(BALL-A 2018)

BALL-A Discussion

There were 3 samples distributed in this B-ALL MRD challenge. Case 1 was sent as a generic FCS file, while cases 2 and 3 were sent as gated PDF's. 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-01 Low Level Positive (0.2%)

This case had a small population of residual leukemic cells. The CD19 gated population in Tube 1 showed normal expression of CD10 positive cells with dim/negative CD45. CD20 and CD38 are negative. CD58 was similar to the brightest background mature B-cells. Plasma cells were clearly visible (CD38 bright, CD10 neg, CD20 neg). In Tube 2, the CD19 gated cells were positive for CD34, dim/negative CD45, negative for CD9 and positive for CD10 and CD13/CD33.

All participants reported this sample as positive; most (94.6%) identified the sample as positive in the 0.1%–0.9% range.

Case BALL-02 Small Hematogone Population

This case did not contain an abnormal population. The CD19 gated population in Tube 1 showed a continuum of CD10 positive cells with variable CD45 and CD20, and constant CD38 expression. CD58 was similar to background mature B-cells. Plasma cells were clearly visible (CD38 bright, CD10 neg, CD20 neg). In Tube 2, the CD19 gated cells were partially positive for CD34, variable CD45, CD9, and CD10, and negative for CD13/CD33.

A total of 58.2% of participants reported this sample as positive, and all identified the sample as positive in the 0.1%–0.9% range. It is possible this is because laboratories were not familiar with the gating strategy, and expression level of the various antibodies and fluorochromes.

Case BALL-03 Positive (0.05%)

This case had a very small population of residual leukemic cells. The CD19 gated population in Tube 1 showed overexpression of CD10 positive cells with dim/negative CD45 and CD20, and negative CD38 expression. CD58 was similar to the brightest background mature B-cells. Plasma cells were clearly visible (CD38 bright, CD10 neg, CD20 neg). In Tube 2, the CD19 gated cells were negative for CD34, dim/negative CD45, bright CD9 and CD10, and negative for CD13/CD33.

A total of 94.5% of participants correctly identified this sample as positive, and most (98.2%) identified the sample as positive in the 0.01%–0.09% range. One participant called the sample positive but in a higher range (0.1%–0.9%).

Some Key Comments:

As this was the first MRD Survey for B-ALL, participant's comments were sought. Between 70%–80% of participants felt that the level of difficulty was "about right," with between 12%–23% finding the Survey "somewhat or much too difficult." Interestingly, 23% of participants found case 2 difficult. 7%–9% of participants felt the Survey was too easy.

BALL-A Discussion (cont.)

A few laboratories had difficulty with Diva software, but did manage to report results.

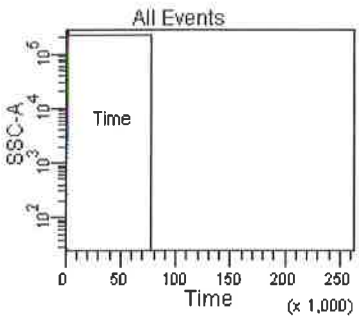
There was some confusion over Tube 3, which is used purely to calculate the denominator. Future Surveys will not require reporting of CD3 or CD71.

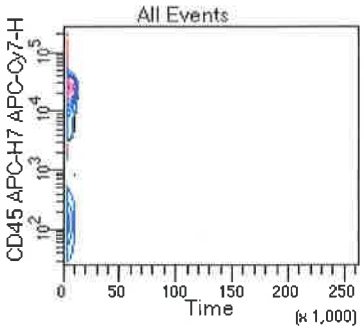
Some laboratories felt that listmode files should be used for all cases whereas others felt that manipulating LMD's was too difficult.

Reporting negatives was confusing for some laboratories. When a sample is negative, the value of abnormal cells is 0.

Overall, this first Survey was successful. There is concern over Case 2 being reported as MRD positive, however it is understood that many laboratories were not familiar with the gating strategy and fluorochromes. Future Surveys will contain a mixture of positive and negative samples including those with regenerating hematogones. Future cases will also include diagnostic plots for comparison.

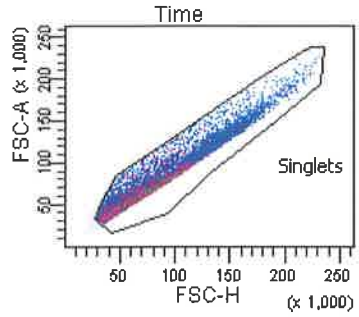
Michael Keeney, ART, FIMLS, FCSMLS(D)
Diagnostic Immunology Resource Committee

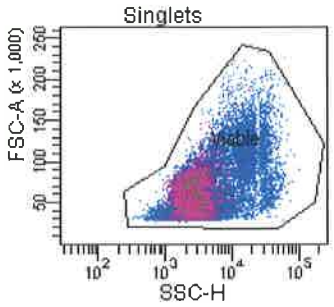


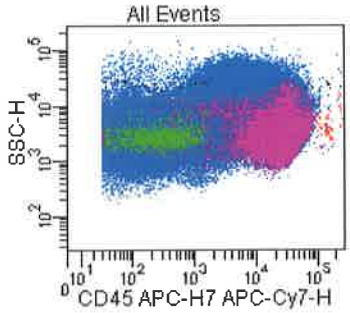


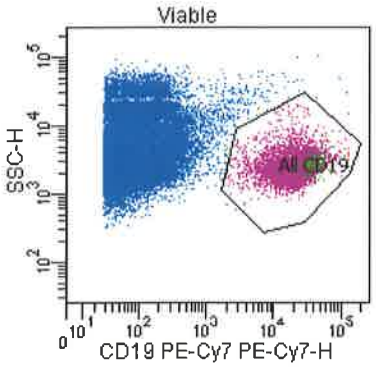
Tube: A (1)

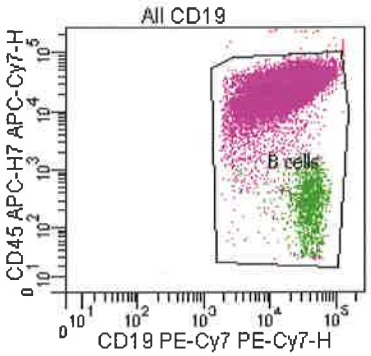
Population	#Events	%Parent	%Total
All Events	672,806	####	100.0
Time	658,484	97.9	97.9
Singlets	521,932	79.3	77.6
Viable	521,849	100.0	77.6
All CD19	70,290	13.5	10.4
B cells	70,222	99.9	10.4
P1	1,304	1.9	0.2

