

**BALL-B Discussion**

There were 3 samples distributed in this B-ALL MRD challenge. Case 04 was sent as a generic "gateable" FCS file, while cases 05 and 06 were sent as gated PDFs. Participants were asked to report on surface marker expression and calculate the percentage MRD positive as a subset of mononuclear cells *in samples that contained an abnormal population*.

**Case BALL-04 List Mode Case Negative for MRD**

Diagnostic files were included in this case, showing a population of cells that were CD45dim, CD10 and CD58bright, CD20 negative, CD38 variable in Tube 1. Tube 2 showed a population that was CD34 and CD13/33 negative, with variable CD9. This case did not contain an abnormal population. However a very small population of hematogones with a classic maturation pattern was present. Plasma cells were clearly visible (CD38 bright, CD10 negative, CD20 negative).

A total of 78.7% of participants reported this case as negative, with 21.3% calling it positive. Of those calling it positive, the majority reported 0.1 - 0.9%.

**Case BALL-05 Positive With Myeloid Markers**

This case had a very small population of residual leukemic cells (0.014%). The CD19 gated population in Tube 1 showed a population separate from mature B cells. This separate population expressed bright CD19, CD10, and was negative for CD38. The level of CD45 and CD20 expression was comparable to that of normal B cells. CD58 was similar to the brightest background seen in the mature B-cells. Plasma cells were clearly visible (CD38 bright, CD10 negative, CD20 negative). In Tube 2, the CD19 gated cells were positive for CD34, CD10, and CD13/CD33. Taken together, these are clearly abnormal cells.

A total of 96.8% of participants correctly identified this sample as positive, and all identified the sample as positive in the 0.01% - 0.09% range. Two participants called the sample negative.

**Case BALL-06 Negative for MRD**

This case did not contain an abnormal population. However, a large population of hematogones was present (hematogone hyperplasia). A subset of CD19 gated population in Tube 1 showed dim CD45 and bright CD10, within a background of CD10 positive cells with variable CD45 and CD20, with constant CD38 expression. CD58 was also bright when compared against the background of maturing B cells. Plasma cells were clearly visible (CD38 bright, CD10 negative, CD20 negative). In Tube 2, the CD19 gated cells were partially positive for CD34, dim CD45, bright CD9, and CD10, and negative for CD13/CD33. This population corresponds to the most immature hematogones (dim CD45, bright CD10, CD34+, CD20 negative, CD38 bright, CD58 bright, and CD9 bright).

A total of 59.7% of participants reported this sample as negative, with 40.3% calling it positive. Of those calling it positive, 78.1% reported in the 1 - 9.9% range, 6.3% reported in the 0.01% - 0.09% range and 15.6% reported positivity in the <0.01% range.

## **BALL-B Discussion (cont.)**

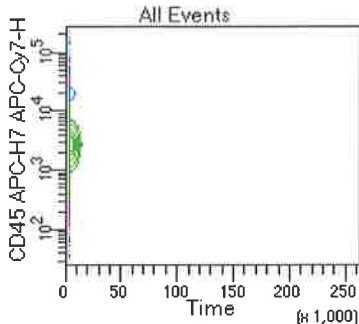
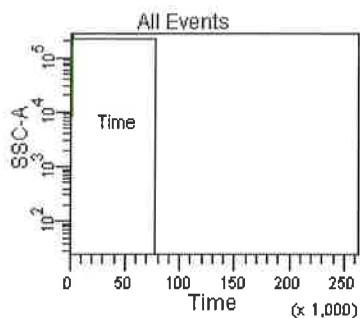
### **Some Key Comments:**

Participants should review case 04 in particular, as the diagnostic phenotype was given and the small hematogone population in the Day 29 sample was clearly different with a normal maturation sequence. Case 06 may have been confusing to some participants as the “population of interest” was a subset of a normal hematogone maturation pattern (CD10+CD34+) corresponding to the most immature hematogones.

A few laboratories had difficulty with BD FACSDiva software, but did manage to report results. Some laboratories felt that listmode files should be used for all cases whereas others felt that manipulating listmode files was too difficult.

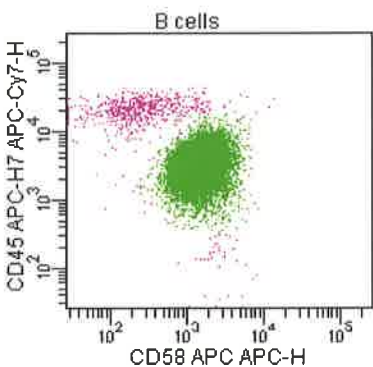
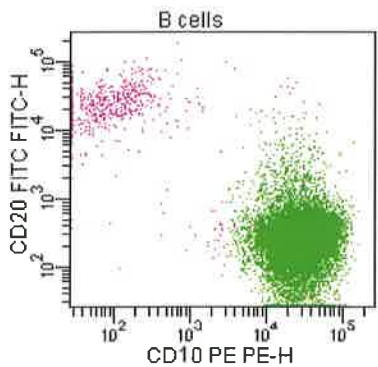
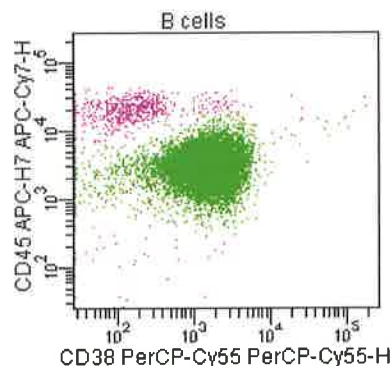
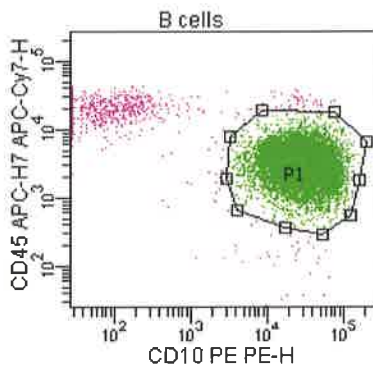
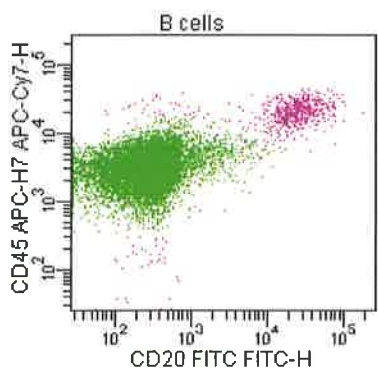
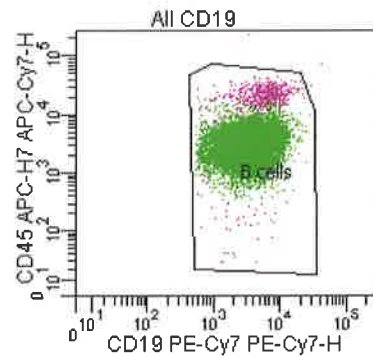
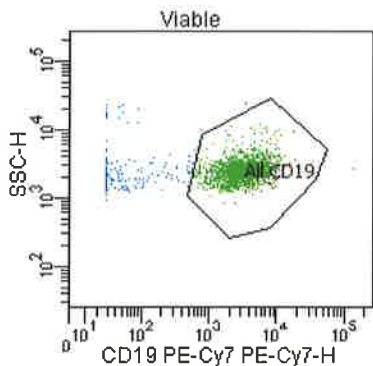
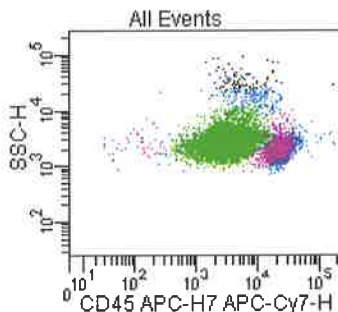
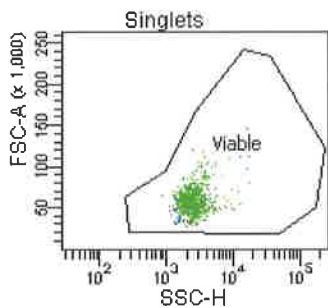
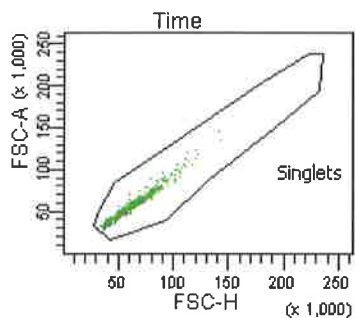
When a sample is negative, the value of abnormal cells is 0 and should not be reported as MRD positive.

**Michael Keeney, ART, FIMLS, FCSMLS(D)  
Diagnostic Immunology Resource Committee**

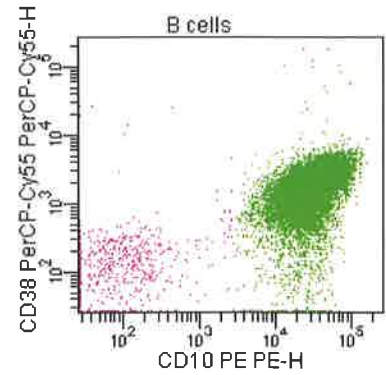
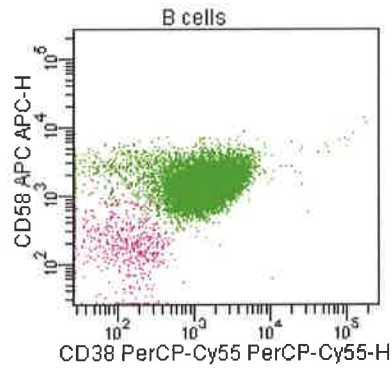
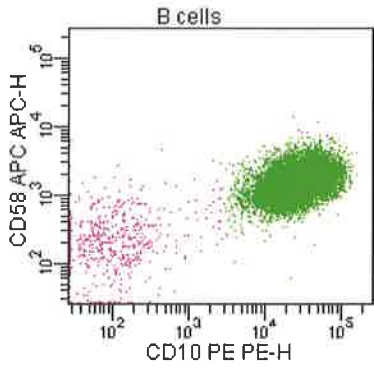
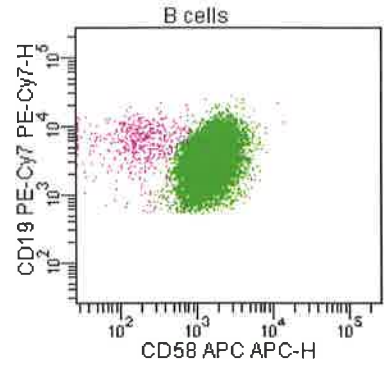
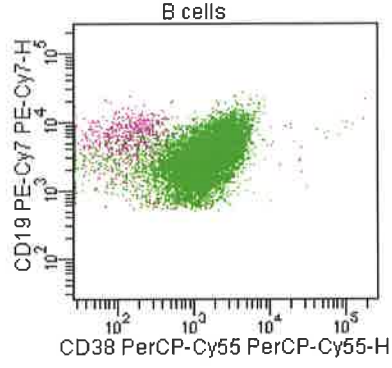
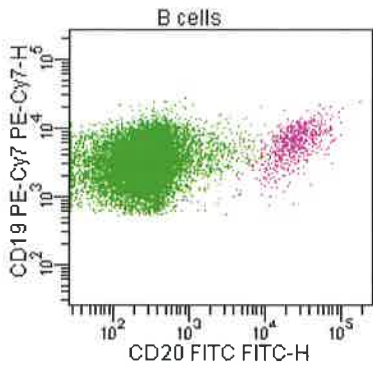
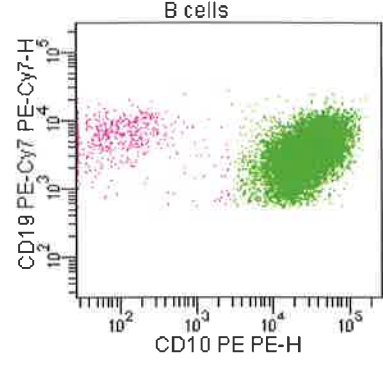
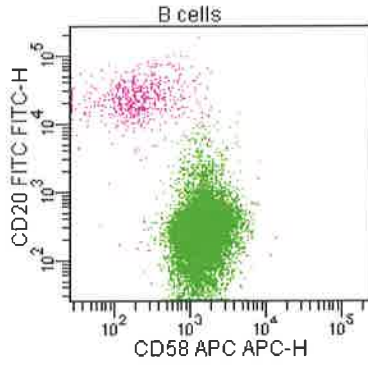
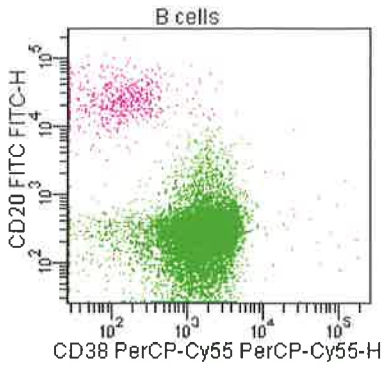


