2019 BALL-A
PARTICIPANT SUMMARY

Evaluation Criteria

Results for the BALL Survey are not formally evaluated; however, statistics will appear in the Participant Summary for your information.

To provide a timely evaluation of your results, statistics presented in this Participant Summary reflect participant data received by the due date.

Cell markers with less than ten reported results were not included in this Participant Summary.

In the event a result is not graded, a numeric code will appear next to your result. A definition of the code will appear on the first page of your evaluation. Please see "Actions laboratories should take when a PT result is not graded" on page 17.

BALL-A Discussion

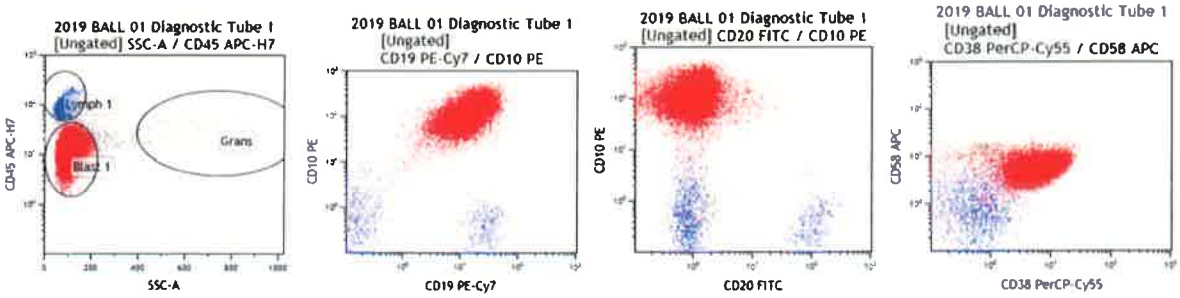
Case BALL-01 List Mode Case Negative for MRD

This case did not contain an abnormal population. However, a small population of hematogones with a classic maturation pattern was present. Plasma cells were also clearly visible (CD38 bright, CD10 negative, CD20 negative). The diagnostic population of cells was CD45 dim, CD10 and CD58 bright, CD20 negative, CD38 variable in Tube 1. Tube 2 showed a diagnostic population that was CD34 and CD13/33 negative, and positive for CD9. The day 29 sample was clearly distinct from the prior diagnostic immunophenotype with weaker CD10, variable CD20, slightly brighter CD45, and weaker CD58 (representative dot plots shown below).

A total of 83.1% (54/65) of participants reported this case as negative, with 16.9% (11/65) calling it positive. Of those calling it positive, the majority reported 0.1 - 0.9%. Participants who reported positive results for this sample should review their analysis procedures.

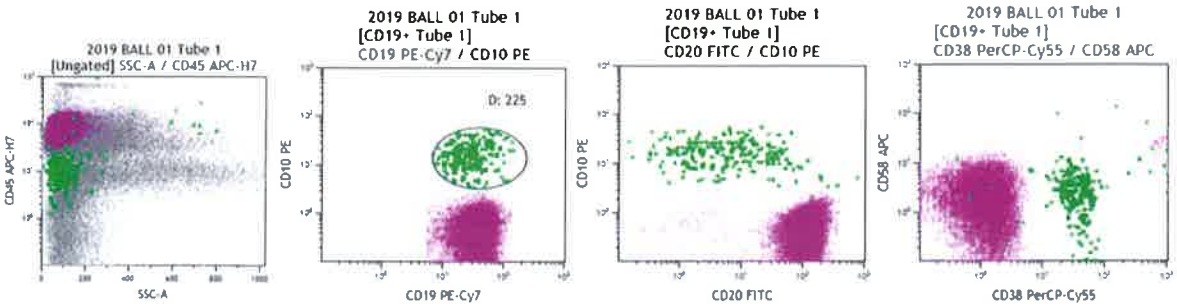
Please note that when a sample is negative, the value of abnormal cells is 0 and should not be reported as MRD positive. An immunophenotype should not be provided if no abnormal population is present.

Diagnostic population shown in red:



BALL-A Discussion (cont.)

Day 29 population (hematogones) shown in green:

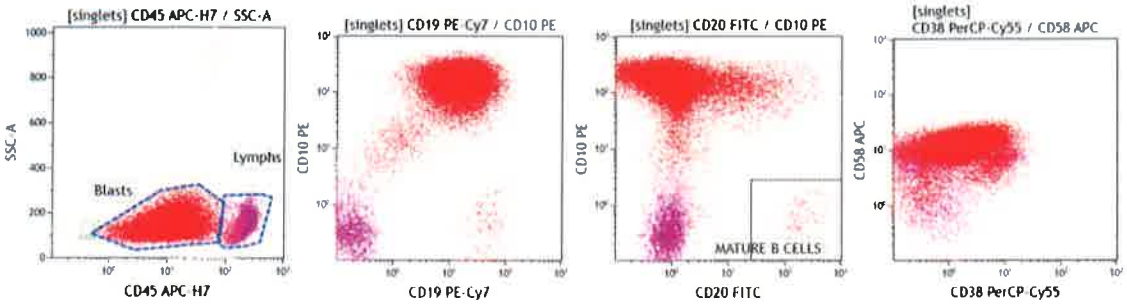


Case BALL-02 List Mode Case Positive for MRD at approximately 0.1%

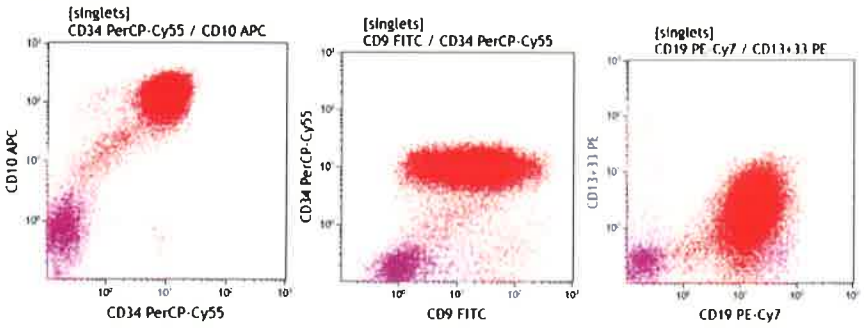
This case contained an abnormal population. The diagnostic population of cells was CD45 dim, CD10 and CD58 bright, CD20 variable, CD38 dim/neg in Tube 1. Tube 2 showed a diagnostic population that was CD34 and CD13/33 positive, and positive for CD9. The day 29 sample showed a shift in CD20 expression from variable to positive. This is not uncommon and has been reported as early as 2005.[1] However, this population had an abnormal phenotype as the cells in Tube 1 were CD10 positive and CD38 negative. The population was also brighter CD19 than the normal lymphocyte population. Tube 2 showed that this CD20+CD10+ population was also positive for CD34 and CD13/33, consistent with the original phenotype.