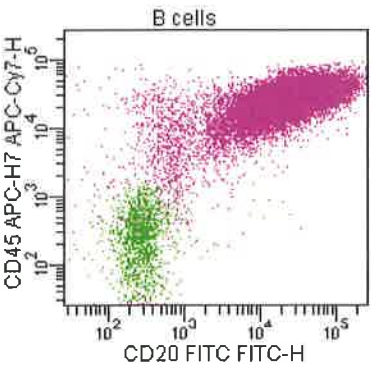


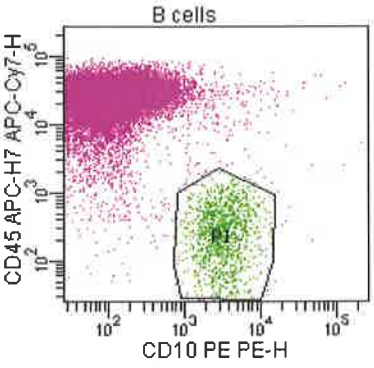


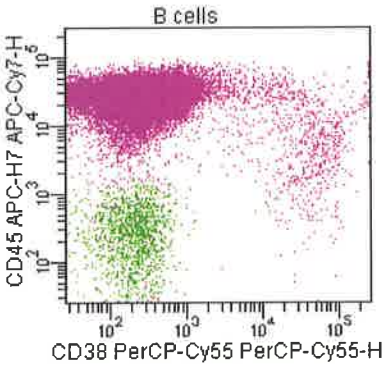


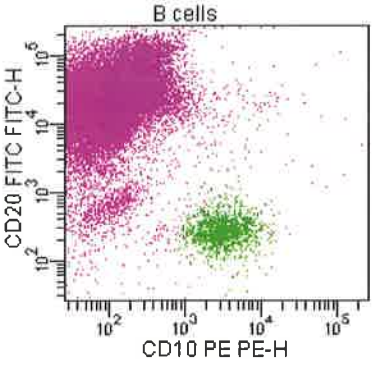


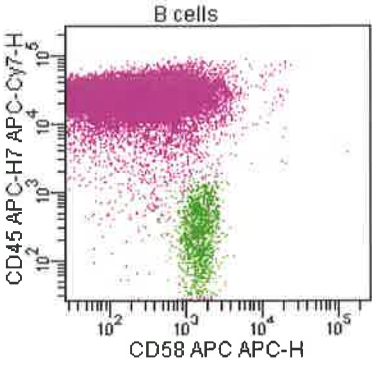


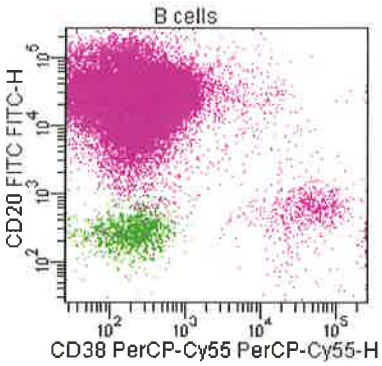


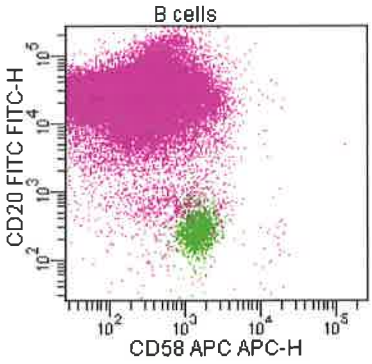


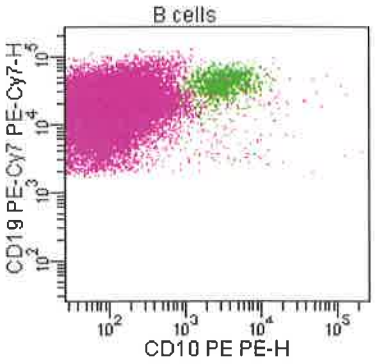


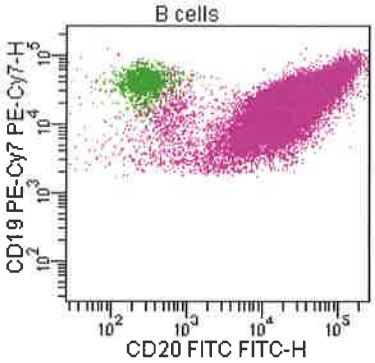


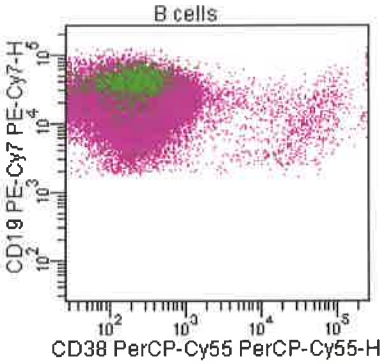


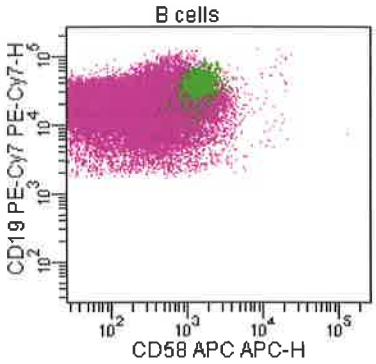


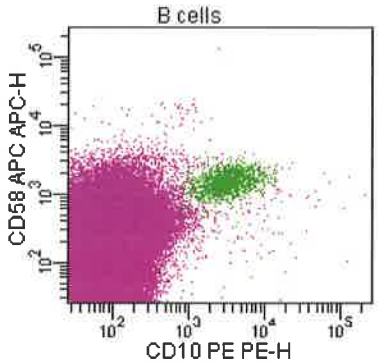


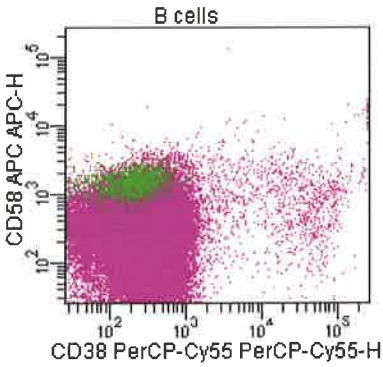


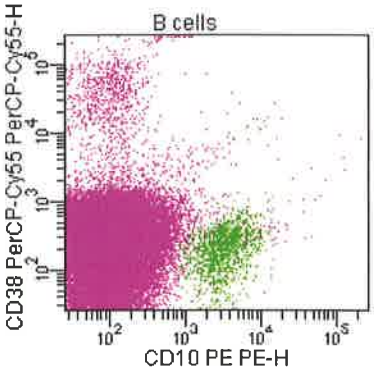












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Specimen Name:	Case 4
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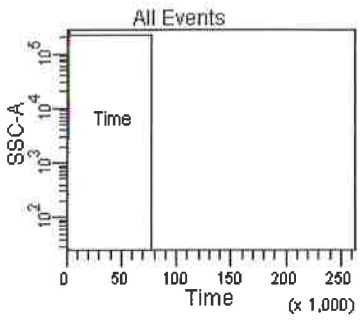
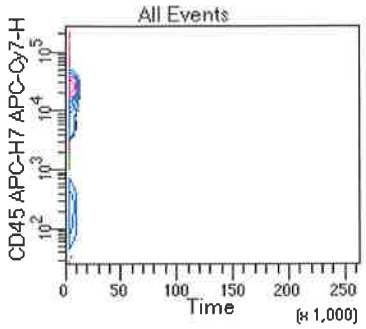
$OP:	Administrator
GUID:	4aa35c30-f6e2-4186-8dbf-c85bfa16bffb
Serial Number:	

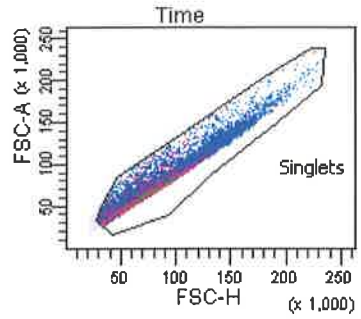
Population	#Events	%Parent	%Grand Parent	%Total
All Events	672,806	####	####	100.0
Time	658,484	97.9	####	97.9
Singlets	521,932	79.3	77.6	77.6
Viable	521,849	100.0	79.3	77.6
All CD19	70,290	13.5	13.5	10.4
B cells	70,222	99.9	13.5	10.4
P1	1,304	1.9	1.9	0.2