Tube: A-PRE

Population	#Events	%Parent	%Total
All Events	18,007	####	100.0
Time	17,917	99.5	99.5
Singlets	17,756	99.1	98.6
Viable	17,756	100.0	98.6
All CD19	15,945	89.8	88.5
B cells	15,940	100.0	88.5
P1	15,269	95.8	84.8

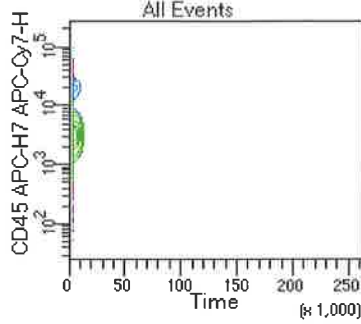
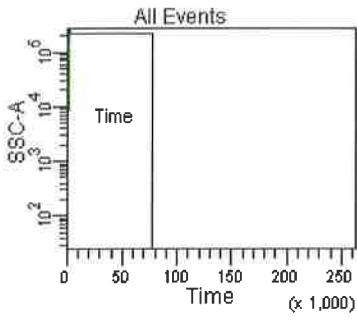


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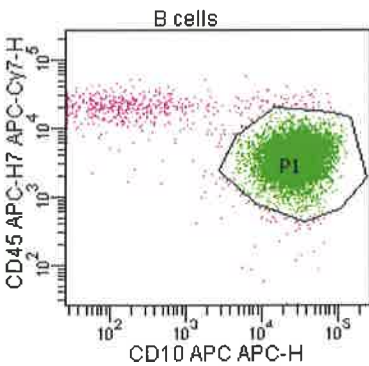
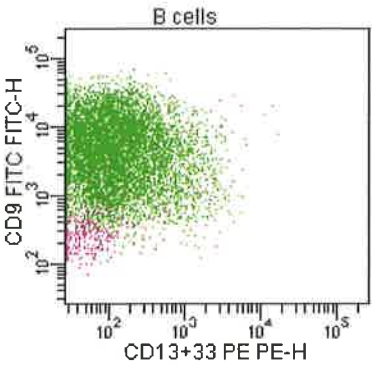
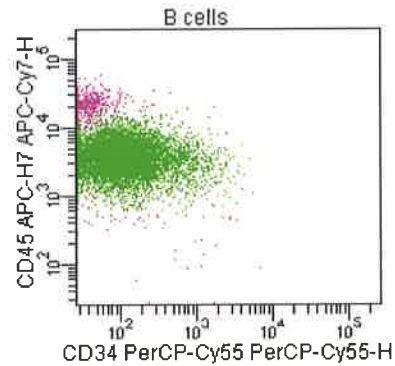
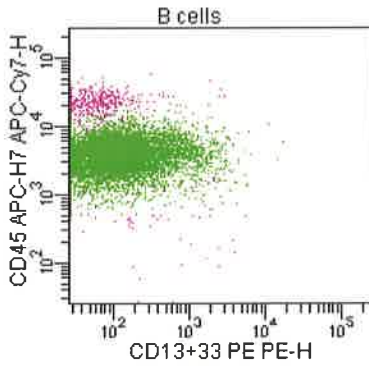
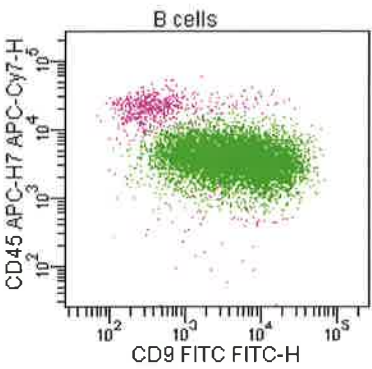
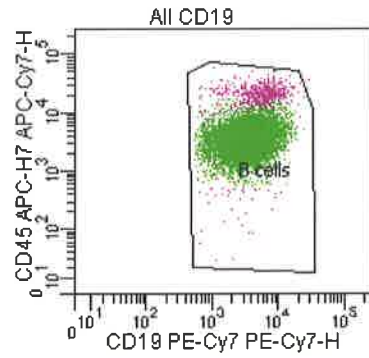
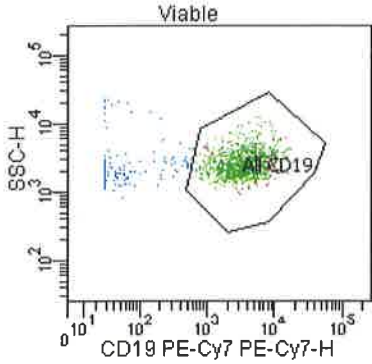
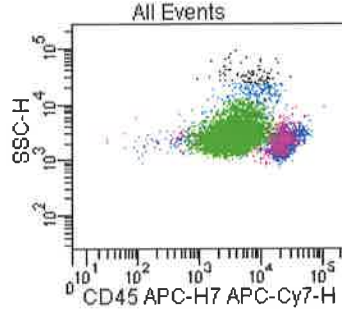
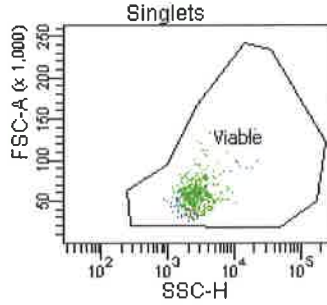
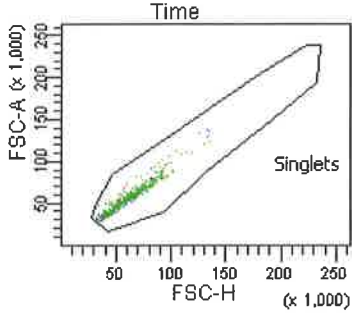
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Time	17,917	99.5	####	99.5
Singlets	17,756	99.1	98.6	98.6
Viable	17,756	100.0	99.1	98.6
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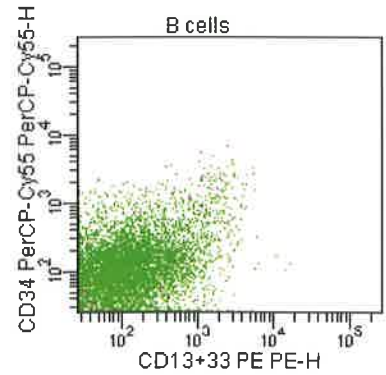
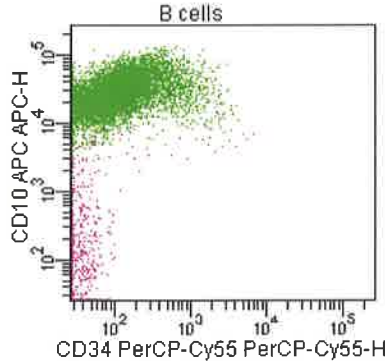
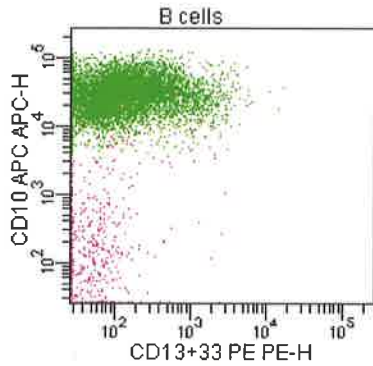
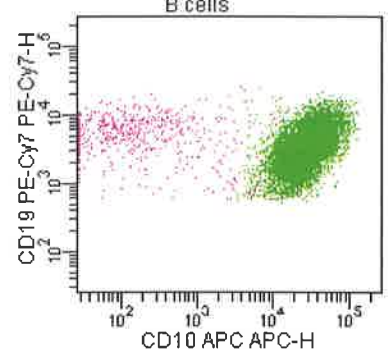
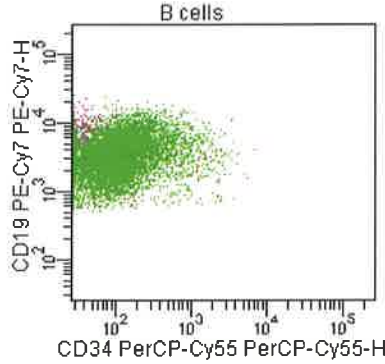
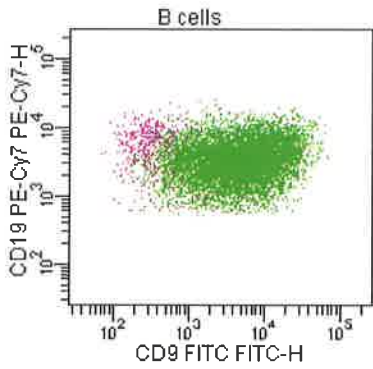
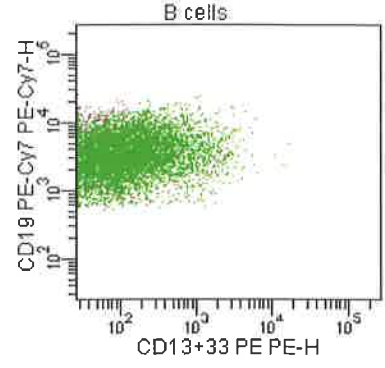
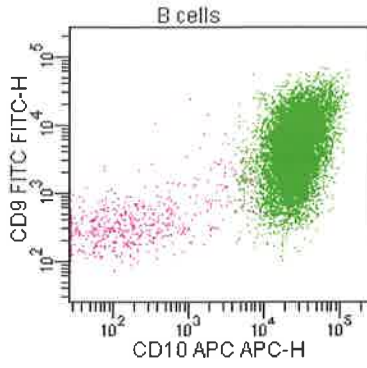
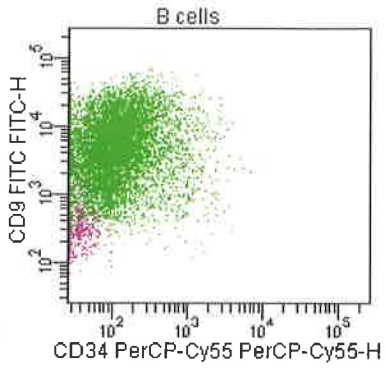


Tube: B-PRE

Population	#Events	%Parent	%Total
All Events	11,941	####	100.0
Time	11,855	99.3	99.3
Singlets	11,658	98.3	97.6
Viable	11,658	100.0	97.6
All CD19	9,929	85.2	83.2
B cells	9,927	100.0	83.1
P1	9,282	93.5	77.7

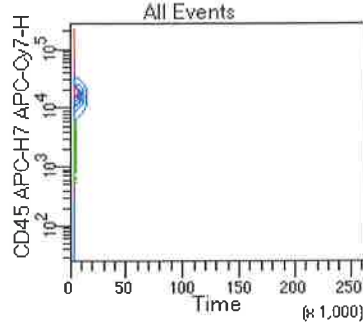
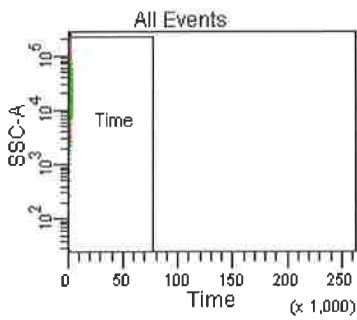