A total of 76.9% (50/65) of participants reported this case as positive, with 23.1% (15/65) calling it negative. Of those calling it positive, the majority reported 0.01 - 0.1%. Participants who reported negative results for this sample should review their analysis procedures and consult recent literature on gating strategy.[2] While this case showed a shift in some of the original markers, the abnormal population continued to express an aberrant phenotype. Comparison of case 1 and case 2 shows how one can distinguish hematogones from residual disease in most cases.

Case 2 Tube 1. Diagnostic population shown in red

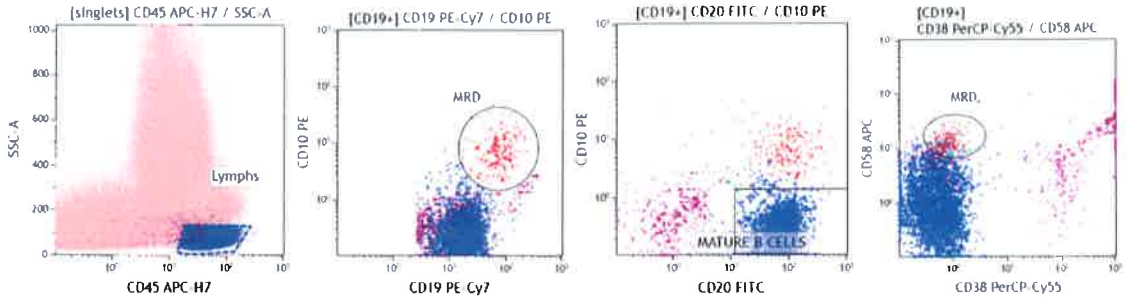


Case 2 Tube 2

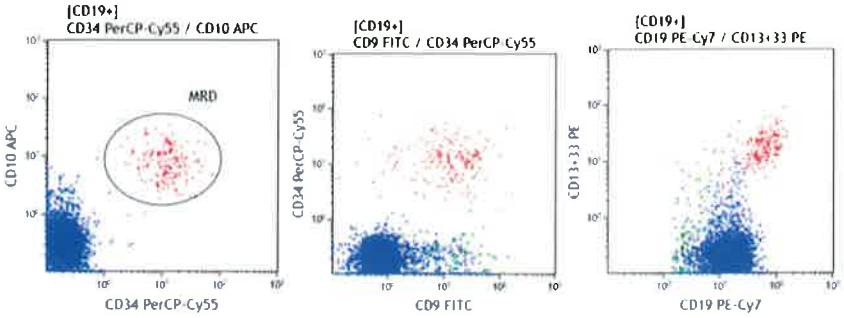


BALL-A Discussion (cont.)

Case 2 Day 29 tube 1



Case 2 Day 29 Tube 2



Case BALL-03 Negative for MRD

Two sets of plots were provided for this case, one representing the diagnostic immunophenotype at onset of disease, and one representing MRD assessment at day 29. The diagnostic case showed the following abnormal immunophenotype: positive for CD9 bright, CD10 bright, CD19 dim, CD34 and CD38 dim/neg, and CD58 bright; and negative for CD20, CD13/33, and CD45. The MRD tubes did not demonstrate a similar abnormal blast population, but rather showed a population of immature B cells (hematogones) with a normal immunophenotype: positive for CD19, CD10, CD38 uniform bright, and CD45 dim, with heterogeneous expression of CD20, CD34, and CD58. A key to identifying these as normal cells is to recognize differences in antigen expression intensity between these cells and the B lymphoblasts seen at diagnosis. For example, the hematogones show reduced expression of CD9, CD10, CD34, and CD58 with increased expression of CD20, CD38, and CD45 compared with the B lymphoblasts seen at diagnosis.

Of the 66 participants who reported a result, 57 (86.4%) correctly reported this sample as negative, with the remaining 9 (13.6%) participants reporting positive. Of those reporting positive, 1 participant reported in the 0.1 - 0.9% range, and 8 participants reported in the 1% - 9.9% range.

BALL-A Discussion (cont.)

References

1. Borowitz MJ, Pullen DJ, Winick N, Martin PL, Bowman WP, Camitta B. Comparison of diagnostic and relapse flow cytometry phenotypes in childhood acute lymphoblastic leukemia: implications for residual disease detection: a report from the children's oncology group. *Cytometry B Clin Cytom.* 2005 Nov;68(1):18-24.
2. Keeney M, Wood BL, Hedley BD, et al. A QA program for MRD testing demonstrates that systematic education can reduce discordance among experienced interpreters. *Cytometry B Clin Cytom.* 2018 Mar;94(2):239-249.

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Michael Keeney, ART, FIMLS, FCSMLS(D)
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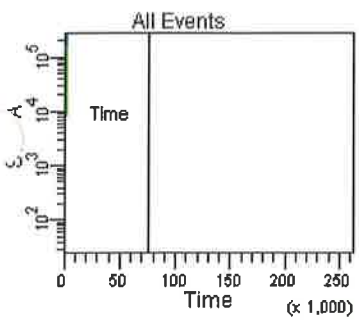
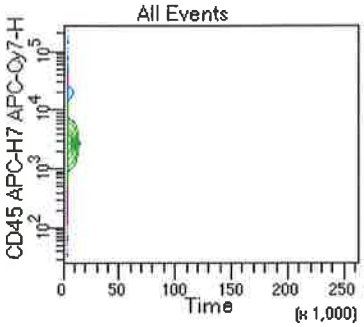
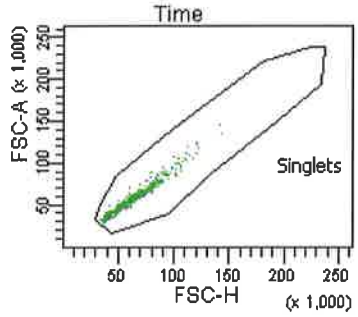
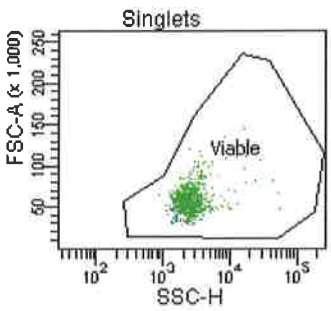


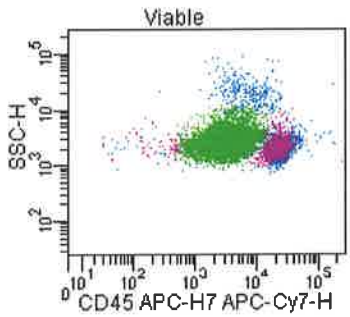


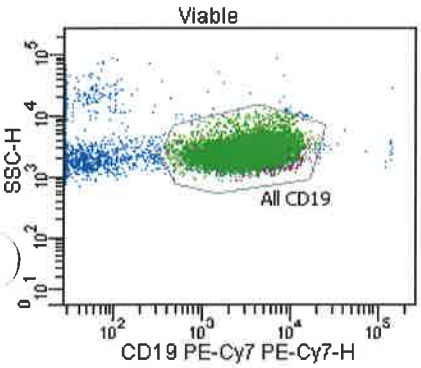
Tube: A-PRE

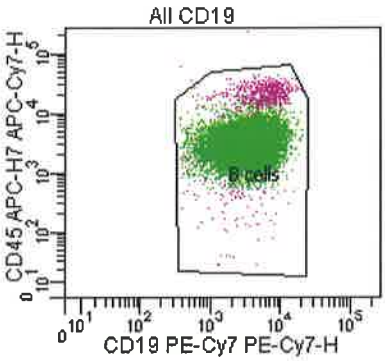
Population	#Events	%Parent	%Total
All Events	18,007	####	100.0
Time	18,007	100.0	100.0
Singlets	17,777	98.7	98.7
Viable	17,776	100.0	98.7
All CD19	15,994	90.0	88.8
B cells	15,990	100.0	88.8
ABNORMAL	15,273	95.5	84.8