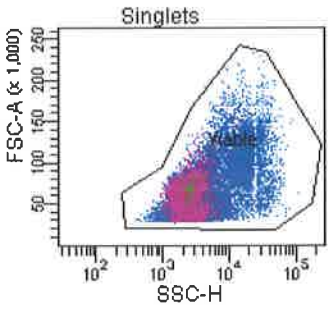


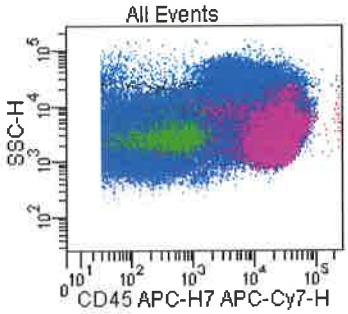


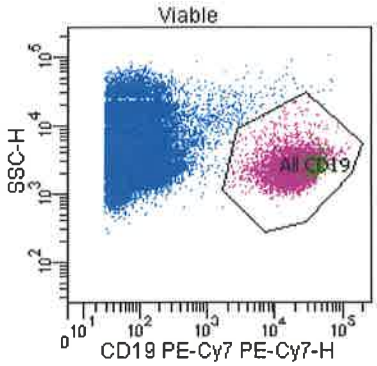
Tube: B (2)

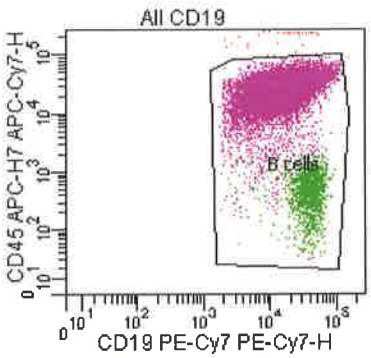
Population	#Events	%Parent	%Total
All Events	725,096	####	100.0
Time	710,018	97.9	97.9
Singlets	562,141	79.2	77.5
Viable	562,062	100.0	77.5
All CD19	76,305	13.6	10.5
B cells	76,264	99.9	10.5
P1	1,516	2.0	0.2