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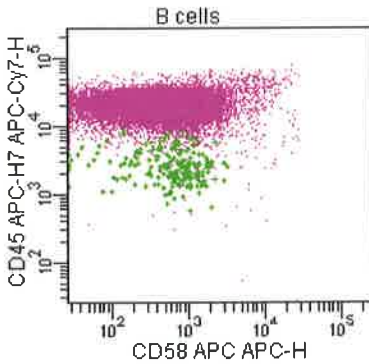
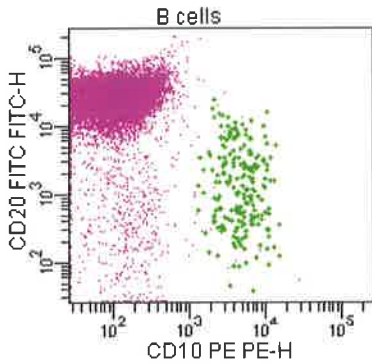
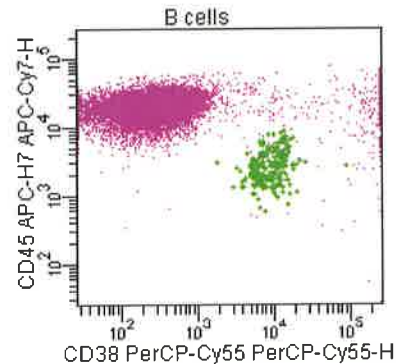
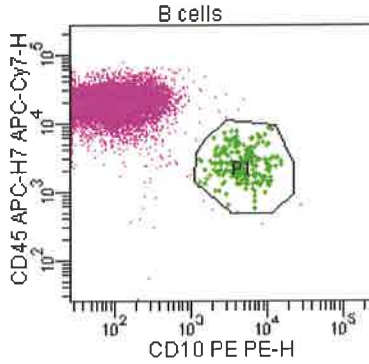
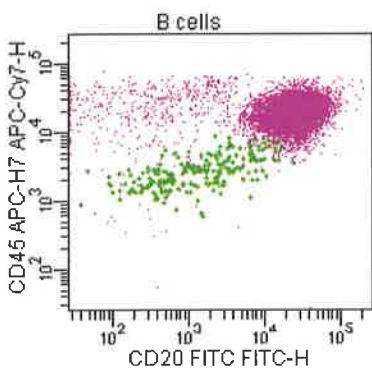
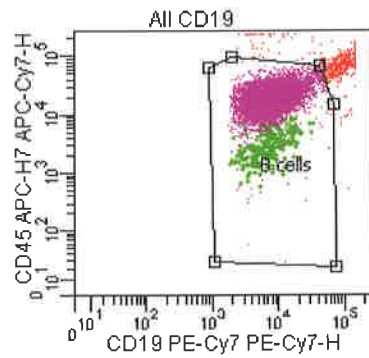
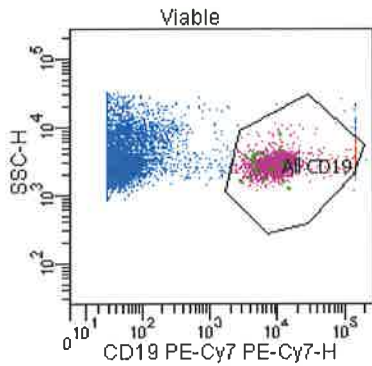
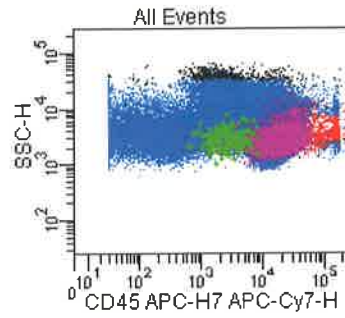
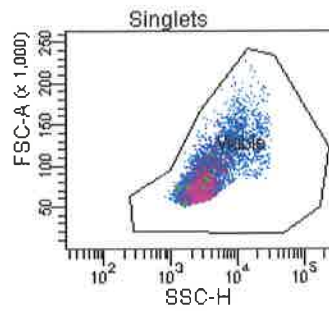
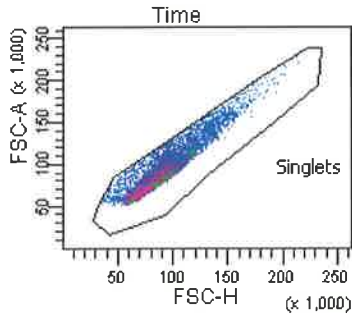
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Population	#Events	%Parent	%Grand Parent	%Total
All Events	11,941	####	####	100.0
Time	11,855	99.3	####	99.3
Singlets	11,658	98.3	97.6	97.6
Viable	11,658	100.0	98.3	97.6
All CD19	9,929	85.2	85.2	83.2
B cells	9,927	100.0	85.2	83.1
P1	9,282	93.5	93.5	77.7

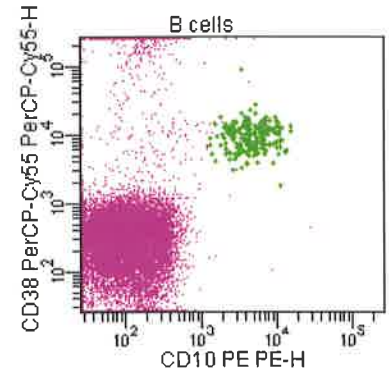
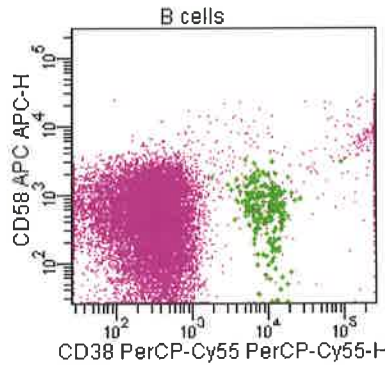
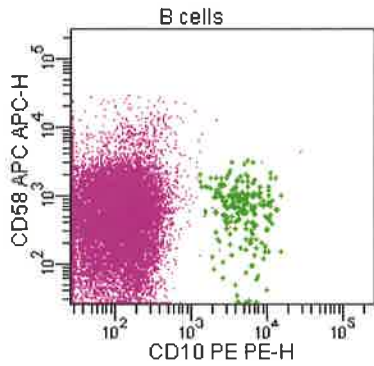
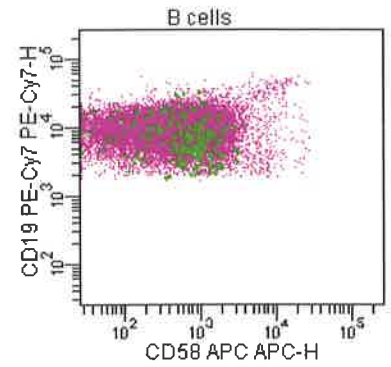
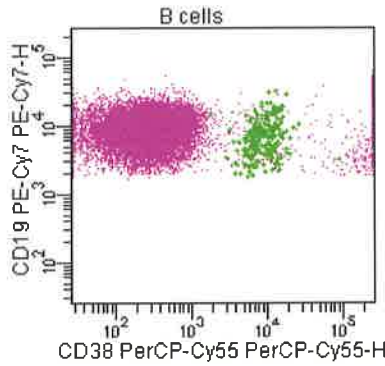
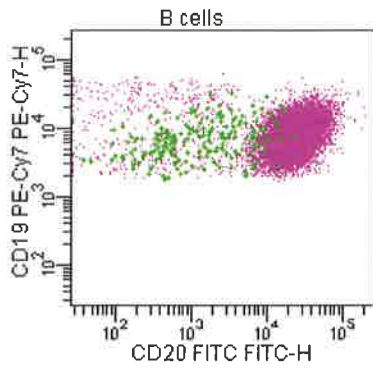
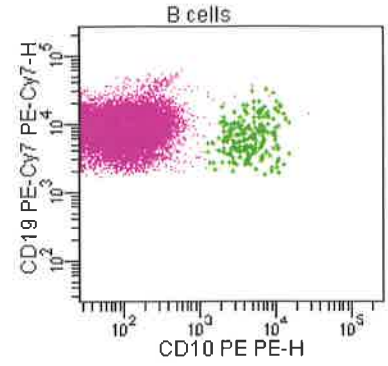
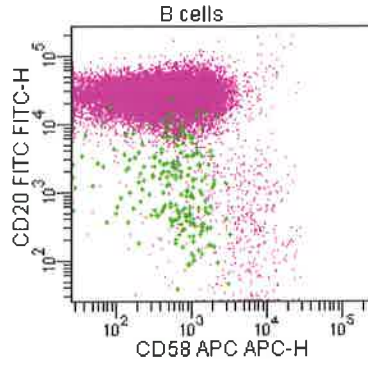
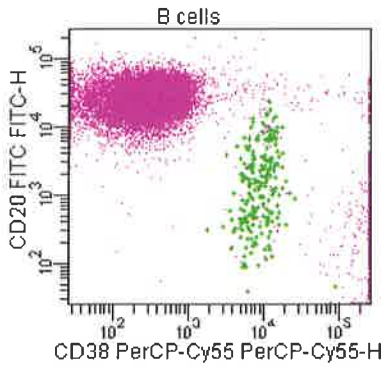


Tube: A-MRD (1)

Population	#Events	%Parent	%Total
All Events	245,292	####	100.0
Time	244,058	99.5	99.5
Singlets	242,472	99.4	98.9
Viable	242,460	100.0	98.8
All CD19	19,827	8.2	8.1
B cells	18,782	94.7	7.7
P1	203	1.1	0.1



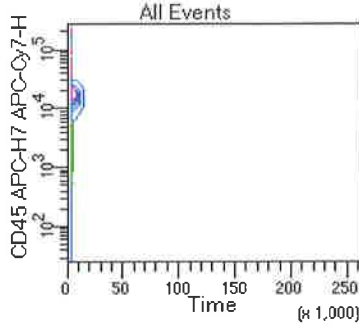
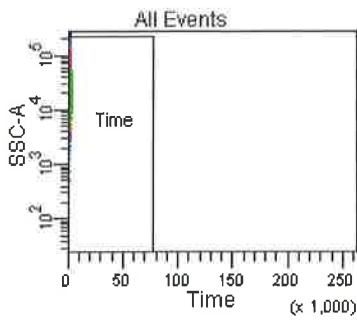
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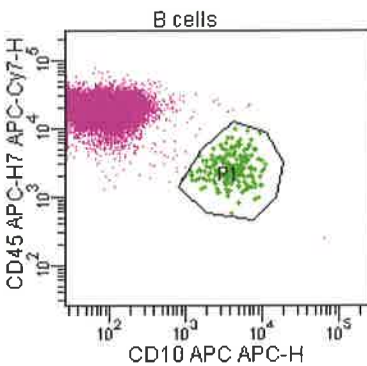
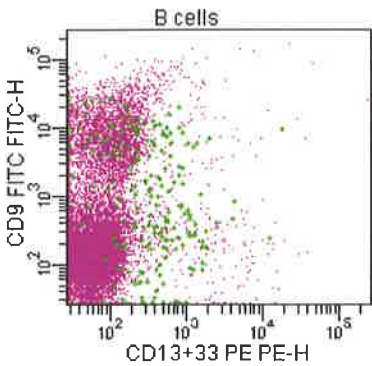
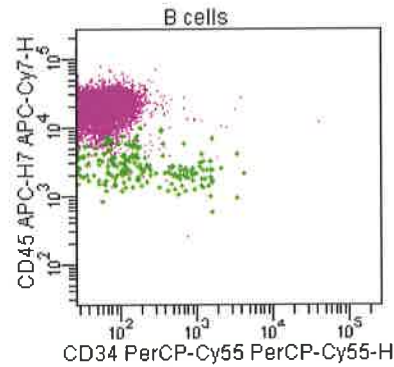
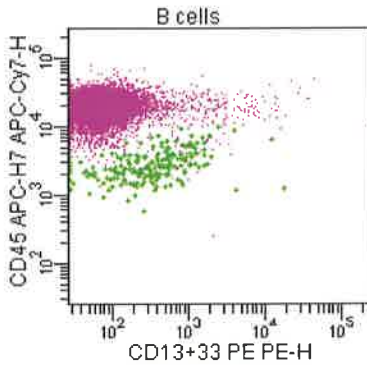
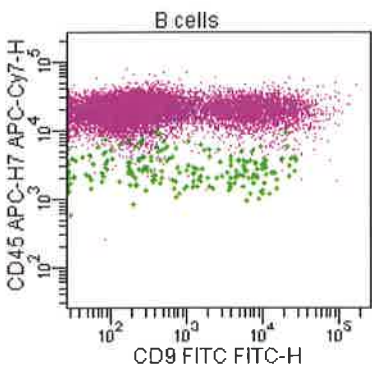
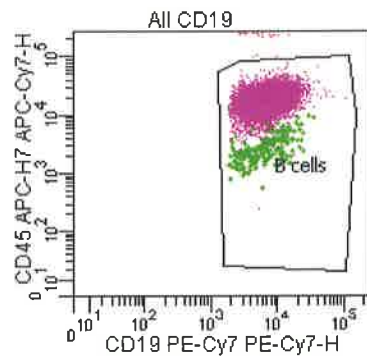
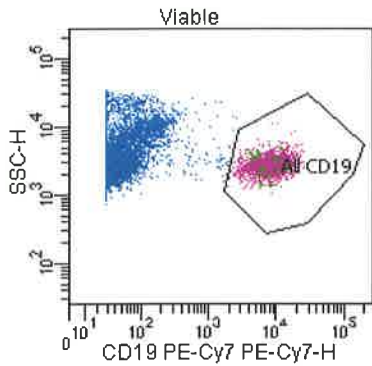
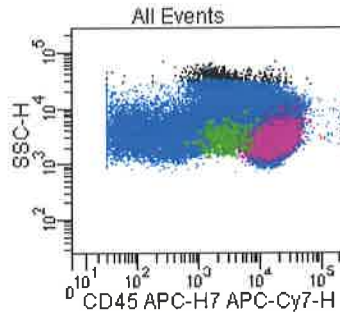
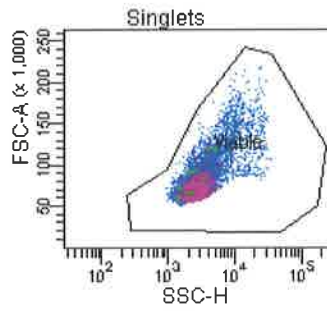
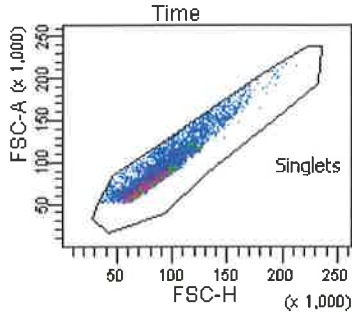
Population	#Events	%Parent	%Grand Parent	%Total
All Events	245,292	####	####	100.0
Time	244,058	99.5	####	99.5
Singlets	242,472	99.4	98.9	98.9
Viable	242,460	100.0	99.3	98.8
All CD19	19,827	8.2	8.2	8.1
B cells	18,782	94.7	7.7	7.7
P1	203	1.1	1.0	0.1