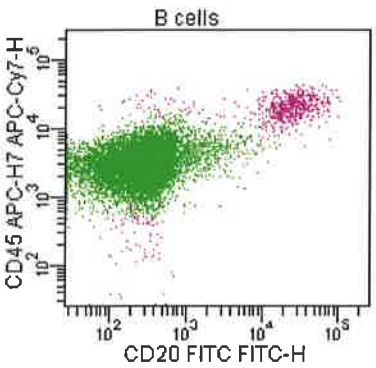


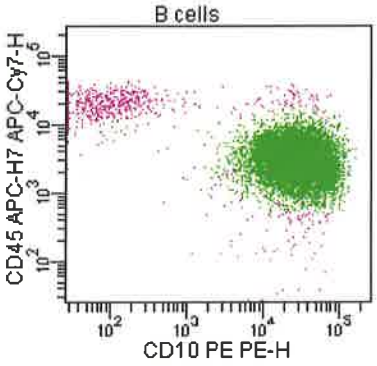


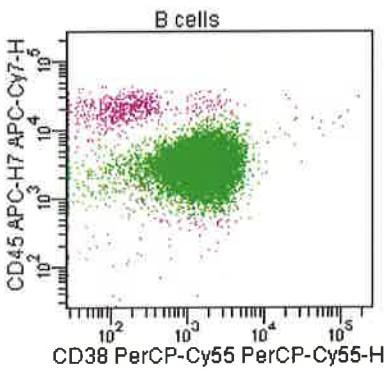


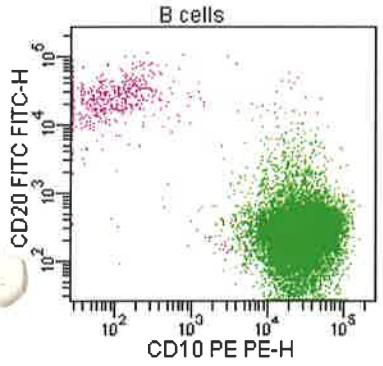


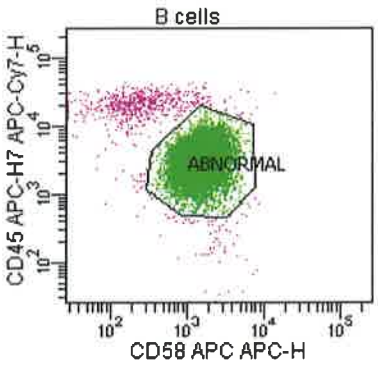


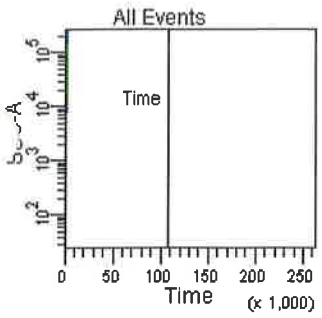


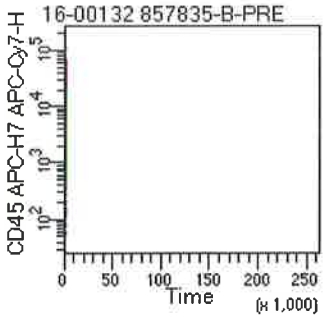


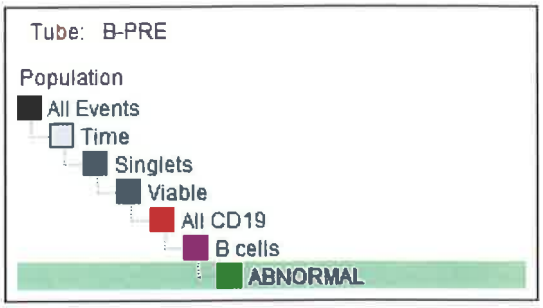


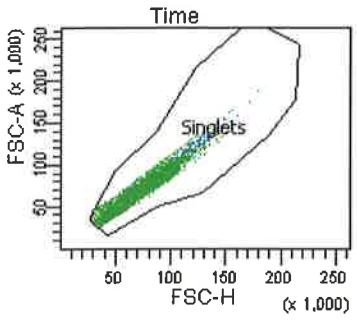


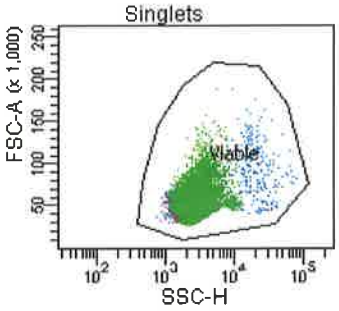


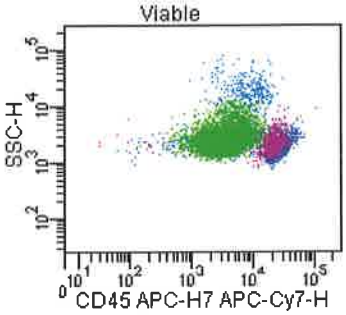

All Events
Time
Time
(x 1,000)

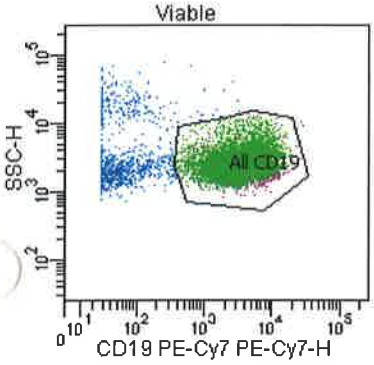

16-00132 857835-B-PRE
CD45 APC-H7 APC-Cy7-H
Time
(x 1,000)

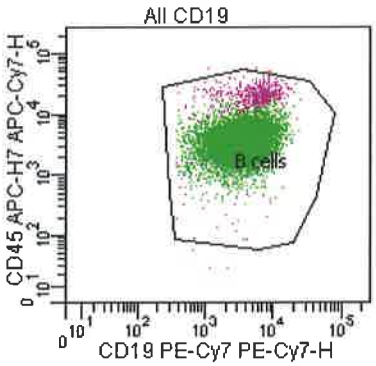

Tube: B-PRE
Population
All Events
Time
Singlets
Viable
All CD19
B cells
ABNORMAL

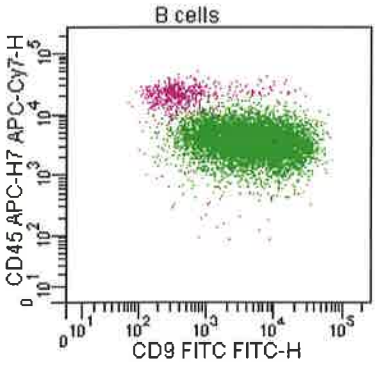

Time
Singlets
FSC-A (x 1,000)
FSC-H
(x 1,000)