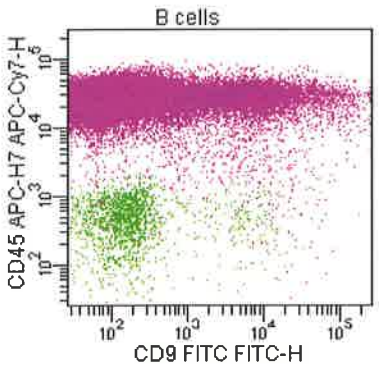


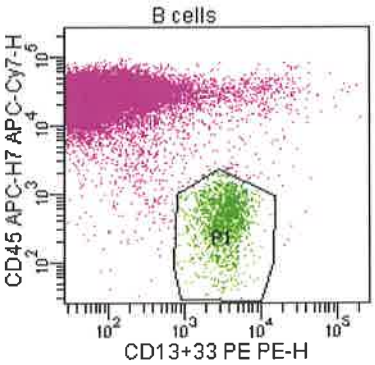


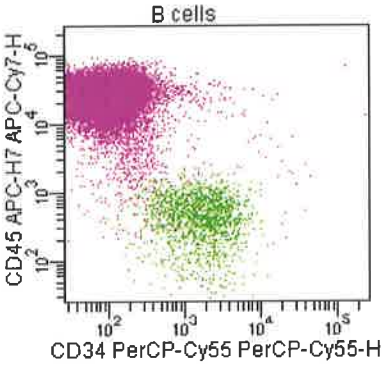


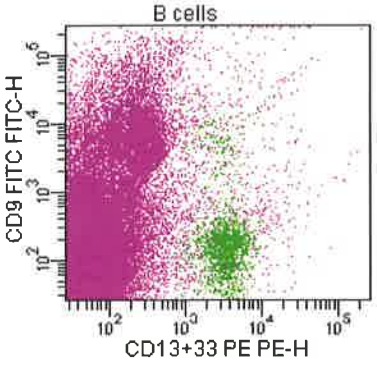


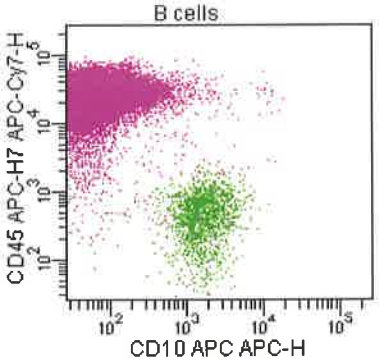


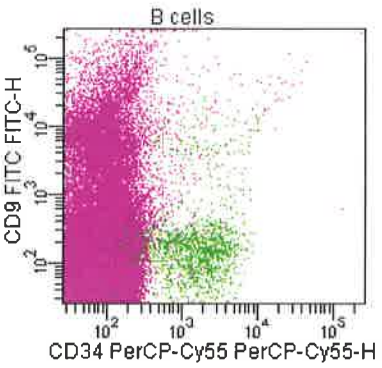


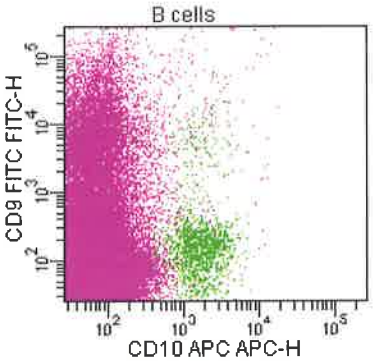


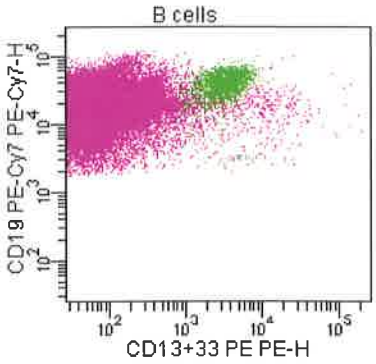


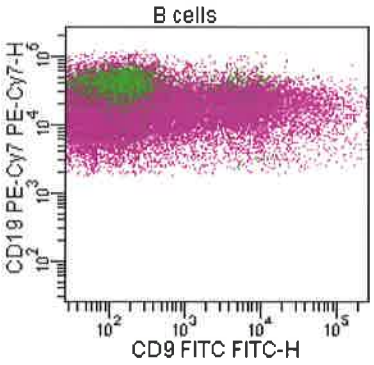


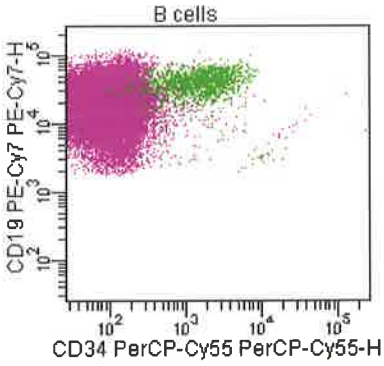


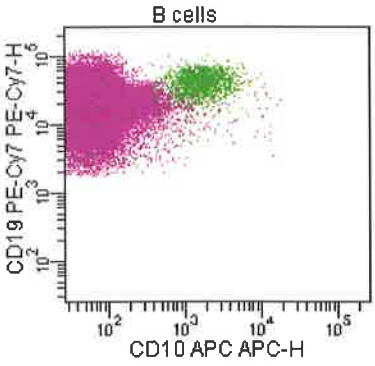


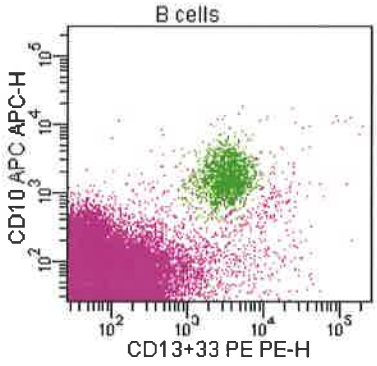


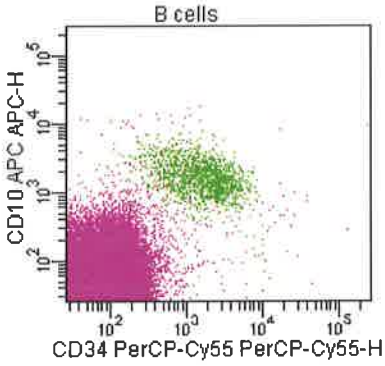


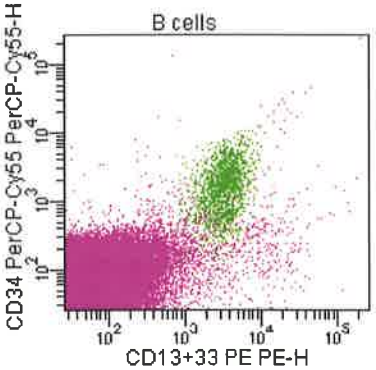












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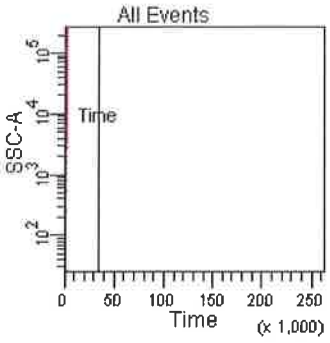
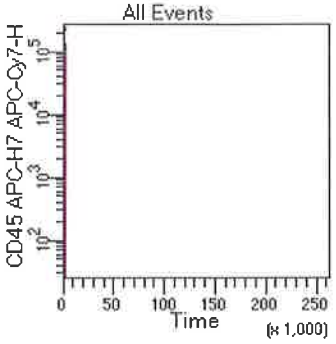
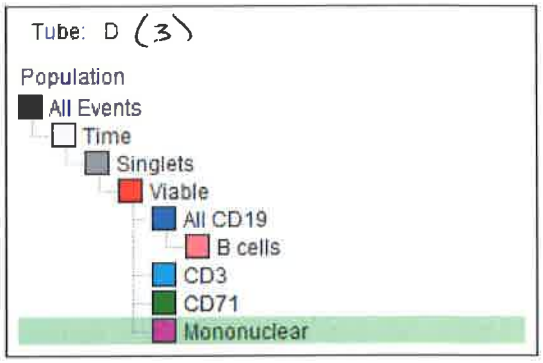
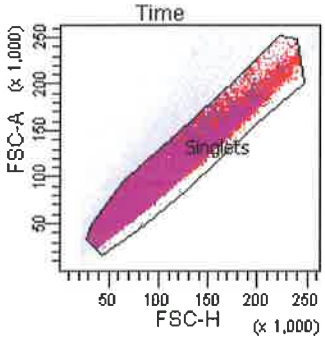
$OP:	Administrator
GUID:	55eafa75-c209-4378-aef9-f23b9e749e76
Serial Number:	

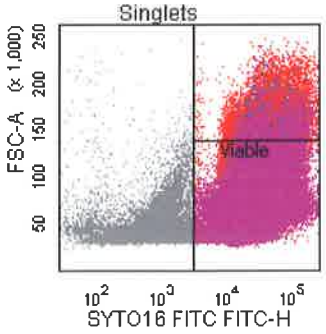
Population	#Events	%Parent	%Grand Parent	%Total
All Events	725,096	####	####	100.0
Time	710,018	97.9	####	97.9
Singlets	562,141	79.2	77.5	77.5
Viable	562,062	100.0	79.2	77.5
All CD19	76,305	13.6	13.6	10.5
B cells	76,264	99.9	13.6	10.5
P1	1,516	2.0	2.0	0.2

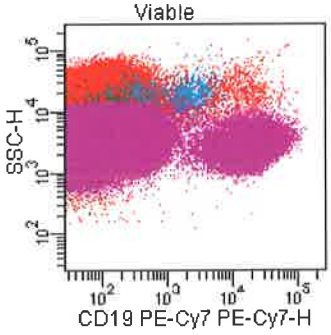


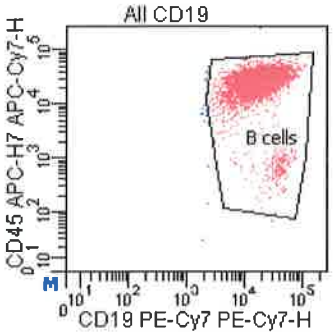


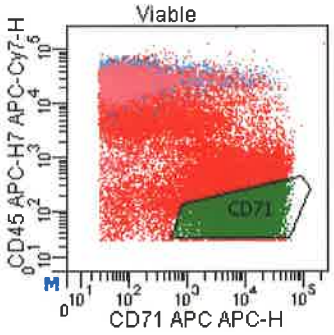


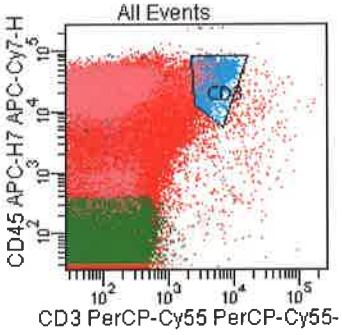


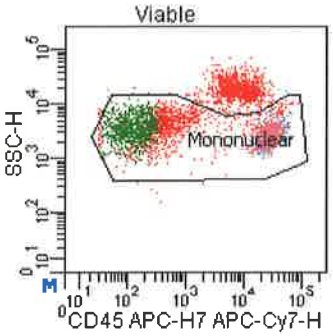


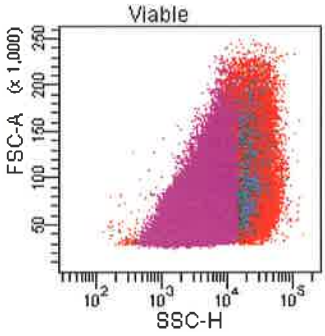






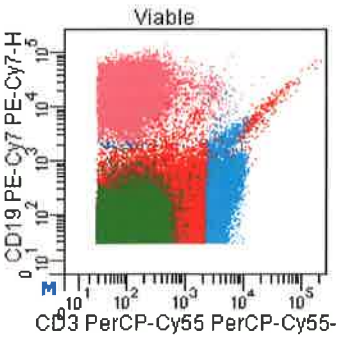


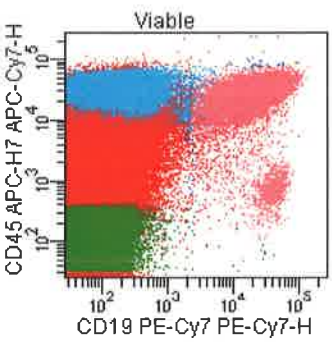




Experiment Name:	BALL Case 1
Specimen Name:	Case 4_003
Tube Name:	D
Record Date:	Nov 29, 2014 4:10:08 PM
$OP:	Administrator
GUID:	054b77c1-1b74-4287-b81e-97ef2...
Serial Number	

Population	#Events	%Parent	%Grand Par...	%Total
All Events	529,760	####	####	100.0
Time	529,760	100.0	####	100.0
Singlets	438,223	82.7	82.7	82.7
Viable	419,592	95.7	79.2	79.2
All CD19	55,444	13.2	12.7	10.5
B cells	55,323	99.8	13.2	10.4
CD3	54,336	12.9	12.4	10.3
CD71	85,340	20.3	19.5	16.1
Mononuclear	319,688	76.2	73.0	60.3





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FLOW CYTOMETRY B-ALL MINIMAL RESIDUAL DISEASE

BALL-A 2018 (BALL-02)

