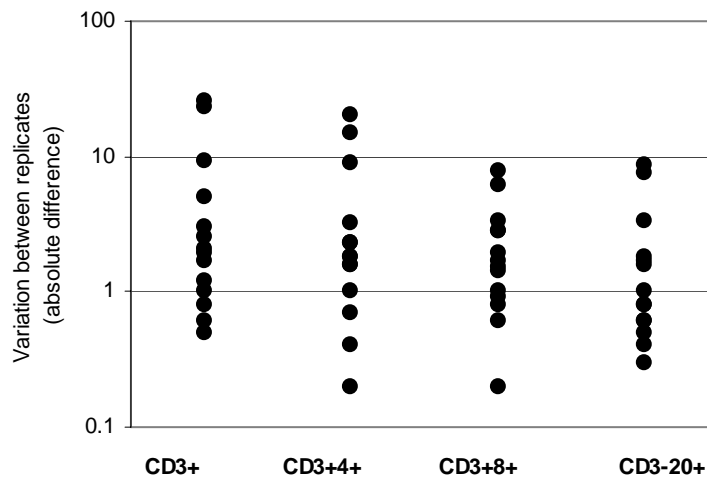
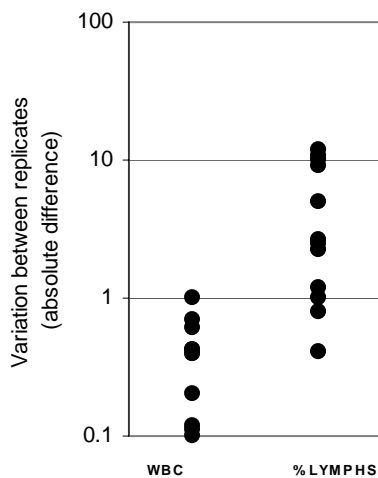
	<b>MEDIAN OF ALL LABS (RANGE)</b>	
	<i>Difference between replicates</i>	<i>% variation <sup>1</sup></i>
<b>WBC</b>	0.4 <b>(0.0-1.0)</b>	6.2% <b>(0%-14%)</b>
<b>% Lymphocytes</b>	2.5 <b>(0.4-12.0)</b>	11% <b>(1%-38%)</b>

<sup>1</sup> (high# - low#) / your median

**Parameter: Lymphocyte subset percentages  
Variation between replicates**

	<b>MEDIAN OF ALL LABS (RANGE)</b>	
	<i>Difference between replicates</i>	<i>% variation <sup>1</sup></i>
<b>%CD3+</b>	2.0 <b>(0.6-25.8)</b>	3% <b>(1%-36%)</b>
<b>%CD3+4+</b>	1.8 <b>(0.2-20.0)</b>	4% <b>(0%-38%)</b>
<b>%CD3+8+</b>	1.6 <b>(0.2-7.9)</b>	7% <b>(1%-34%)</b>
<b>%CD3-20%</b>	0.9 <b>(0.4-8.7)</b>	5% <b>(2%-107%)</b>