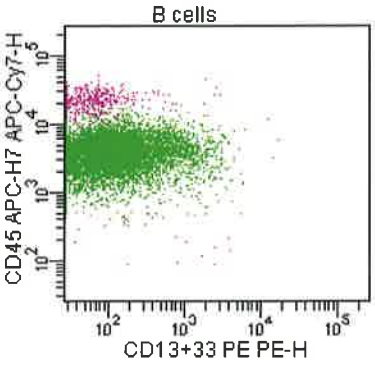

Singlets
Viable
FSC-A (x 1,000)
SSC-H

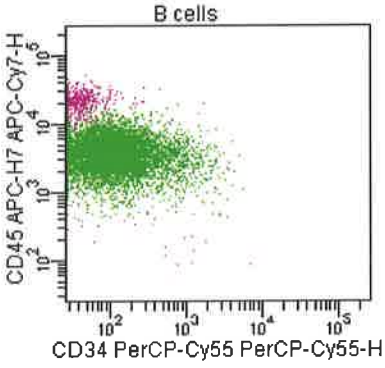

Viable
SSC-H
CD45 APC-H7 APC-Cy7-H

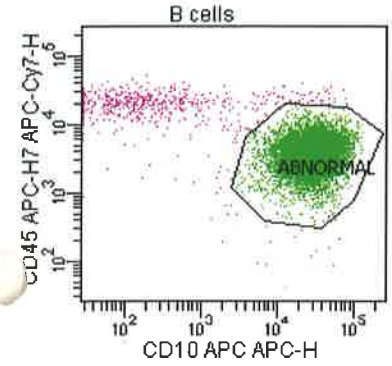

Viable
All CD19
SSC-H
CD19 PE-Cy7 PE-Cy7-H

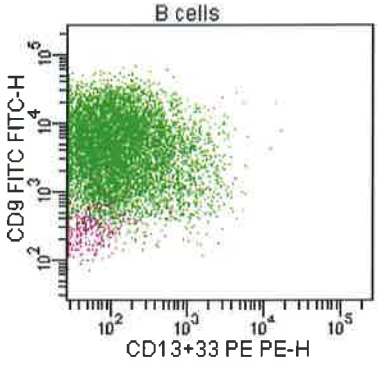

All CD19
B cells
CD45 APC-H7 APC-Cy7-H
CD19 PE-Cy7 PE-Cy7-H

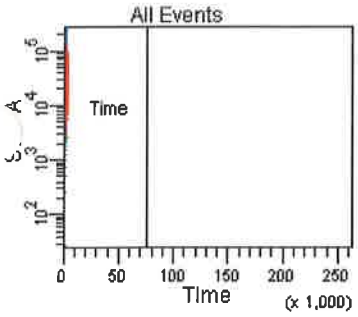

B cells
CD45 APC-H7 APC-Cy7-H
CD9 FITC FITC-H

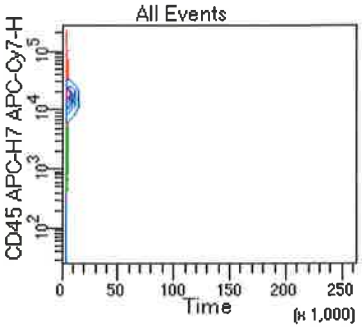

B cells
CD45 APC-H7 APC-Cy7-H
CD13+33 PE PE-H


B cells
CD45 APC-H7 APC-Cy7-H
CD34 PerCP-Cy55 PerCP-Cy55-H


B cells
ABNORMAL
CD45 APC-H7 APC-Cy7-H
CD10 APC APC-H

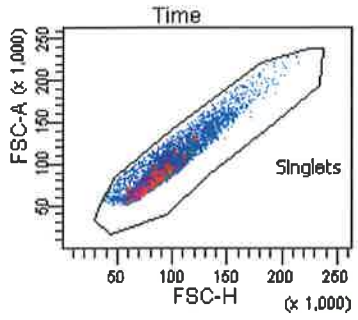

B cells
CD9 FITC FITC-H
CD13+33 PE PE-H

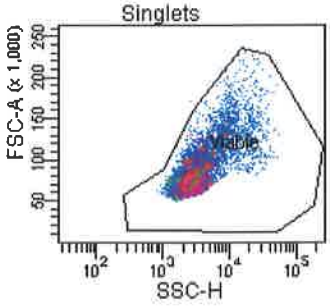


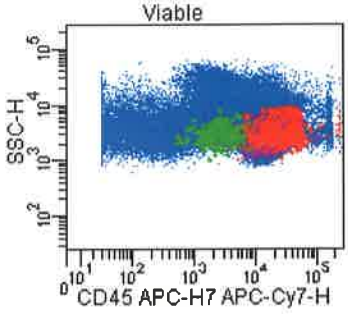


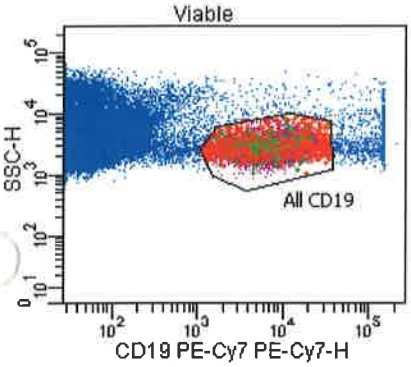
Tube: A-MRD

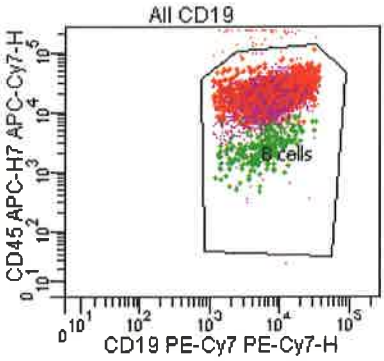
Population	#Events	%Parent	%Total
All Events	245,292	####	100.0
Time	245,292	100.0	100.0
Singlets	244,739	99.8	99.8
Viable	244,659	100.0	99.7
All CD19	19,042	7.8	7.8
B cells	18,996	99.8	7.7
P1	229	1.2	0.1
P2	376	2.0	0.2