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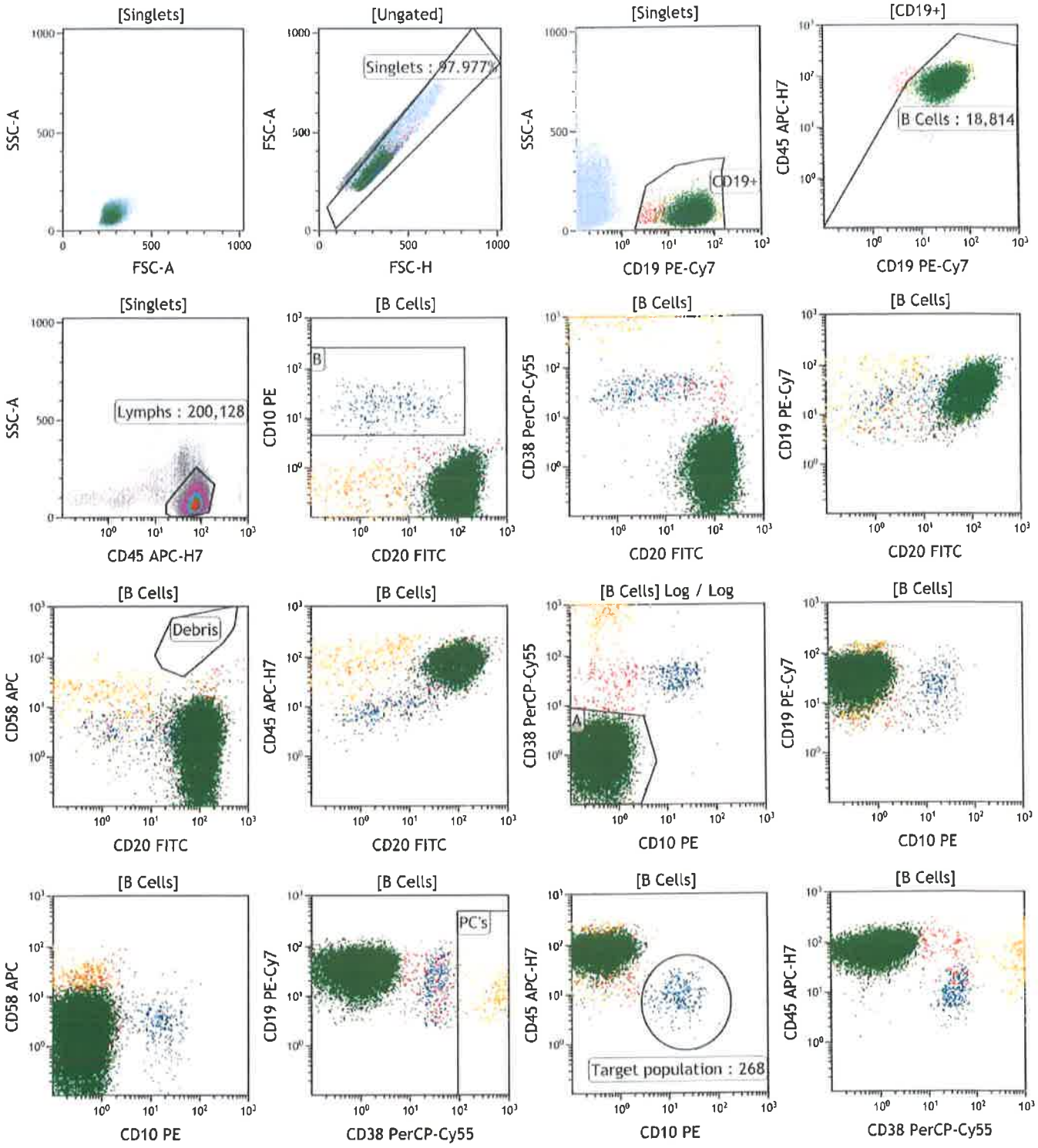
Day 29 - Tube 1



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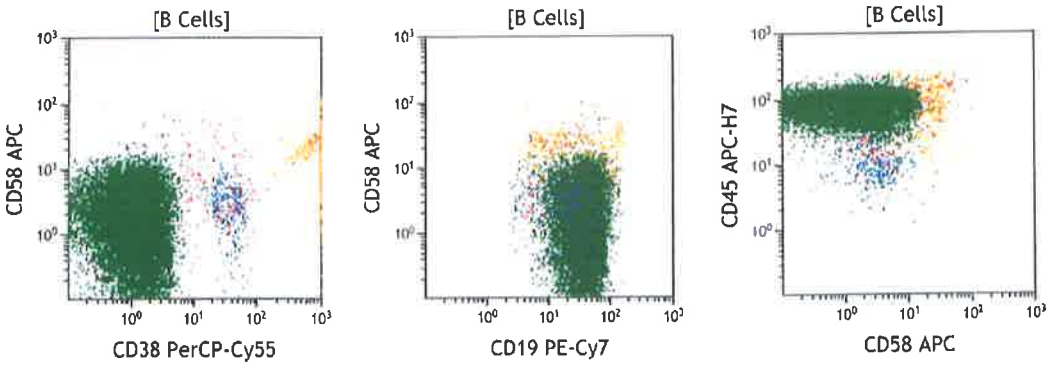

[B Cells] Number 18,814
Target population 268
[Lymphs] Number 200,128

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FLOW CYTOMETRY B-ALL MINIMAL RESIDUAL DISEASE

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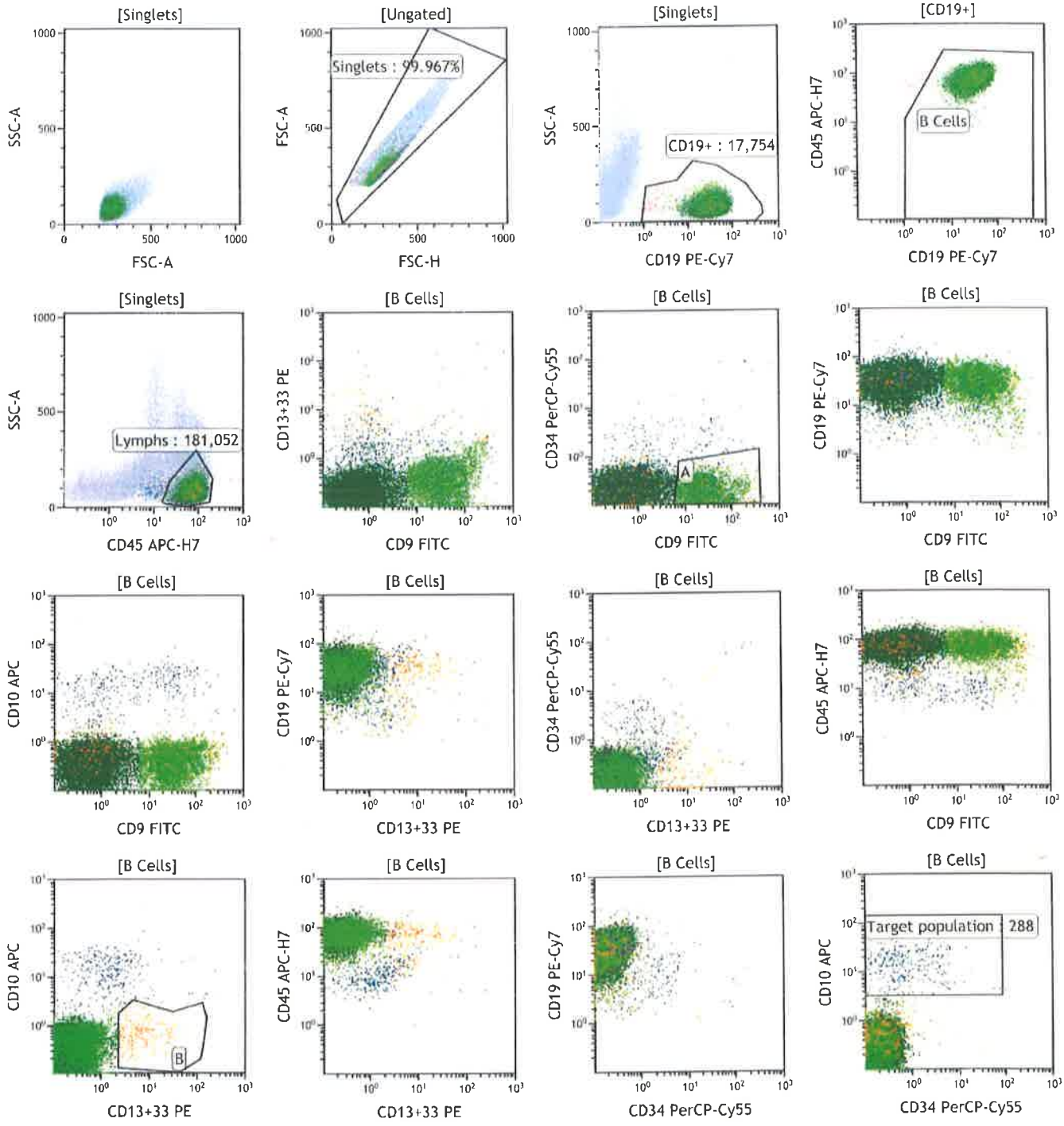