<sup>1</sup> (high# - low#) / your median



Specimen 1

Lab Name	WBC	%Lymphs	# Lymphs	%CD3+	# CD3+	%CD3+4+	# CD3+4+	%CD3+8+	# CD3+8+	%CD3-20+	# CD3-20+
1	6.2	28.2	1730	45.5	787	33.4	578	16.1	279	10.5	182
2	7.6	17.5	1300	47.6	619	32.6	424	22.4	291	8.1	105
3	7.0	32.0	2240	67.7	1516	47.5	1064	26.4	591	18.4	412
4	7.0	38.5	2695	75.8	2043	56.6	1525	24.0	647	17.1	461
5	5.6	35.2	1960	71.5	1409	53.3	1051	21.8	430	19.7	388
6	6.7	30.0	2010	75.8	1524	53.9	1083	27.4	551	15.0	302
7	6.3	25.9	1632	69.4	1132	51.2	835	22.9	374	19.1	311
8				71.1	1215	53.3	856	23.8	382	19.3	330
9				64.8	1976	51.6	1431	18.9	353	17.4	489
10	7.3	26.0	1898	76.2	1446	52.4	997	29.5	559	16.6	315
11	6.5	29.0	1885	74.6	1405	55.4	1045	19.5	369	17.9	339
12	6.6	25.1	1650	70.6	1165	52.7	870	15.0	248	17.7	292
13				72.8	1382	52.7	1000	25.3	480	9.2	175
14	7.2	27.0	1940								
15	5.9	41.0	2419	70.5	1705	51.7	1250	17.4	420	18.6	450
<b>Median</b>	<b>6.7</b>	<b>28.6</b>	<b>1919</b>	<b>70.9</b>	<b>1407</b>	<b>52.6</b>	<b>1023</b>	<b>22.7</b>	<b>401</b>	<b>17.6</b>	<b>323</b>
<b>High</b>	<b>7.6</b>	<b>41</b>	<b>2695</b>	<b>76.2</b>	<b>2043</b>	<b>56.6</b>	<b>1525</b>	<b>29.5</b>	<b>647</b>	<b>19.7</b>	<b>489</b>
<b>Low</b>	<b>5.6</b>	<b>17.5</b>	<b>1300</b>	<b>45.5</b>	<b>619</b>	<b>32.6</b>	<b>424</b>	<b>15.0</b>	<b>248</b>	<b>8.1</b>	<b>105</b>

Specimen 2

Lab Name	WBC	%Lymphs	#Lymphs	%CD3+	# CD3+	%CD3+4+	# CD3+4+	%CD3+8+	# CD3+8+	%CD3-20+	# CD3-20+
1	8.3	33.3	2780	56.8	1579	29.4	817	32.6	906	29.5	820
2	9.9	20.8	2100	65.9	1384	29.8	626	45.0	945	24.0	504
3	10.0	37.0	3700	56.9	2105	26.2	969	35.2	1302	31.3	1158
4	9.3	38.7	3599	60.7	2185	33.1	1191	31.8	1145	32.6	1173
5	8.7	33.7	2930	56.7	1662	29.4	862	31.5	924	36.1	1058
6	9.6	33.4	3206	63.2	2026	28.8	923	38.3	1228	29.7	952
7	9.2	33.1	3045	56.6	1724	28.9	879	32.4	986	33.5	1019
8				58.8	1599	29.4	826	32.7	918	34.5	939
9				55.1	1379	30.8	549	18.9	593	17.4	663
10	8.3	34.0	2822	72.2	2038	31.7	895	44.1	1253	24.5	691
11	9.7	35.0	3395	57.8	1963	30.3	1028	28.6	972	34.5	1171
12	9.2	30.3	2790	56.6	1579	30.2	843	24.5	684	34.7	968
13				67.5	1905	33.8	954	38.5	1086	14.1	398
14	9.9	33.0	3280								
15	7.2	54.0	3888	57.9	2251	29.6	1150	26.7	1040	32.8	1277
<b>Median</b>	<b>9.25</b>	<b>33.55</b>	<b>3125.5</b>	<b>57.9</b>	<b>1815</b>	<b>29.7</b>	<b>887</b>	<b>32.5</b>	<b>979</b>	<b>32.0</b>	<b>960</b>
<b>High</b>	<b>10</b>	<b>54</b>	<b>3888</b>	<b>72.2</b>	<b>2251</b>	<b>33.8</b>	<b>1191</b>	<b>45</b>	<b>1302</b>	<b>36.1</b>	<b>1277</b>
<b>Low</b>	<b>7.2</b>	<b>20.8</b>	<b>2100</b>	<b>55.1</b>	<b>1379</b>	<b>26.2</b>	<b>549</b>	<b>18.9</b>	<b>593</b>	<b>14.1</b>	<b>398</b>