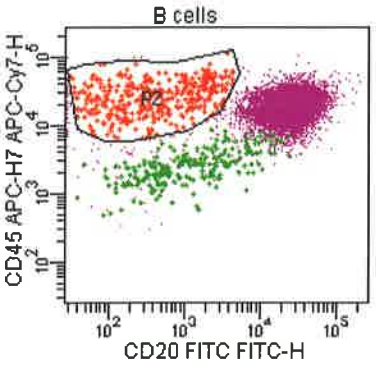


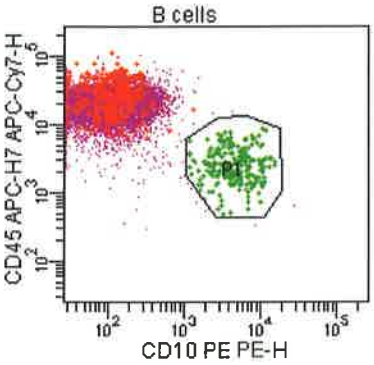


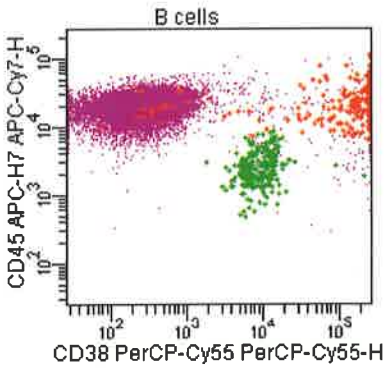


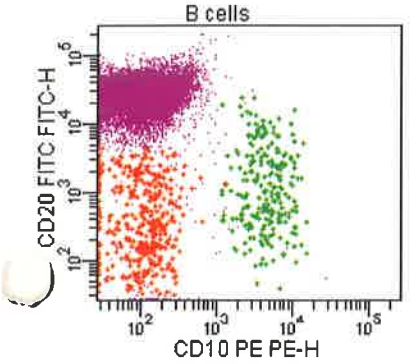


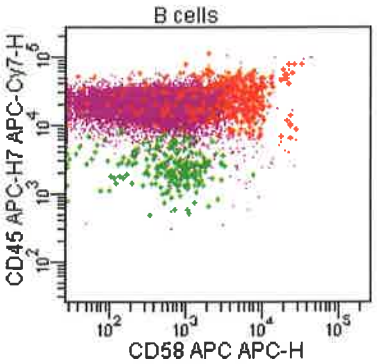


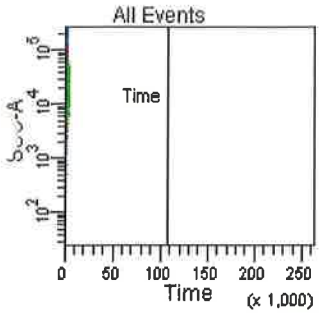


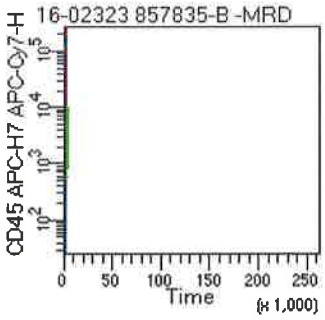


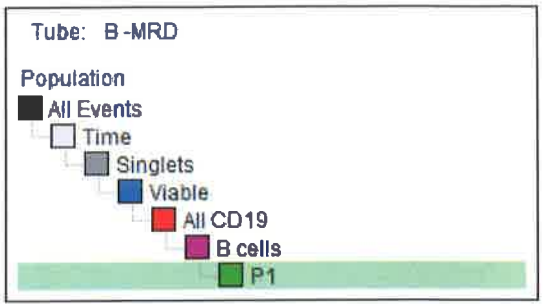


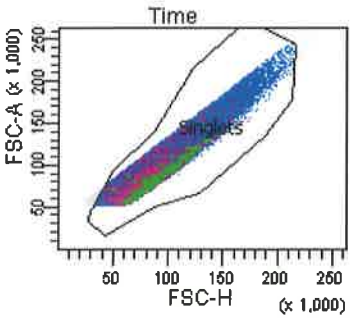


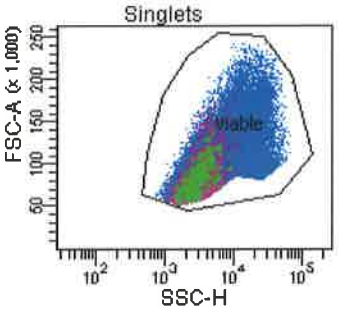


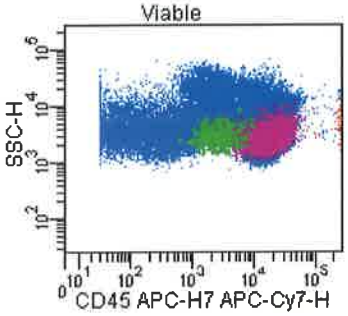

All Events
Time
Time
(x 1,000)

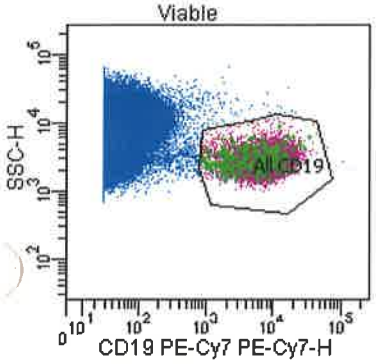

16-02323 857835-B -MRD
CD45 APC-H7 APC-Cy7-H
Time
(x 1,000)

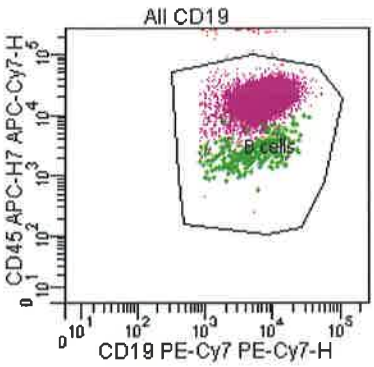

Tube: B -MRD
Population
All Events
Time
Singlets
Viable
All CD19
B cells
P1

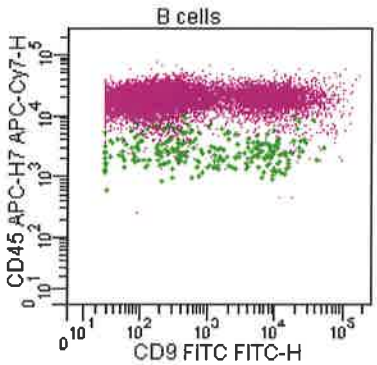

Time
FSC-A (x 1,000)
Singlets
FSC-H
(x 1,000)