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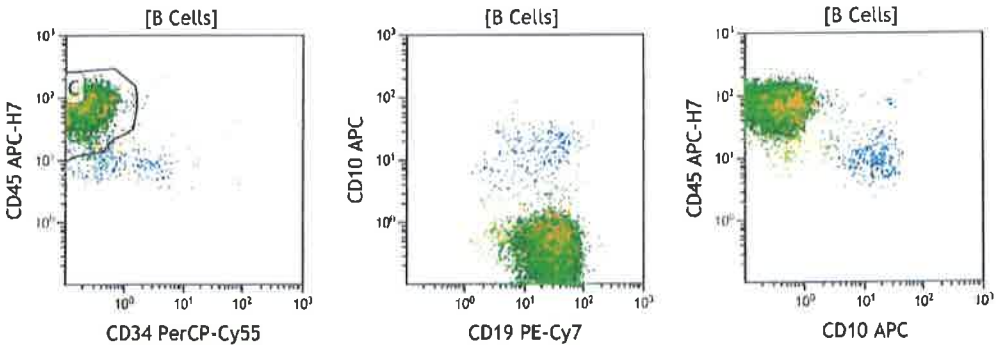
Day 29 - Tube 2, cont'd



[B Cells] Number 17,575
Target Population 288
[Lymphs] Number 181,052

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FLOW CYTOMETRY B-ALL MINIMAL RESIDUAL DISEASE

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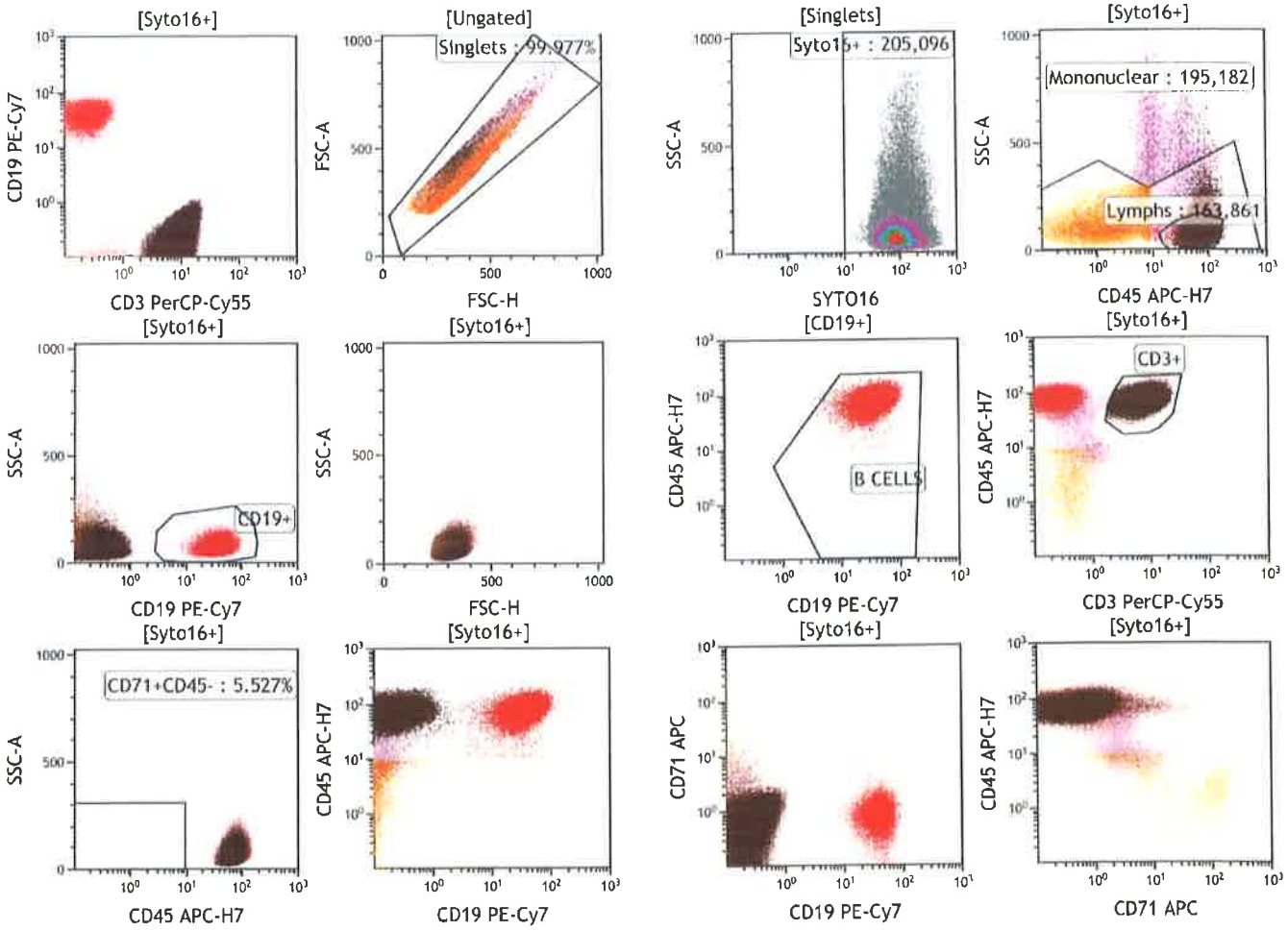

[B CELLS] Number	15,879
[Mononuclear] Number	195,182
[Syto16+] Number	205,096
[CD71+CD45-] % Gated	5.527

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FLOW CYTOMETRY B-ALL MINIMAL RESIDUAL DISEASE

BALL-A 2018 (BALL-03)

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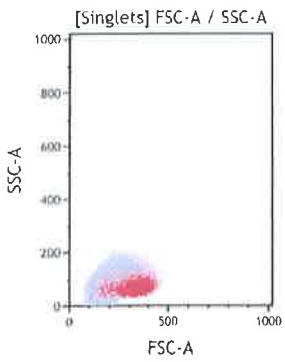
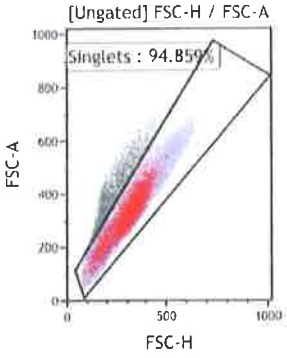
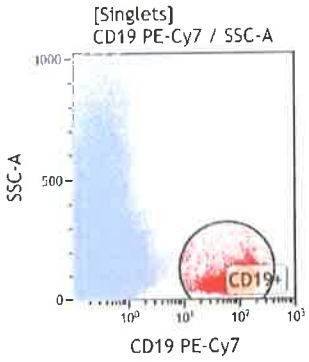
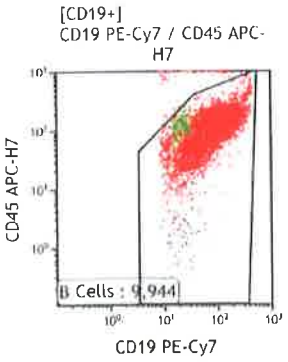
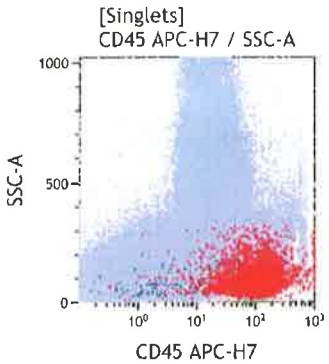
Day 29 - Tube 1