Specimen 3

Lab Name	WBC	%Lymphs	# Lymphs	%CD3+	# CD3+	%CD3+4+	# CD3+4+	%CD3+8+	# CD3+8+	%CD3-20+	# CD3-20+
1	6.3	26.7	1620	62.3	1009	48.5	786	14.8	240	18.9	306
2	6.8	17.2	1200	66.0	792	48.1	577	18.0	216	12.7	152
3	6.5	42.0	2730	61.3	1673	44.8	1223	15.4	420	20.7	565
4	6.3	42.9	2703	69.5	1878	54.1	1462	14.8	400	21.4	578
5	5.6	38.3	2140	67.8	1454	53.1	1139	14.7	315	21.1	453
6	6.4	29.3	1875	65.0	1219	50.0	938	16.6	311	16.1	302
7	5.9	27.1	1599	62.3	996	47.4	758	14.9	239	21.1	339
8				65.6	997	49.6	759	15.5	237	21.4	326
9				53.1	718	43.6	553	9.7	97	19.6	277
10	6.0	29.0	1740	75.9	1320	55.3	961	18.1	314	17.3	301
11	6.6	40.0	2640	69.3	1829	54.0	1426	14.9	388	20.0	528
12	6.3	26.3	1650	62.5	1031	48.7	804	9.7	160	21.1	348
13				54.7	952	41.9	729	12.0	209	10.2	177
14	6.6	26.0	1740								
15	6.6	60.0	3960	64.6	2558	49.7	1967	11.7	463	19.8	783
<b>Median</b>	<b>6.4</b>	<b>29.2</b>	<b>1807.5</b>	<b>64.8</b>	<b>1125</b>	<b>49.2</b>	<b>871</b>	<b>14.9</b>	<b>276</b>	<b>19.9</b>	<b>333</b>
<b>High</b>	<b>6.8</b>	<b>60</b>	<b>3960</b>	<b>75.9</b>	<b>2558</b>	<b>55.3</b>	<b>1967</b>	<b>18.1</b>	<b>463</b>	<b>21.4</b>	<b>783</b>
<b>Low</b>	<b>5.6</b>	<b>17.2</b>	<b>1200</b>	<b>53.1</b>	<b>718</b>	<b>41.9</b>	<b>553</b>	<b>9.7</b>	<b>97</b>	<b>10.2</b>	<b>152</b>

Specimen 4

Lab Name	WBC	%Lymphs	# Lymphs	%CD3+	# CD3+	%CD3+4+	# CD3+4+	%CD3+8+	# CD3+8+	%CD3-20+	# CD3-20+
1	5.6	28.9	1560	71.3	1112	53.4	833	23.5	367	18.0	281
2	7.0	15.3	1100	67.8	746	45.6	502	26.5	292	0.7	8
3	7.0	42.0	2940	64.9	1908	45.9	1349	24.5	720	18.4	541
4	6.6	39.5	2607	76.1	1984	56.5	1473	24.0	626	16.5	430
5	5.6	24.6	1360	72.9	1004	54.5	751	22.7	313	18.9	260
6	6.6	30.4	2006	73.9	1482	54.4	1091	24.1	483	17.2	345
7	6.3	25.1	1581	69.8	1104	51.9	820	22.8	360	18.8	298
8				72.8	1219	55.6	906	24.0	391	19.4	324
9				65.1	835	50.4	576	21.7	191	19.1	259
10	7.3	26.0	1898	76.2	1446	50.6	960	29.7	563	16.2	307
11	6.9	28.0	1932	72.5	1401	53.6	1035	20.4	394	19.7	380
12	6.6	23.9	1590	71.8	1142	52.6	836	16.7	266	18.1	288
13				69.2	1313	50.5	958	23.4	444	7.6	144
14	7.1	22.0	1600								
15	5.5	47.0	2585	71.1	1838	52.3	1353	17.3	447	18.1	469
<b>Median</b>	<b>6.6</b>	<b>27.0</b>	<b>1749</b>	<b>71.6</b>	<b>1266</b>	<b>52.5</b>	<b>932</b>	<b>23.5</b>	<b>393</b>	<b>18.1</b>	<b>303</b>
<b>High</b>	<b>7.3</b>	<b>47</b>	<b>2940</b>	<b>76.2</b>	<b>1984</b>	<b>56.5</b>	<b>1473</b>	<b>29.7</b>	<b>720</b>	<b>19.7</b>	<b>541</b>
<b>Low</b>	<b>5.5</b>	<b>15.3</b>	<b>1100</b>	<b>64.9</b>	<b>746</b>	<b>45.6</b>	<b>502</b>	<b>16.7</b>	<b>191</b>	<b>0.7</b>	<b>8</b>