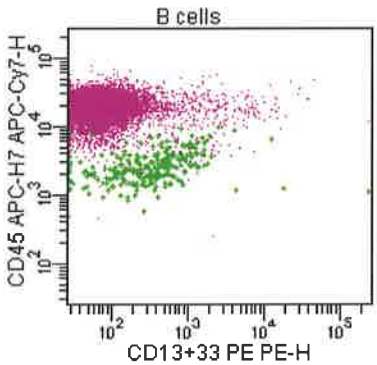

Singlets
FSC-A (x 1,000)
Viable
SSC-H

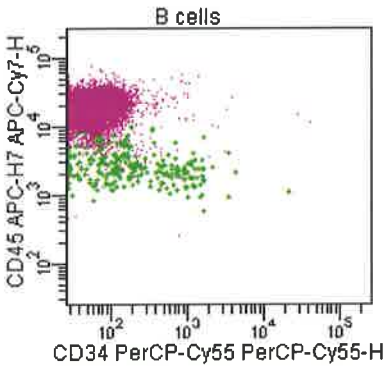

Viable
SSC-H
CD45 APC-H7 APC-Cy7-H

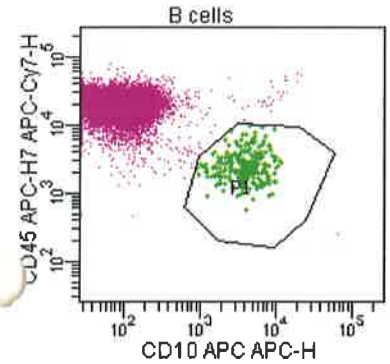

Viable
SSC-H
All CD19
CD19 PE-Cy7 PE-Cy7-H

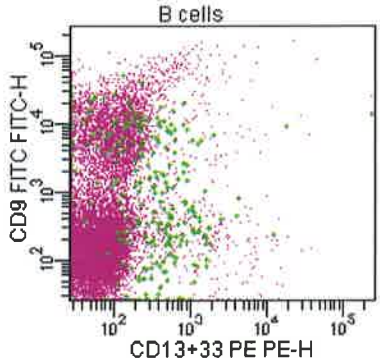

All CD19
CD45 APC-H7 APC-Cy7-H
B cells
CD19 PE-Cy7 PE-Cy7-H

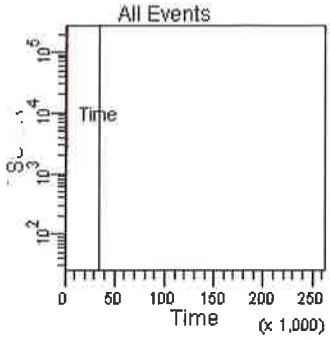

B cells
CD45 APC-H7 APC-Cy7-H
CD9 FITC FITC-H

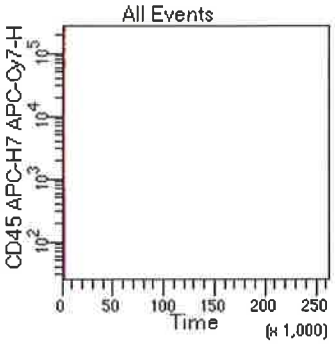

B cells
CD45 APC-H7 APC-Cy7-H
CD13+33 PE PE-H

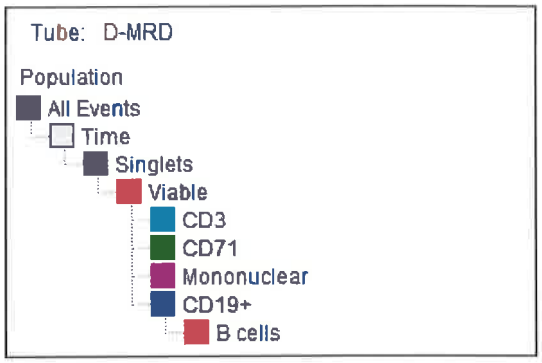

B cells
CD45 APC-H7 APC-Cy7-H
CD34 PerCP-Cy55 PerCP-Cy55-H

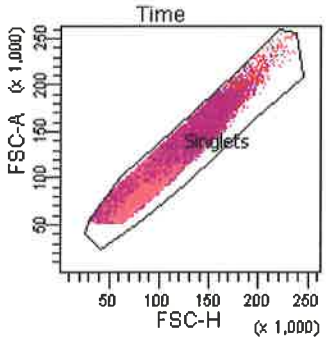

B cells
CD45 APC-H7 APC-Cy7-H
P1
CD10 APC APC-H

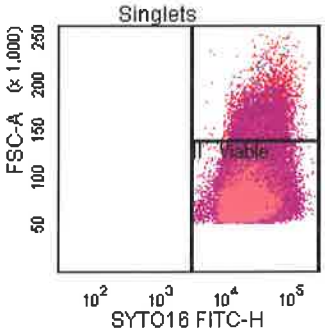

B cells
CD9 FITC FITC-H
CD13+33 PE PE-H

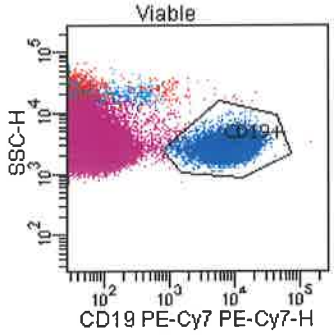


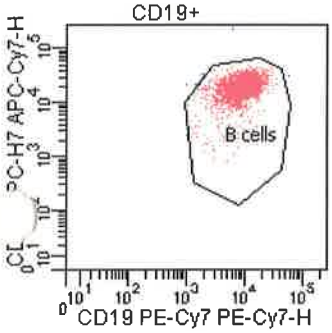


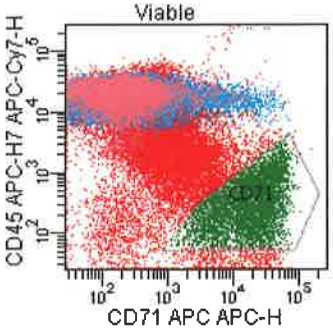


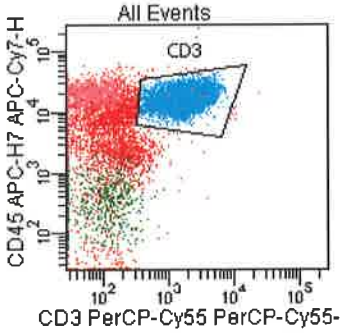


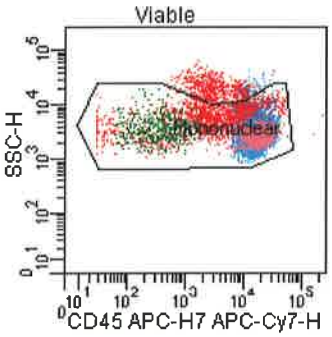


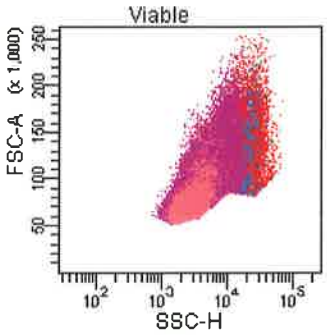






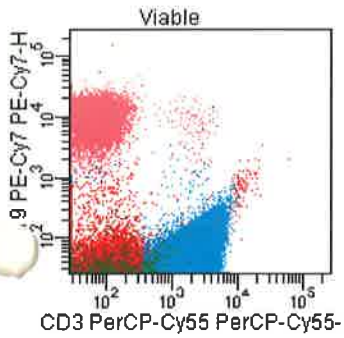