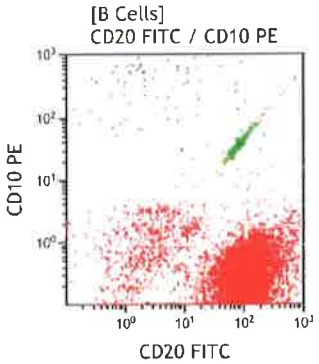


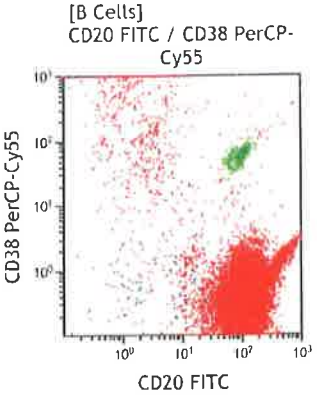


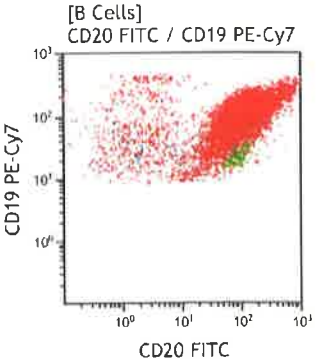


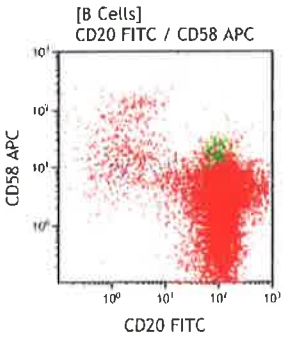


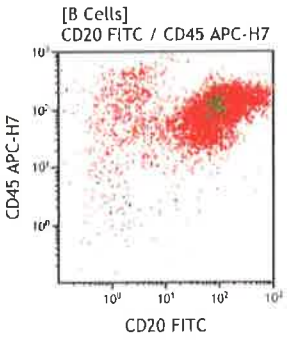


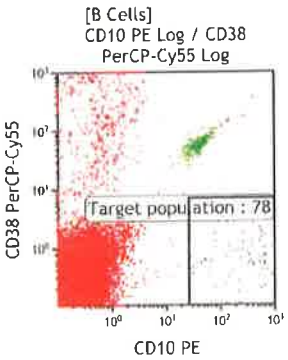


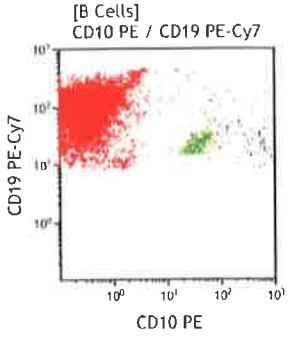


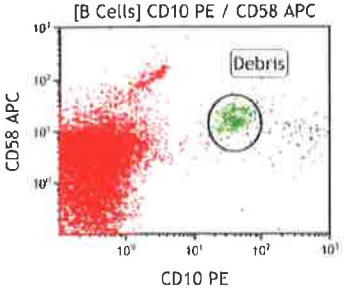


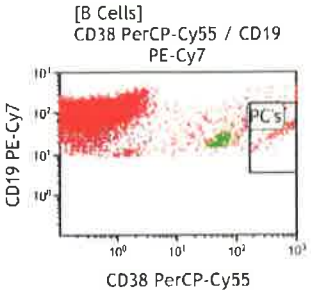


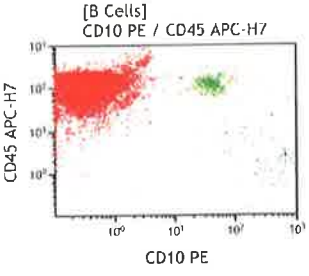


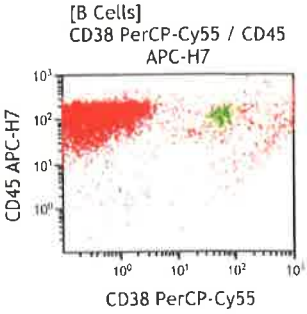












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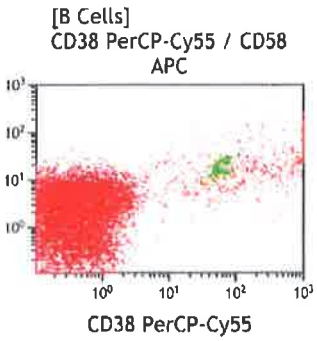

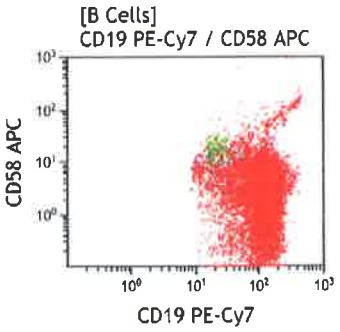

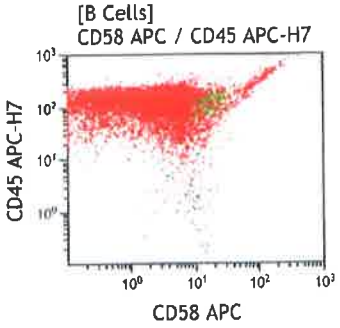

[B Cells] Number	9,944
[Target population] Nu...	78

COLLEGE of AMERICAN PATHOLOGISTS

FLOW CYTOMETRY B-ALL MINIMAL RESIDUAL DISEASE

BALL-A 2018 (BALL-03)