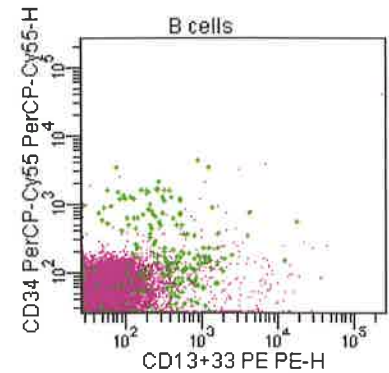
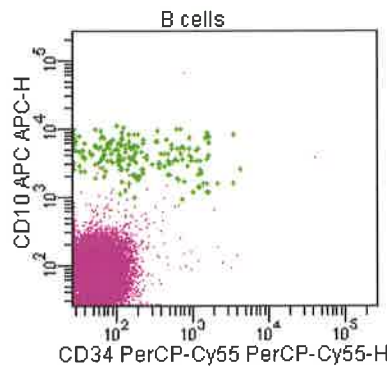
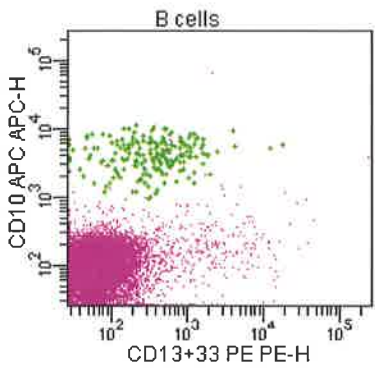
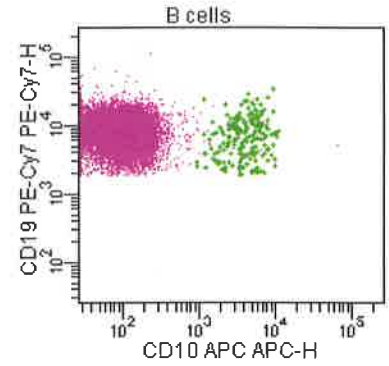
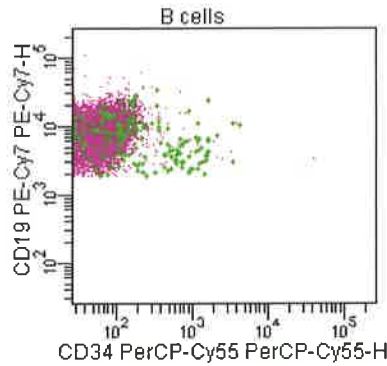
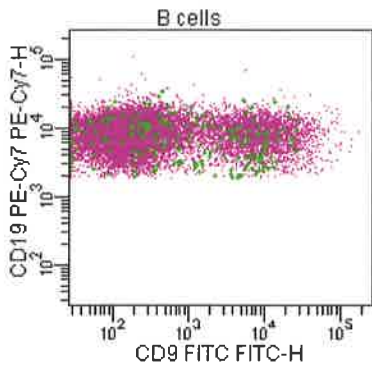
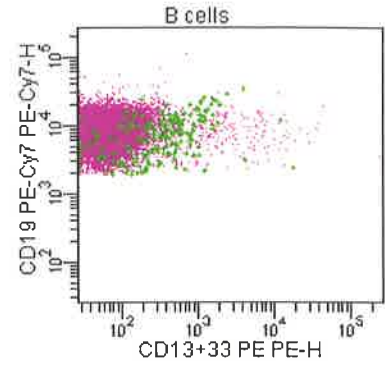
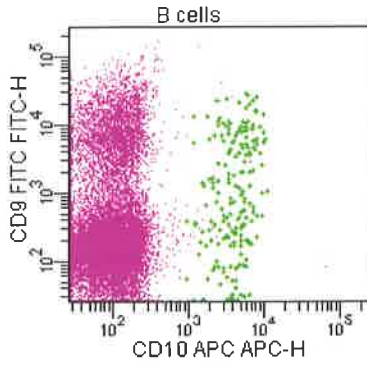
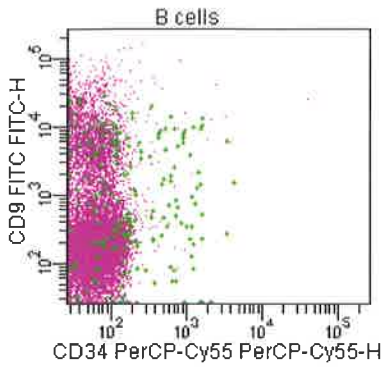


Tube: B-MRD (2)

Population	#Events	%Parent	%Total
All Events	217,748	####	100.0
Time	216,644	99.5	99.5
Singlets	214,411	99.0	98.5
Viable	214,401	100.0	98.5
All CD19	17,193	8.0	7.9
B cells	17,173	99.9	7.9
P1	192	1.1	0.1

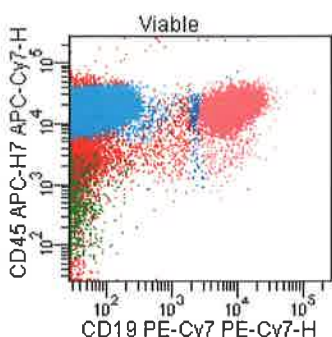
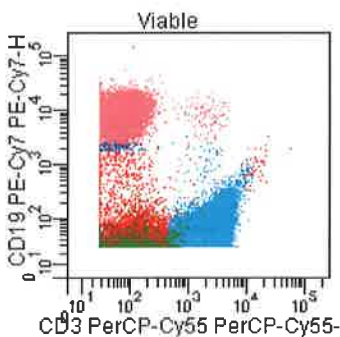
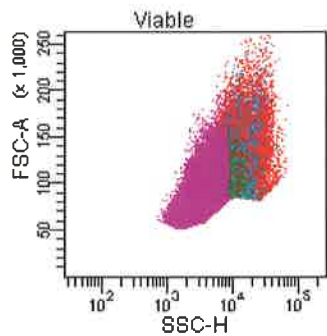
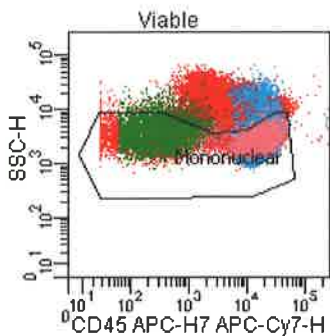
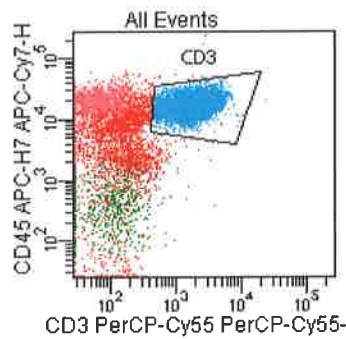
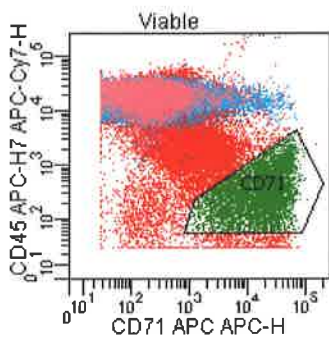
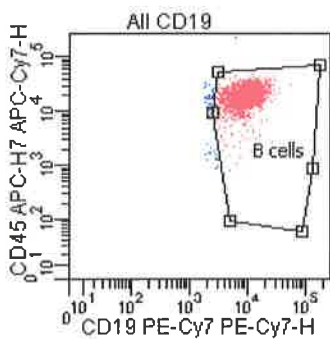
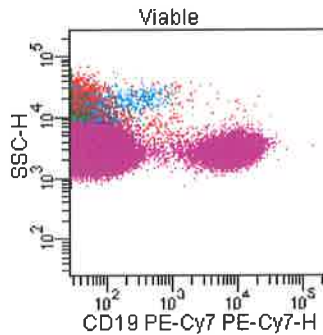
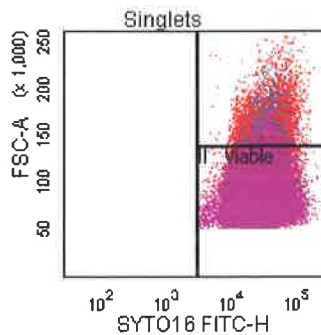
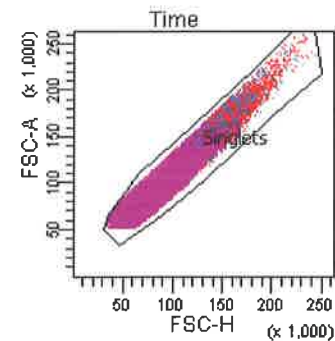
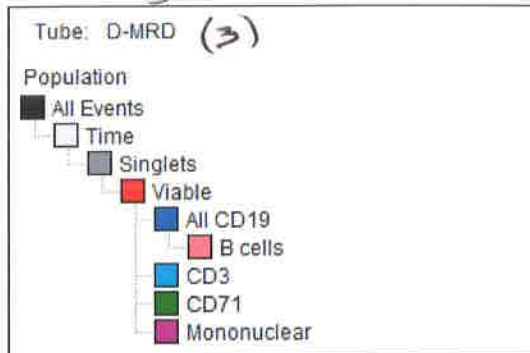
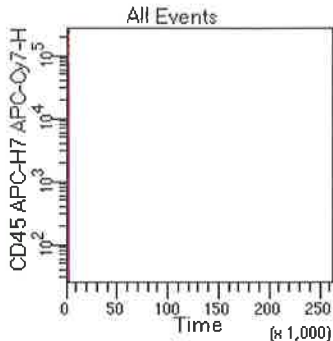
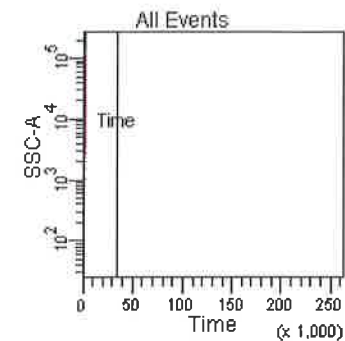


Children's Hospitals and Clinics



Experiment Name: CAP BALL-04 JULY 2018  
 Specimen Name: 16-02323 857835\_001  
 Tube Name: B-MRD  
 Record Date: Feb 3, 2016 2:32:41 PM  
 \$OP: HPDevelopmental  
 GUID: b8cd7bef-f247-4a9e-bb73-a6061fe01ab9  
 Serial Number:

Population	#Events	%Parent	%Grand Parent	%Total
All Events	217,748	####	####	100.0
Time	216,644	99.5	####	99.5
Singlets	214,411	99.0	98.5	98.5
Viable	214,401	100.0	99.0	98.5
All CD19	17,193	8.0	8.0	7.9
B cells	17,173	99.9	8.0	7.9
P1	192	1.1	1.1	0.1



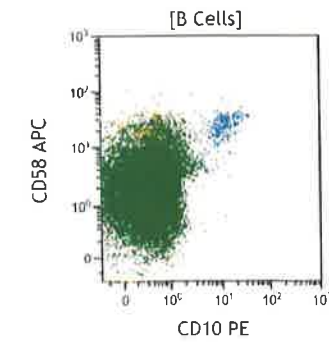
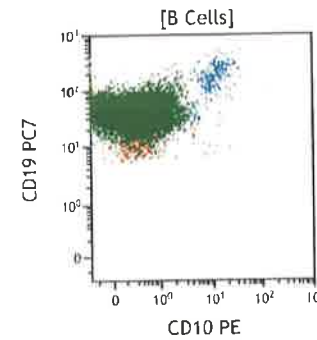
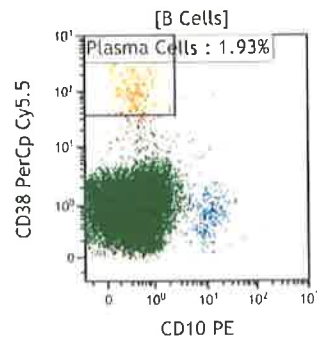
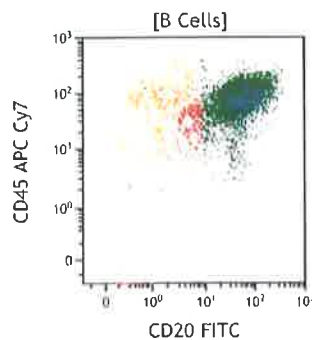
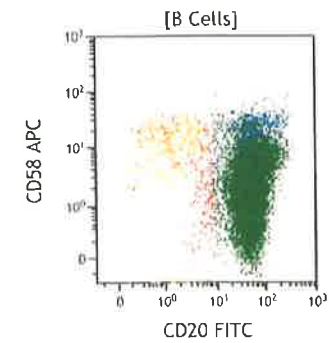
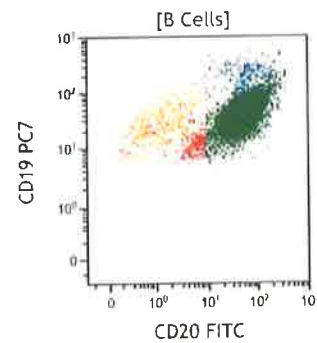
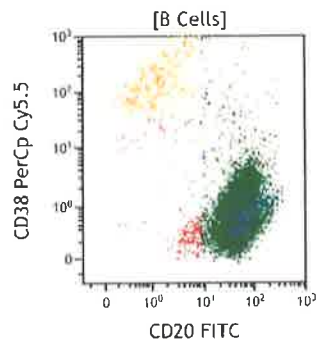
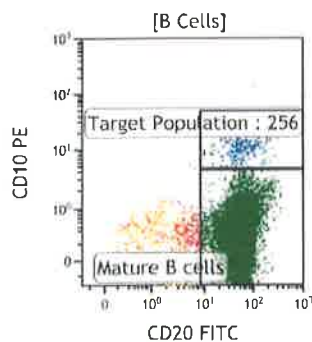
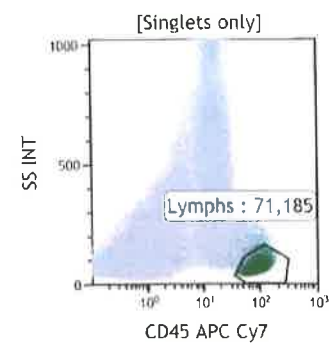
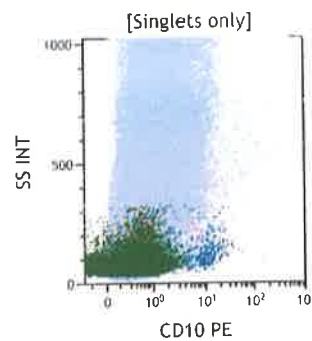
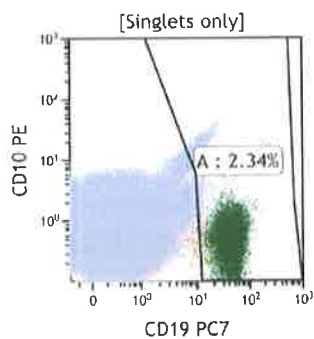
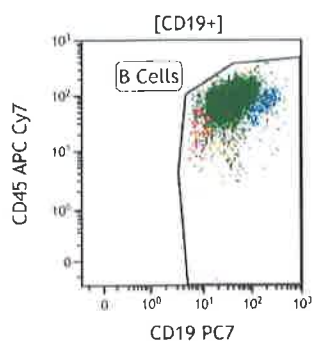
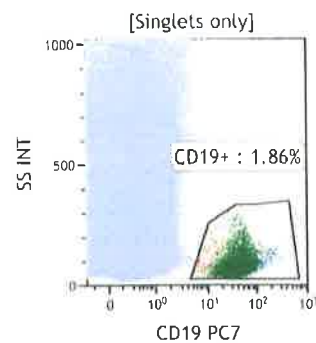
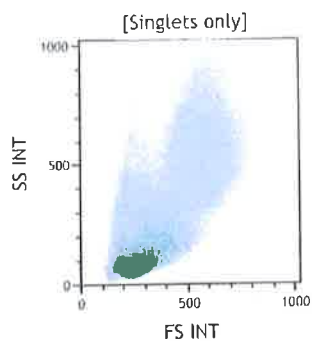
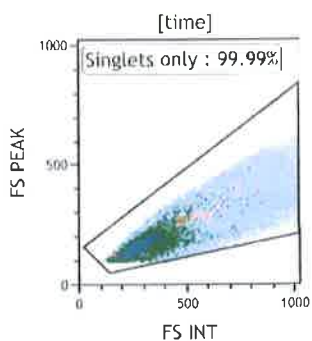
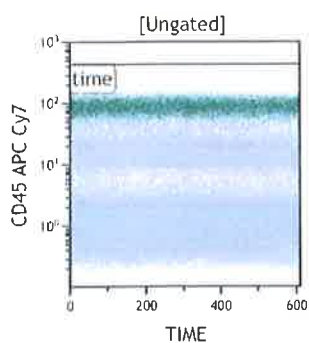
Experiment Name:	CAP BALL-04 JULY 2018			
Specimen Name:	16-02323 857835_002			
Tube Name:	D-MRD			
Record Date:	Feb 3, 2016 2:35:15 PM			
\$OP:	HPDevelopmental			
GUID:	6ffabc82-270e-4a66-93ba-118657...			
Serial Number:				

Population	#Events	%Parent	%Grand Par...	%Total
All Events	205,143	####	####	100.0
Time	205,143	100.0	####	100.0
Singlets	205,080	100.0	100.0	100.0
Viable	205,080	100.0	100.0	100.0
All CD19	15,610	7.6	7.6	7.6
CD3	150,322	73.3	73.3	73.3
CD71	5,592	2.7	2.7	2.7
B cells	15,406	98.7	7.5	7.5
Mononuclear	181,075	88.3	88.3	88.3