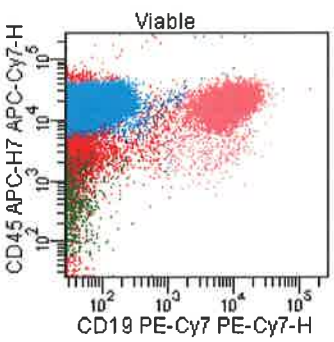


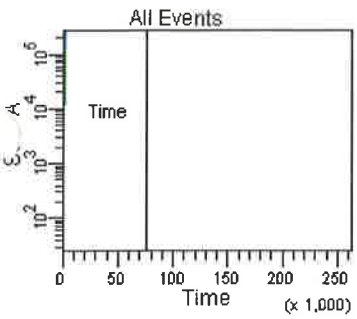


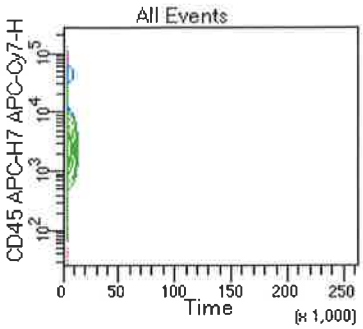
Experiment Name:	BALL-A 2019 CASE 1
Specimen Name:	16-02323 857835
Tube Name:	D-MRD
Record Date:	Feb 3, 2016 2:35:15 PM
$OP:	HPDevelopmental
GUID:	6ffabc82-270e-4a66-93ba-118657...
Serial Number	

Population	#Events	%Parent	%Grand Par...	%Total
All Events	205,143	####	####	100.0
Time	205,143	100.0	####	100.0
Singlets	205,054	100.0	100.0	100.0
Viable	205,054	100.0	100.0	100.0
CD3	151,465	73.9	73.9	73.8
CD71	5,146	2.5	2.5	2.5
Mononuclear	197,541	96.3	96.3	96.3
CD19+	15,939	7.8	7.8	7.8
B cells	15,884	99.7	7.7	7.7



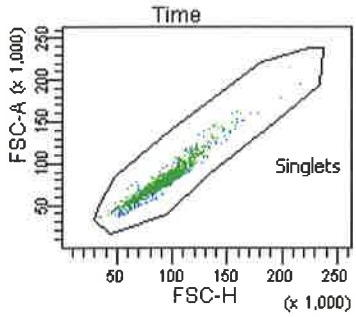


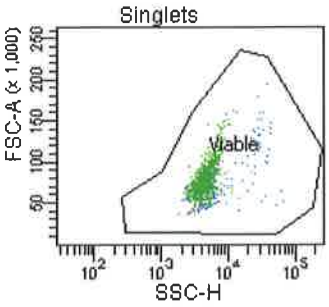


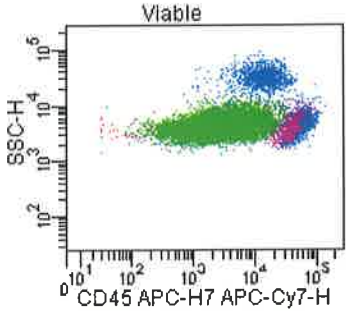


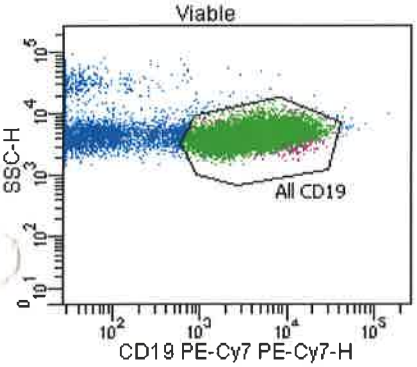
Tube: A-PRE

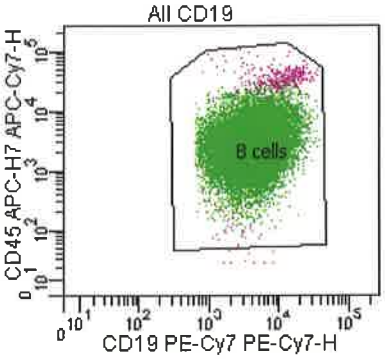
Population	#Events	%Parent	%Total
All Events	27,376	####	100.0
Time	27,376	100.0	100.0
Singlets	27,371	100.0	100.0
Viable	27,370	100.0	100.0
All CD19	22,871	83.6	83.5
B cells	22,859	99.9	83.5
ABNORMAL	22,360	97.8	81.7