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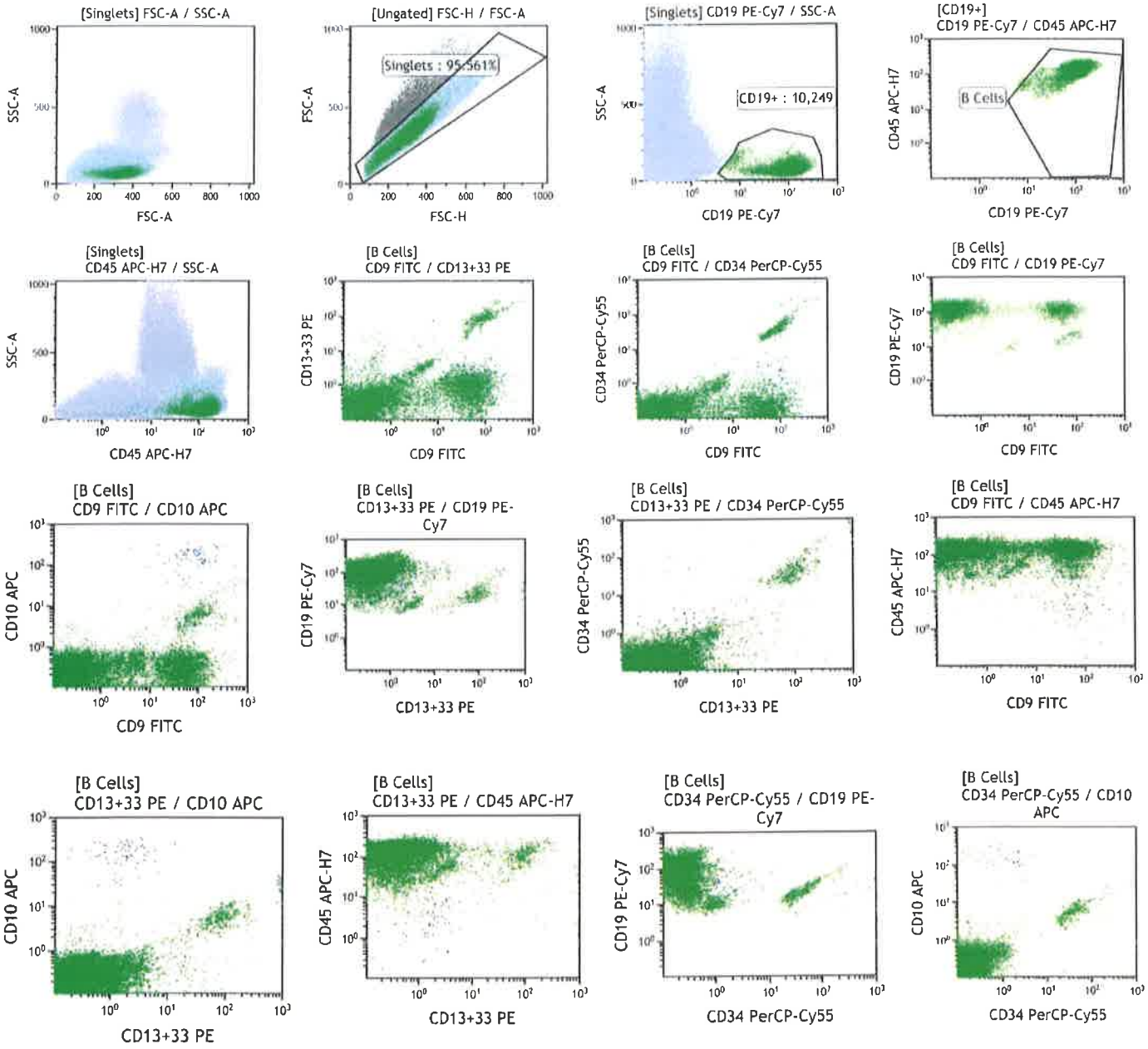
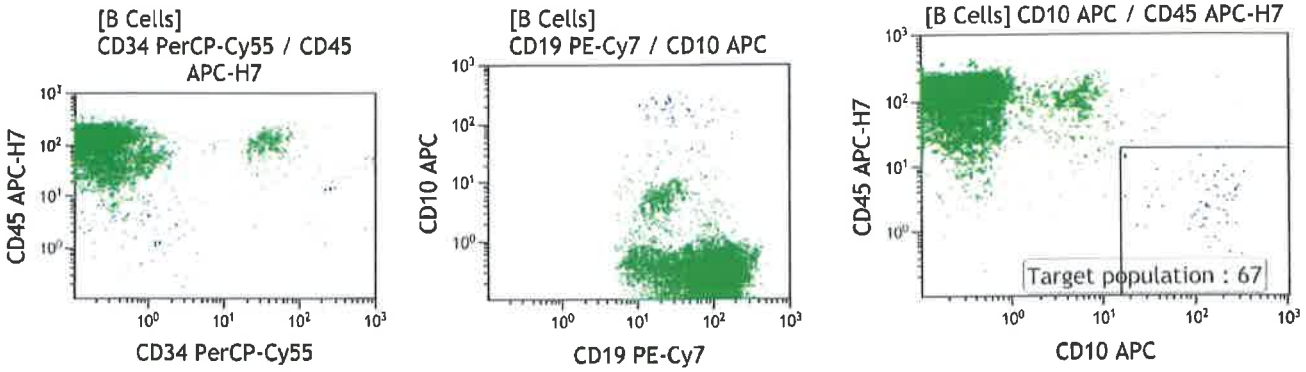


Day 29 - Tube 2, cont'd



[B Cells] Number	9,817
[Target population] Nu...	67

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FLOW CYTOMETRY B-ALL MINIMAL RESIDUAL DISEASE

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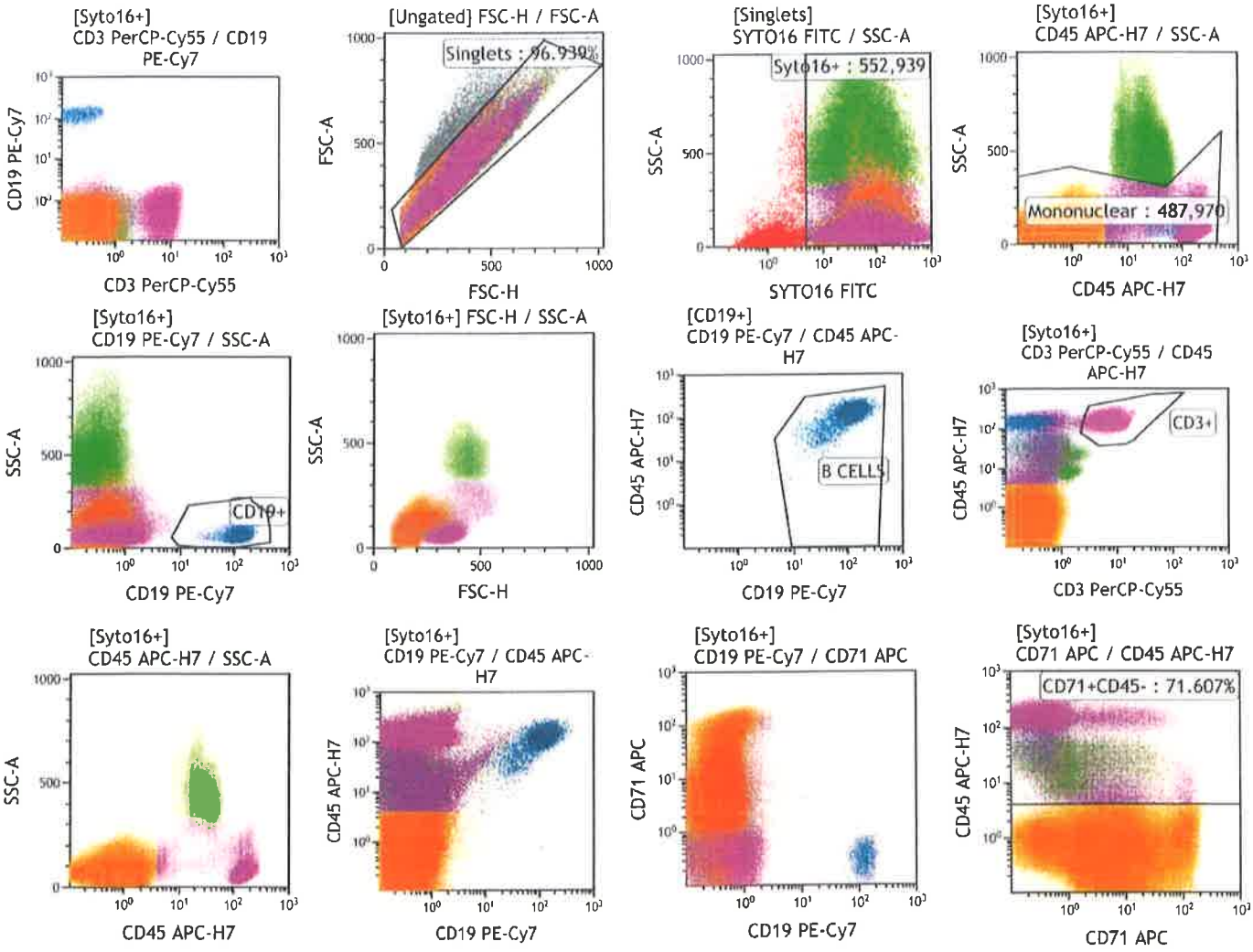

Gate	Number
All	579,747
B CELLS	9,154
Syto16+	552,939
CD71+CD45-	395,943