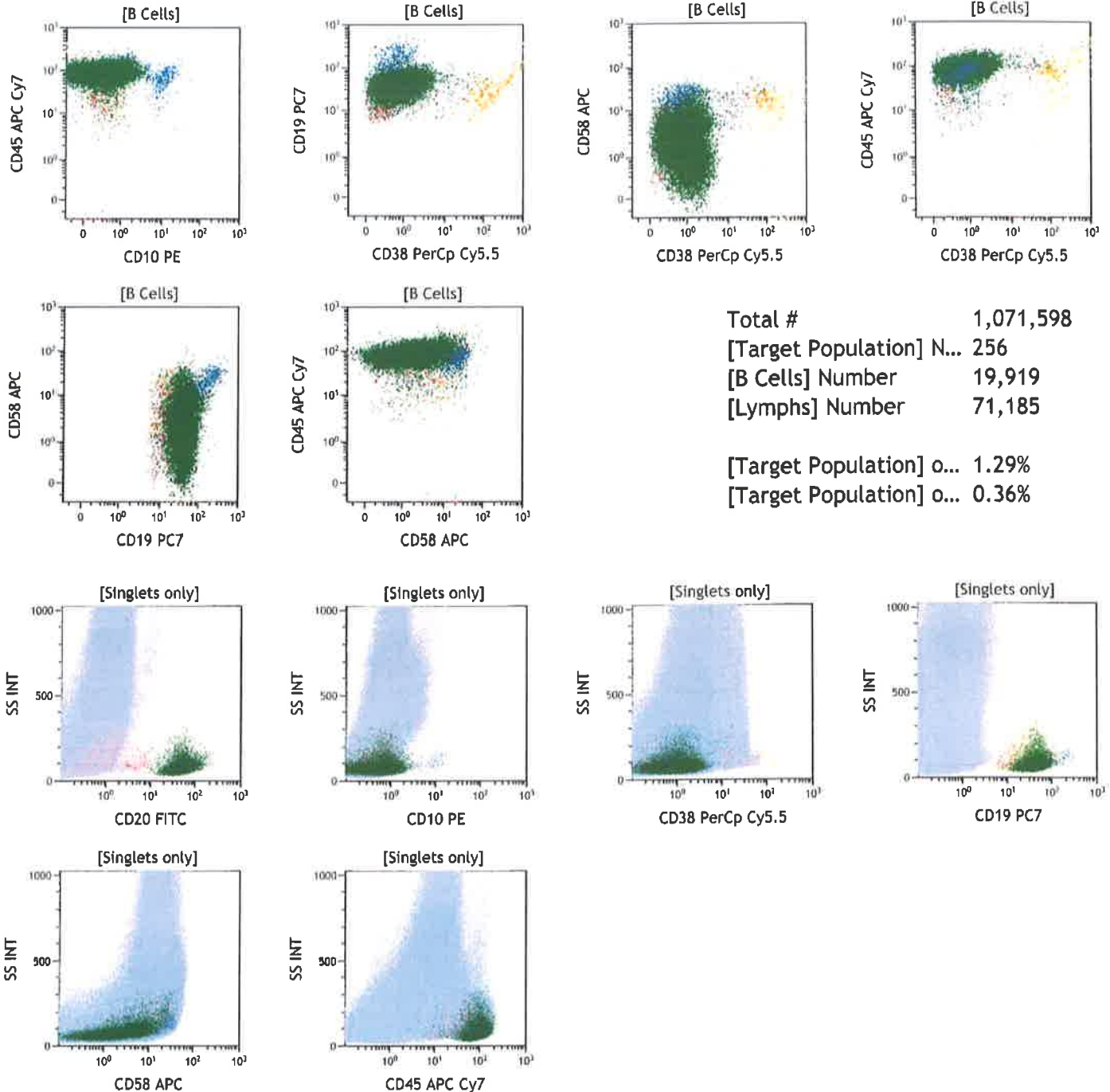


**Day 29 - Tube 1**



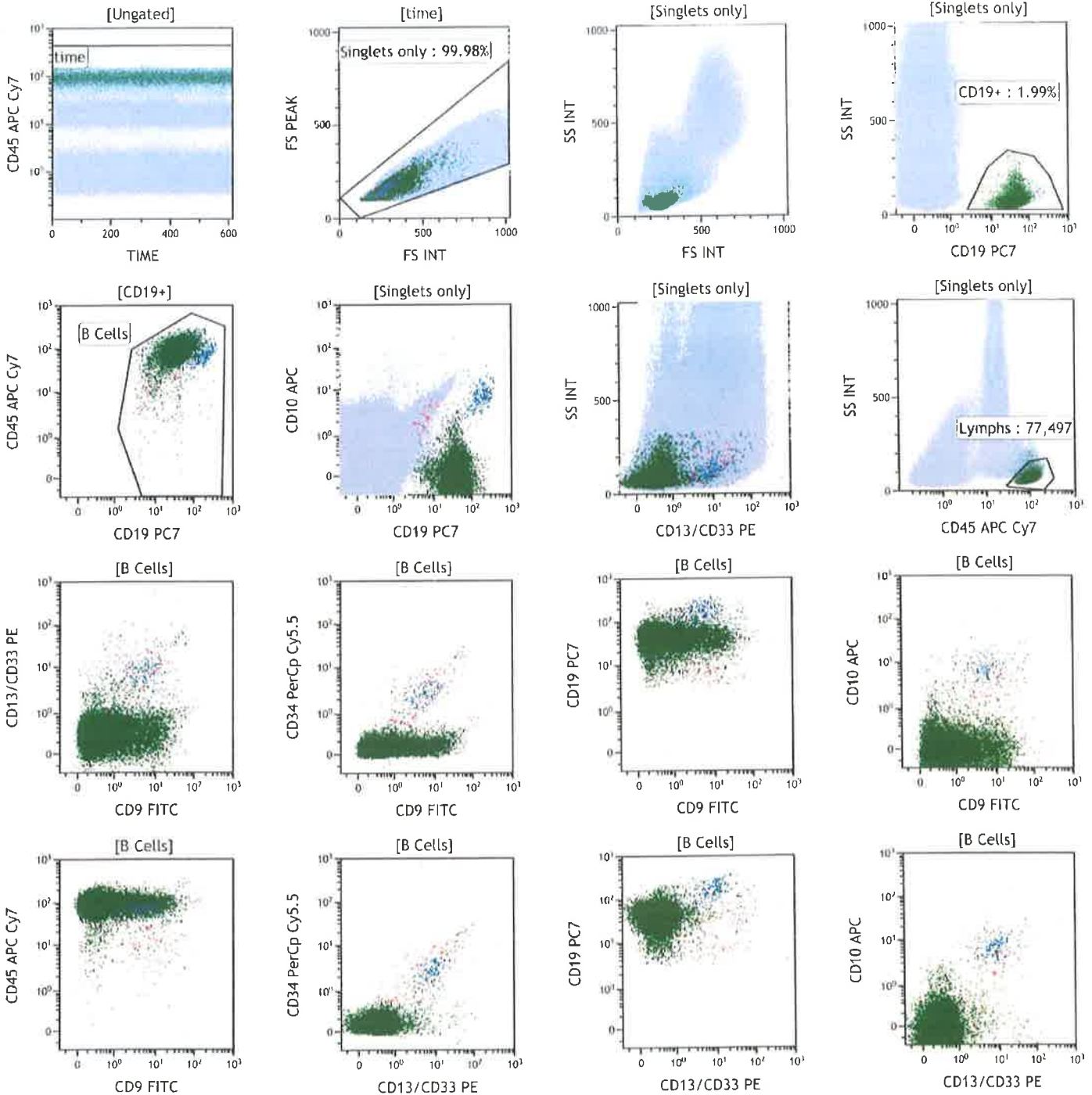


Day 29 - Tube 1, cont'd



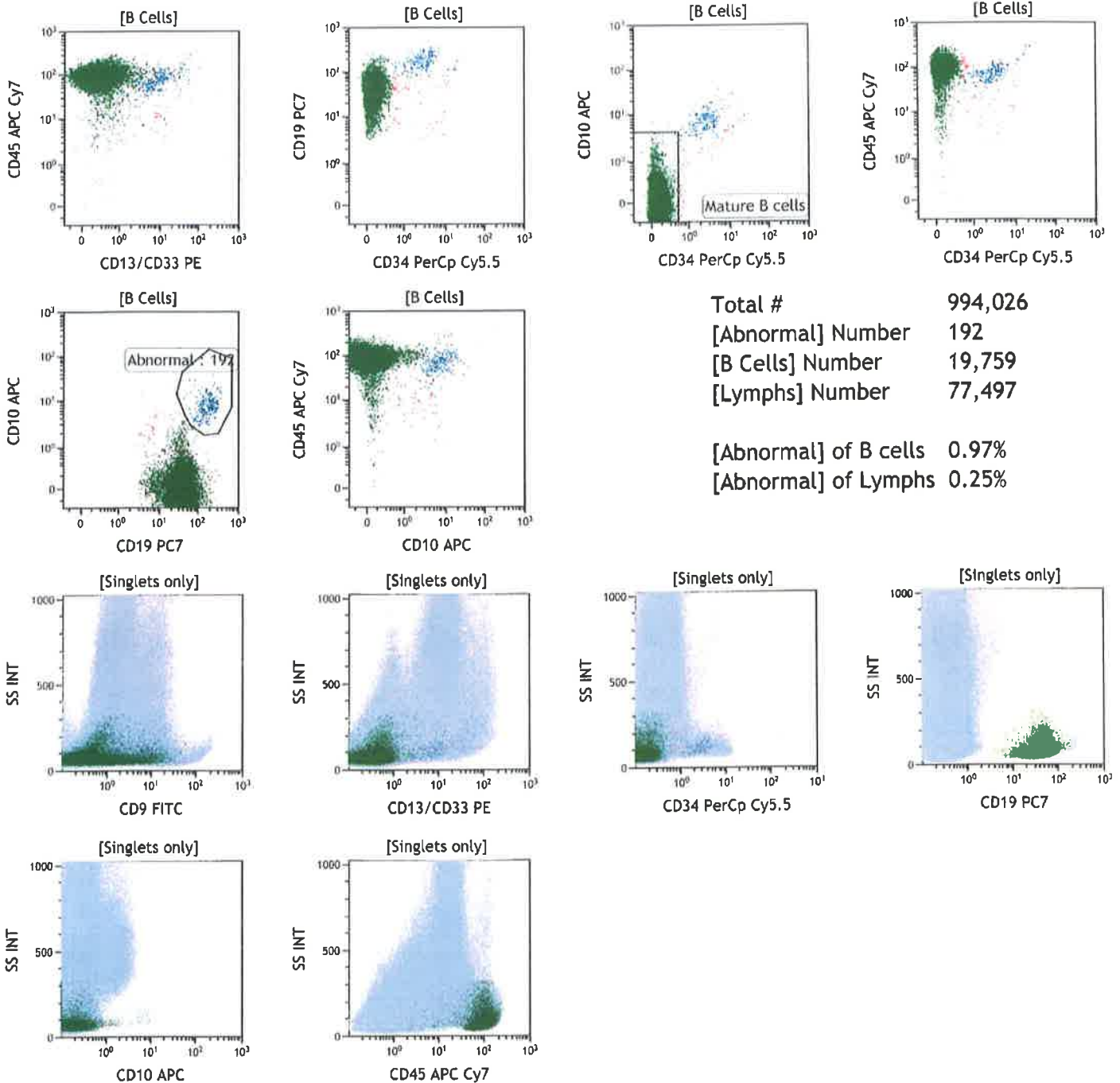


Day 29 - Tube 2



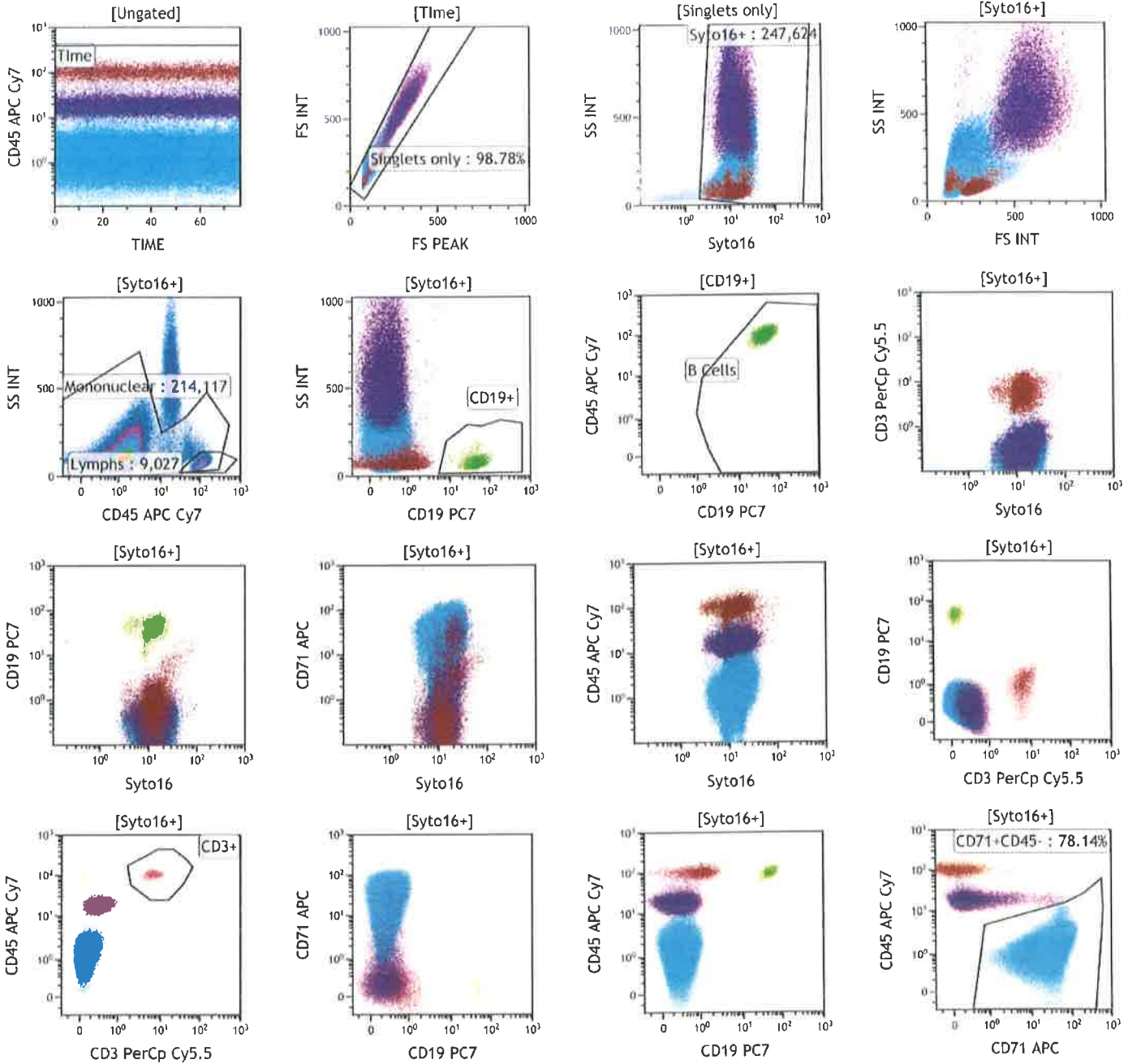


Day 29 - Tube 2, cont'd





**Day 29 - Tube 3**

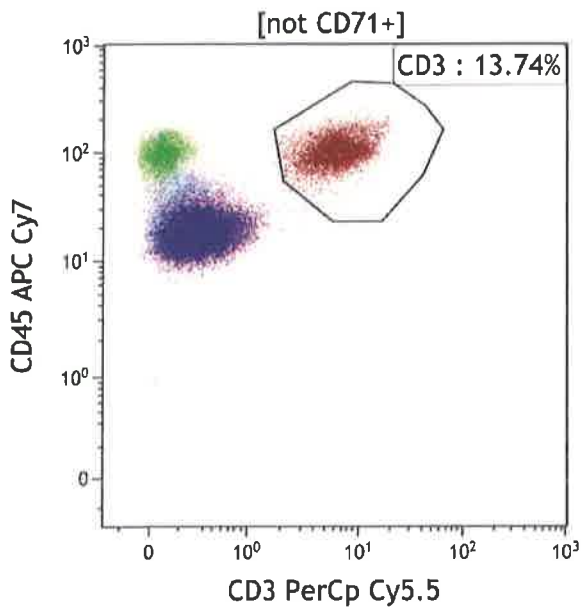
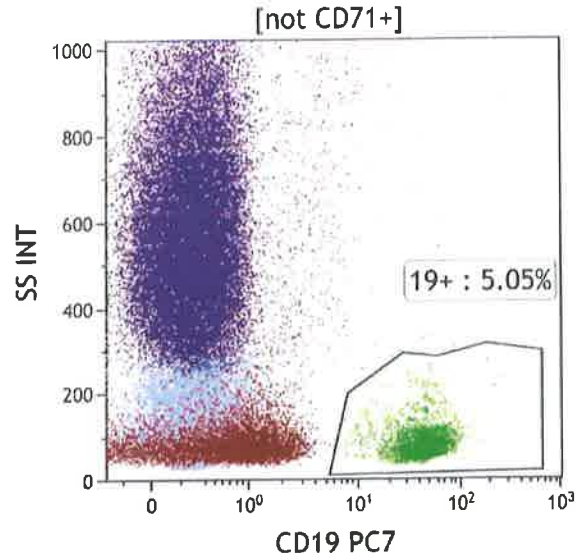
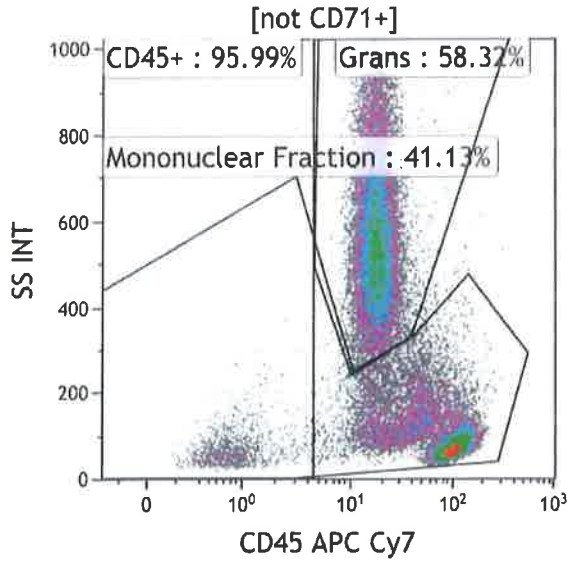