Specimen 5

Lab Name	WBC	%Lymphs	# Lymphs	%CD3+	# CD3+	%CD3+4+	# CD3+4+	%CD3+8+	# CD3+8+	%CD3-20+	# CD3-20+
1	6.3	26.4	1670	70.9	1184	53.1	887	24.0	401	16.7	279
2	7.0	16.8	1200	70.6	847	47.7	572	28.5	342	9.4	113
3	7.0	30.0	2100	67.9	1426	47.5	997	26.1	548	19.2	403
4	6.6	37.0	2442	75.5	1844	56.7	1385	23.0	562	17.0	415
5	6.0	29.5	1750	74.0	1310	54.9	972	23.2	411	18.7	331
6	6.8	30.1	2047	74.1	1517	52.1	1066	27.0	553	13.9	285
7	6.7	25.8	1729	70.4	1217	51.9	897	23.4	405	18.8	325
8				72.7	1220	54.8	834	23.8	362	18.6	312
9				55.9	960	42.6	589	19.2	222	18.4	322
10	6.3	25.0	1575	76.7	1200	50.9	802	31.0	488	16.3	257
11	6.9	37.0	2553	72.8	1859	54.0	1381	19.7	503	18.9	484
12	6.7	24.6	1650	71.7	1183	53.0	875	16.4	271	18.3	302
13				67.8	1068	49.5	780	22.5	354	8.2	129
14	7.2	25.0	1780								
15	5.6	37.0	2072	71.3	1477	52.7	1092	16.6	344	18.3	380
<b>Median</b>	<b>6.7</b>	<b>28.0</b>	<b>1765</b>	<b>71.5</b>	<b>1219</b>	<b>52.4</b>	<b>892</b>	<b>23.3</b>	<b>403</b>	<b>18.3</b>	<b>317</b>
<b>High</b>	<b>7.2</b>	<b>37</b>	<b>2553</b>	<b>76.7</b>	<b>1859</b>	<b>56.7</b>	<b>1385</b>	<b>31</b>	<b>562</b>	<b>19.2</b>	<b>484</b>
<b>Low</b>	<b>5.6</b>	<b>16.8</b>	<b>1200</b>	<b>55.9</b>	<b>847</b>	<b>42.6</b>	<b>572</b>	<b>16.4</b>	<b>222</b>	<b>8.2</b>	<b>113</b>

Lab Name	Colors	CD3 Clone	CD3 FC	CD4 Clone	CD4 FC	CD8 Clone	CD8 FC	CD20 Clone	CD20 FC
1		4 SP34-2	APC	L200	PERCP CY5.5	SK1	PE	L27	FITC
2		3 SP34	FITC	SK3	PE	SK1	PERCP	B9E9	PE
3		4 SP34	FITC	L200	PERCP	SK1	PE	L27	APC
4		4 SP34-2	ALEXAFLUOR-700	L200	PERCP CY5.5	3B5	FITC	2H7	APC
5		4 SP34-2	FITC	MT477	PE	B911	PERCP CY5.5	B9E9	ECD
6		3 SP34-2	FITC	L200	PERCP CY5.5	SK1	APC	L27	PERCP CY5.5
7		4 SP34-2	FITC	L200	APC	SK1	PERCP	L27	PE
8		3 SP34	FITC	L200	PE	B911	PERCP CY5.5	B9E9	PERCP CY5.5
9		4 SP34-2	APC CY 7	OKT4	PCC BLUE	RPAT8	APC CY5.5	2H7	PE CY7
10		3 SP34	FITC	SK3	PE	SK1	PERCP	L27	PE
11		4 SP34-2	FITC	L200	PE	SK1	PERCP	L27	APC
12		4 SP34-2	PERCP	MT477	PE	RPAT8	APC	2H7	FITC
13		4 SP34-2	PE CY7	L200	APC	RPAT8	APC CY 7	2H7	FITC
14		4 SP34-2	APC	L200	PERCP CY5.5	RPAT8	FITC	2H7	PE
15		4 SP34	FITC	MT477	PE	SK1	PERCP	L27	APC