Total #	258,301
[B Cells] Number	2,728
[Lymphs] Number	9,027
[Mononuclear] Number	214,117
[CD3+] Number	7,436





Day 29 - Tube 3, cont'd

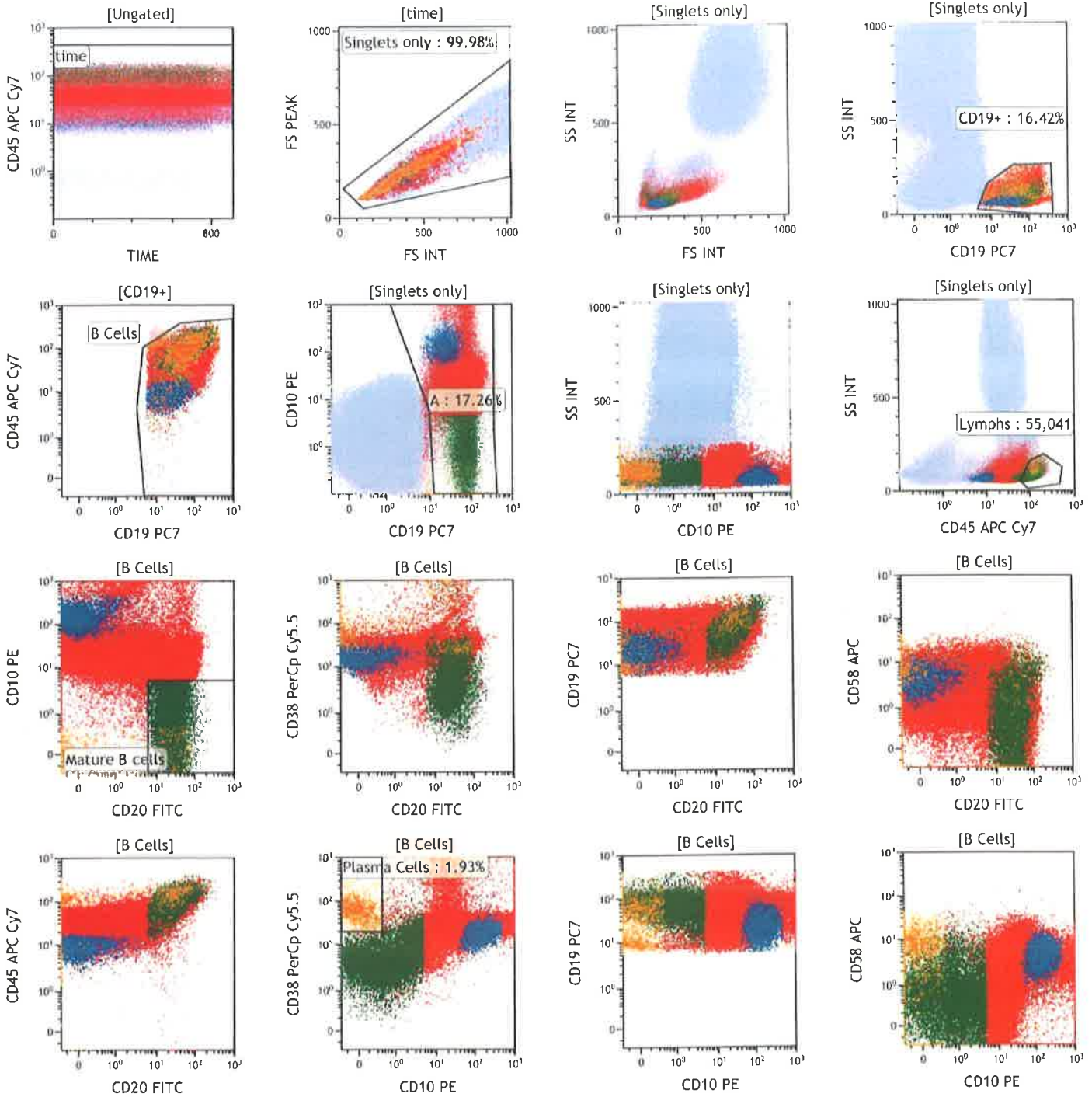


Differential for reporting

Total Lymphocytes	18.78 % of Total Leukocytes
Total Grans	58.32 % of Total Leukocytes
Total of rest	22.90 % of Total Leukocytes

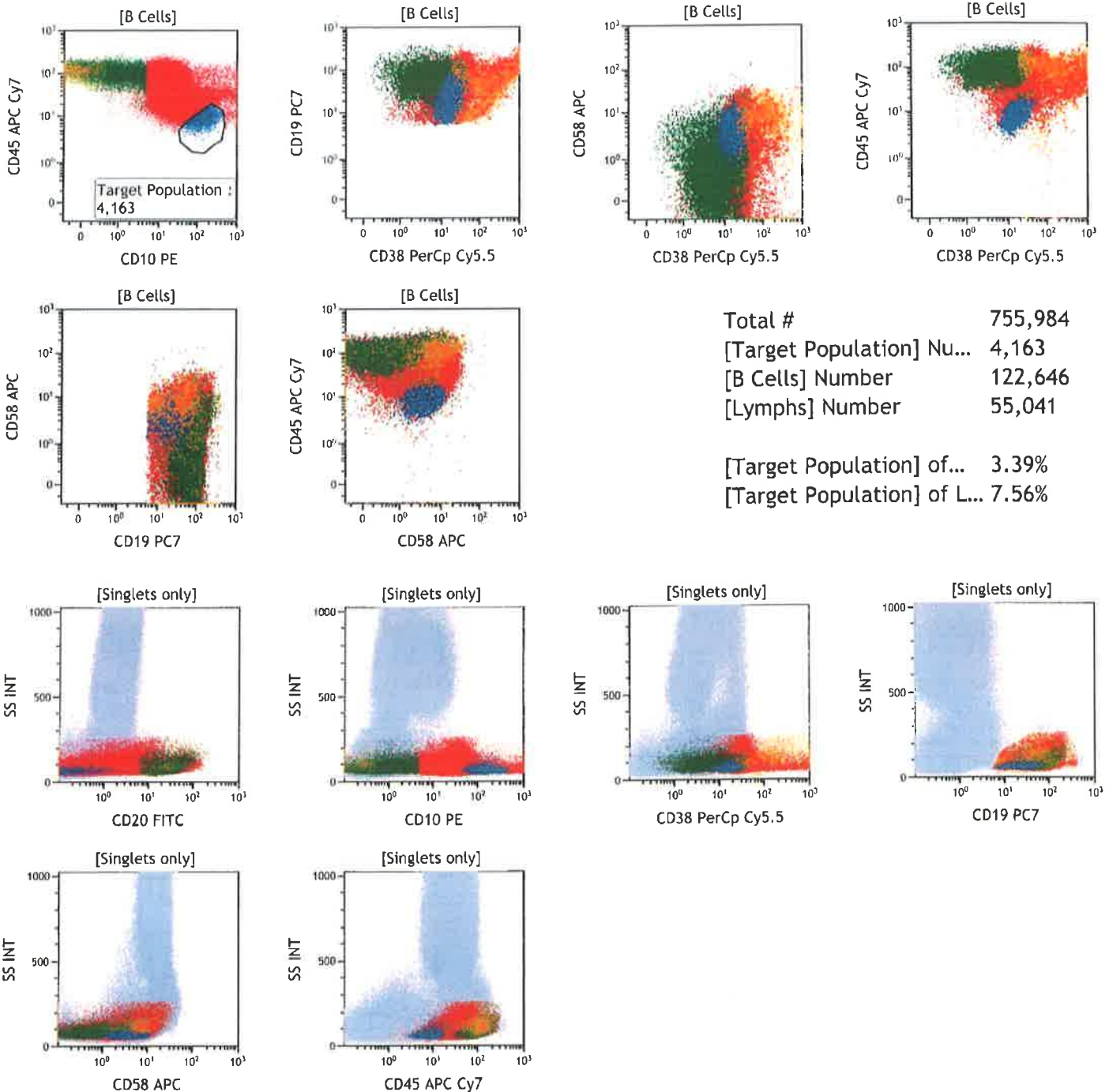


**Day 29 - Tube 1**



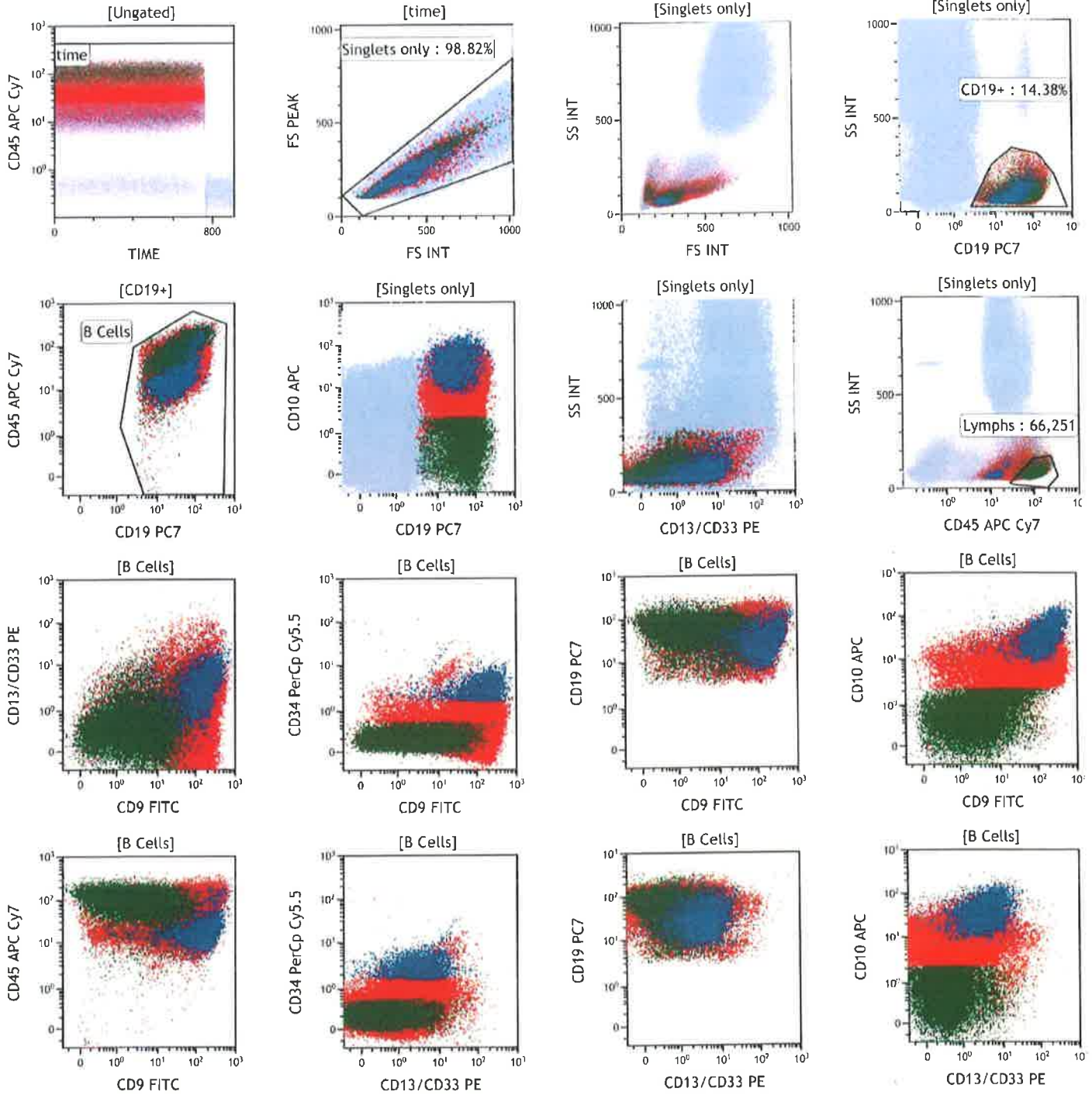


Day 29 - Tube 1, cont'd



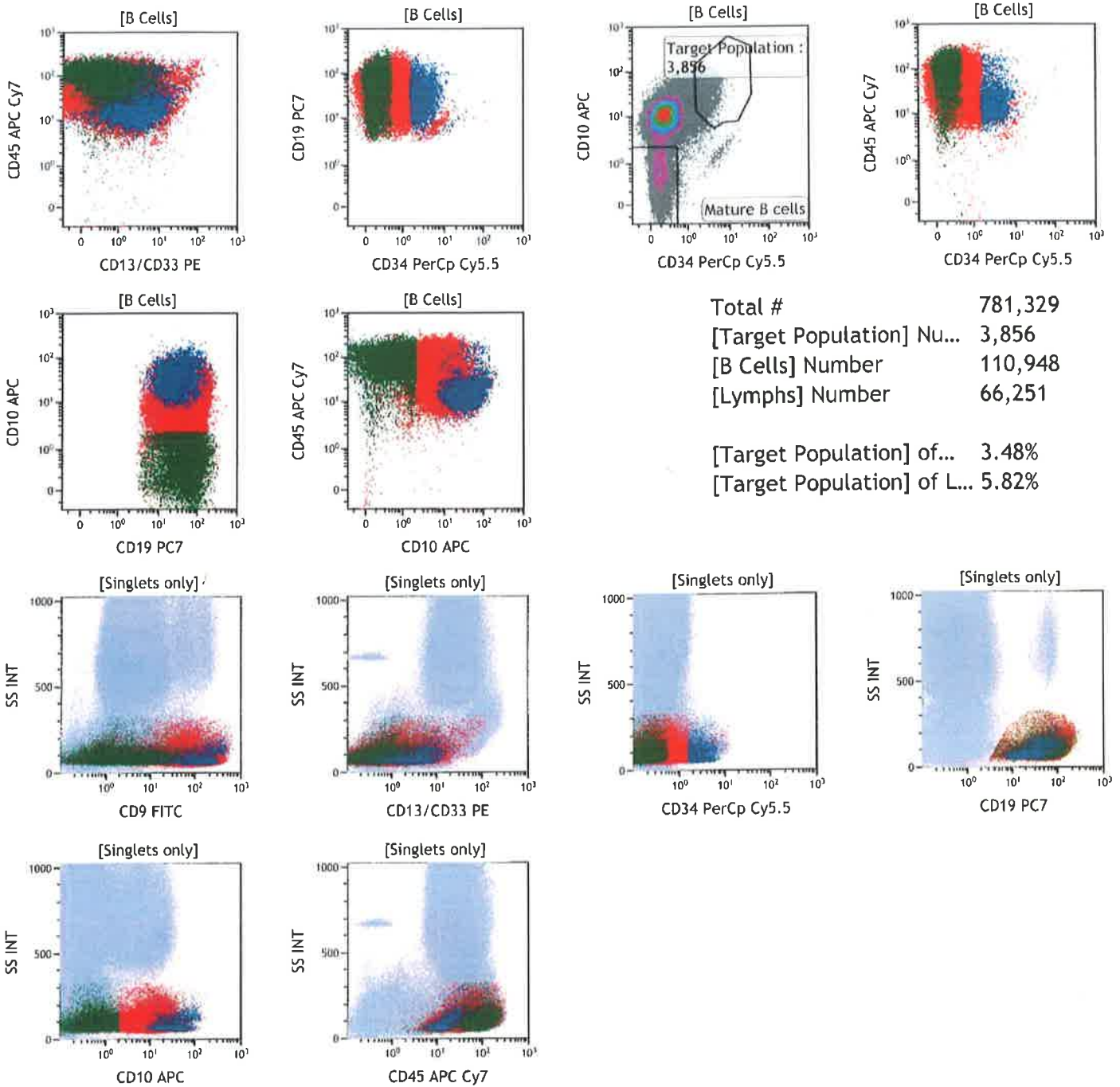


**Day 29 - Tube 2**



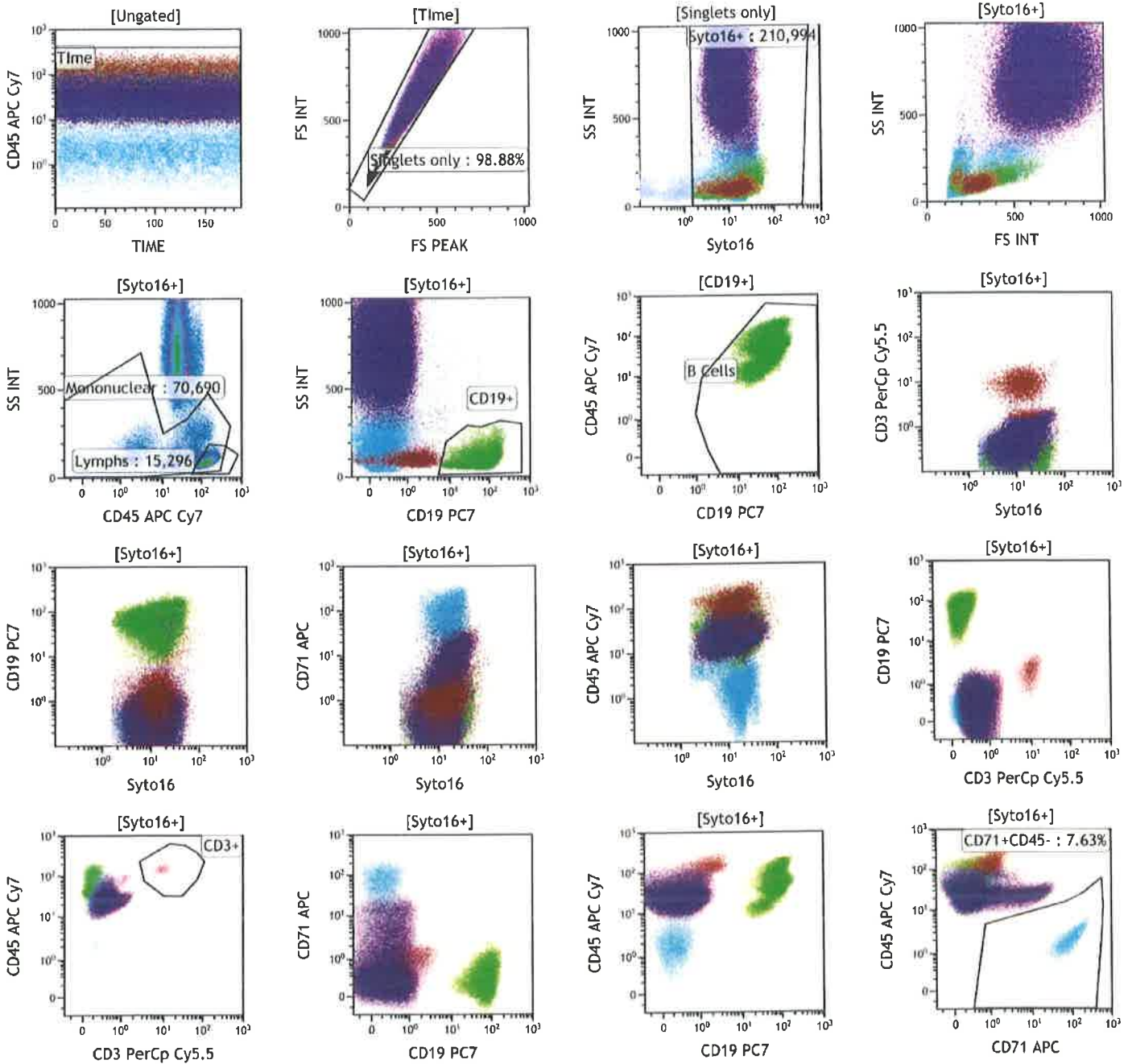


Day 29 - Tube 2, cont'd





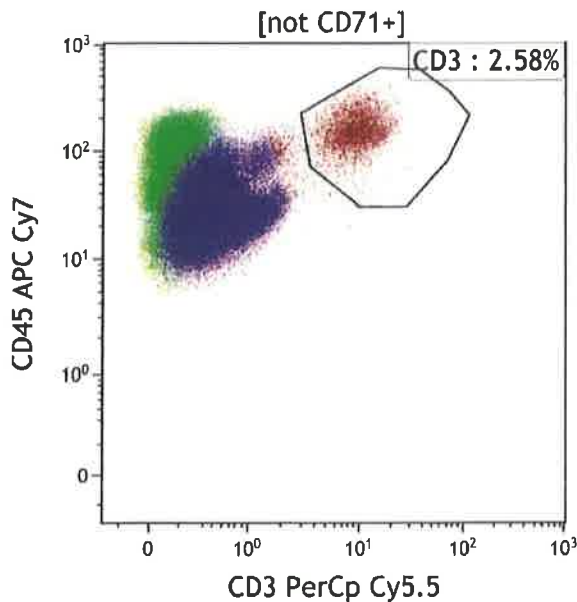
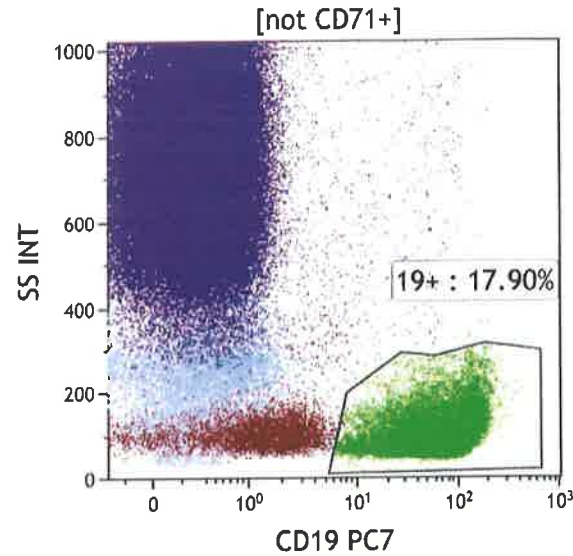
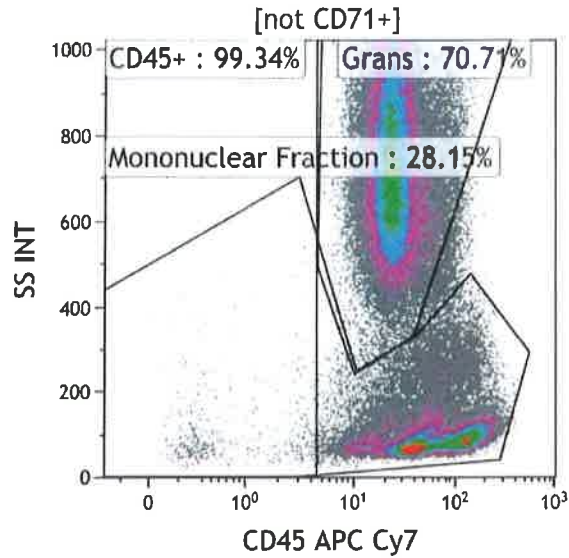
**Day 29 - Tube 3**



Total #	221,443
[B Cells] Number	34,801
[Lymphs] Number	15,296
[Mononuclear] Number	70,690
[CD3+] Number	5,026



Day 29 - Tube 3, cont'd



Differential for reporting

Total Lymphocytes	20.48 % of Total Leukocytes
Total Grans	70.71 % of Total Leukocytes
Total of rest	8.80 % of Total Leukocytes