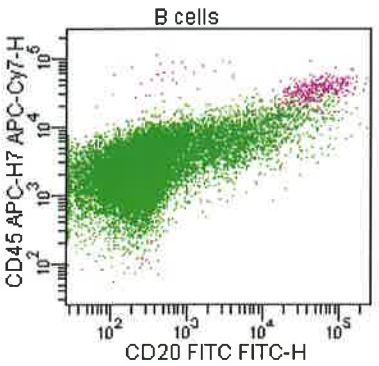


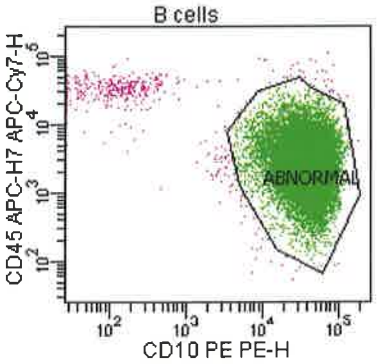


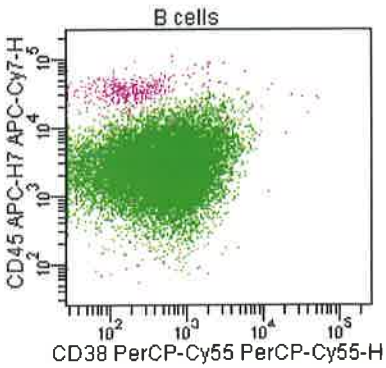


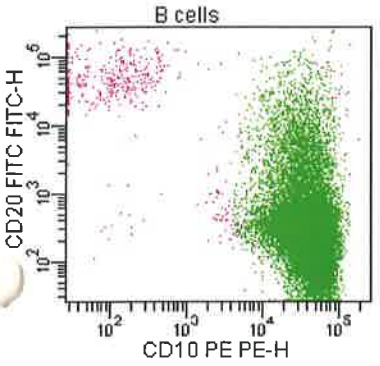


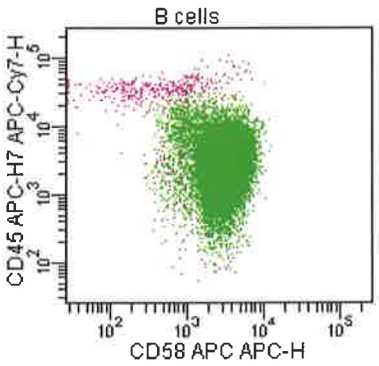


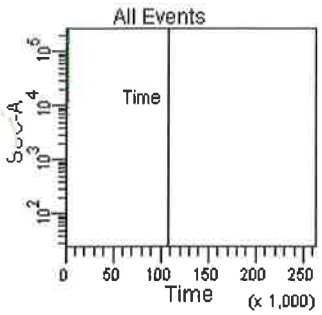


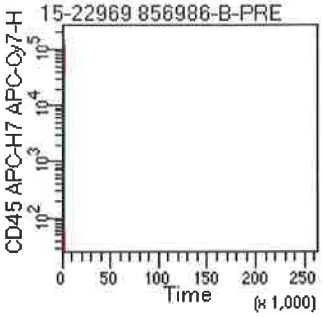


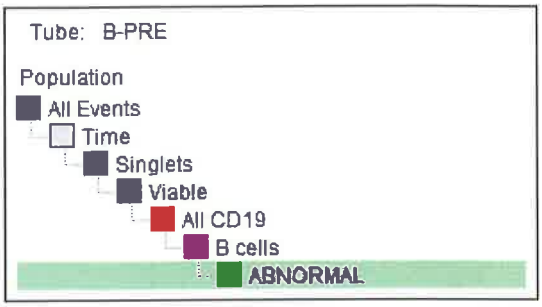


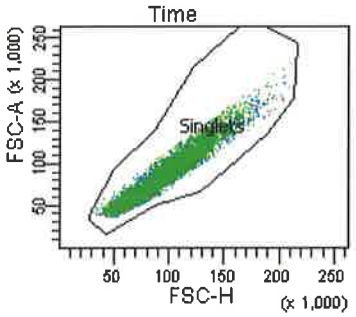


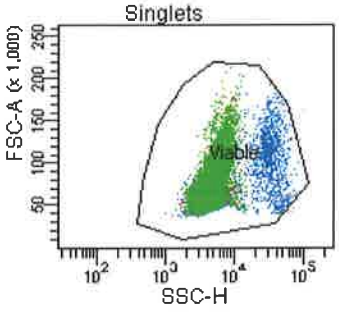


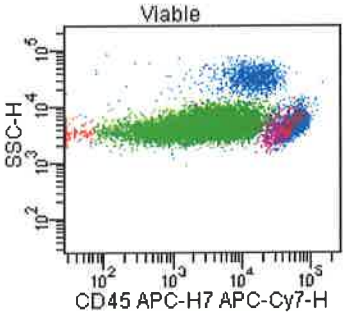

All Events
Time
Time
(x 1,000)

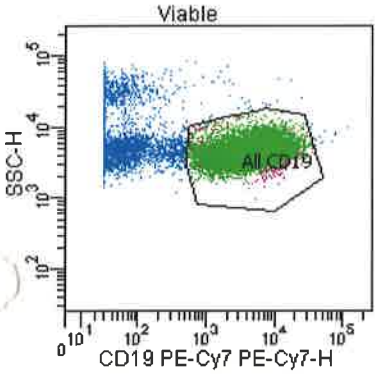

15-22969 856986-B-PRE
CD45 APC-H7 APC-Cy7-H
Time
(x 1,000)

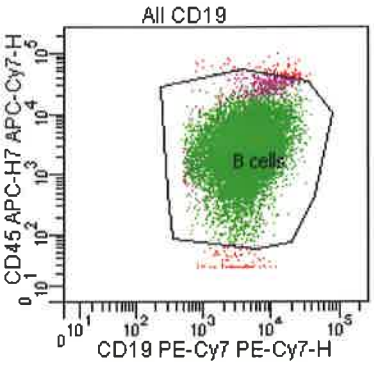

Tube: B-PRE
Population
All Events
Time
Singlets
Viable
All CD19
B cells
ABNORMAL

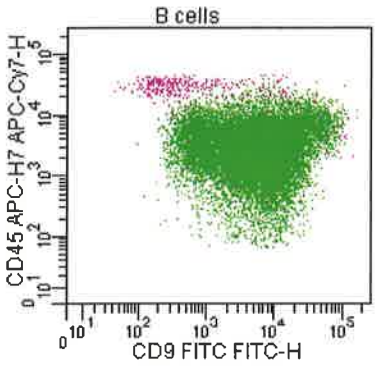

Time
FSC-A (x 1,000)
Singlets
FSC-H
(x 1,000)

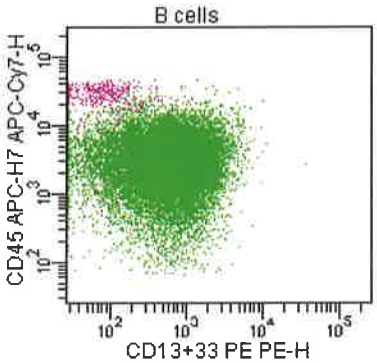

Singlets
FSC-A (x 1,000)
Viable
SSC-H

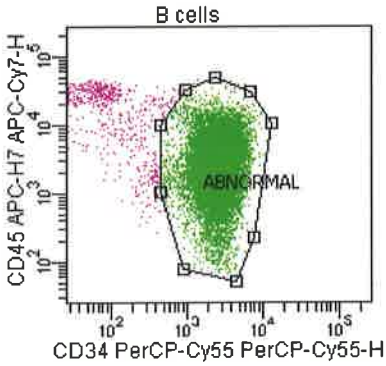

Viable
SSC-H
CD45 APC-H7 APC-Cy7-H

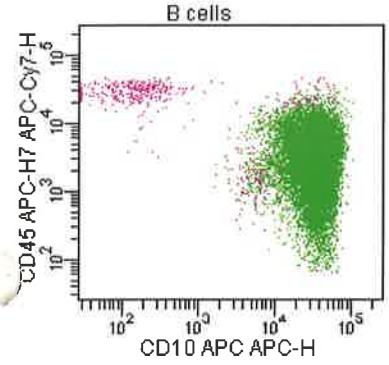

Viable
SSC-H
All CD19
CD19 PE-Cy7 PE-Cy7-H

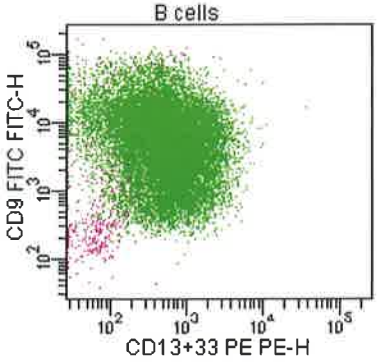

All CD19
CD45 APC-H7 APC-Cy7-H
B cells
CD19 PE-Cy7 PE-Cy7-H

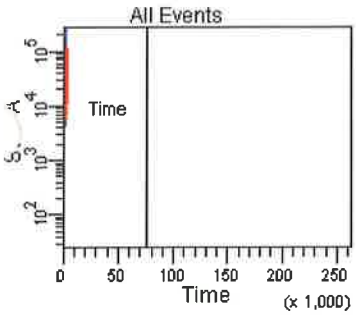

B cells
CD45 APC-H7 APC-Cy7-H
CD9 FITC FITC-H

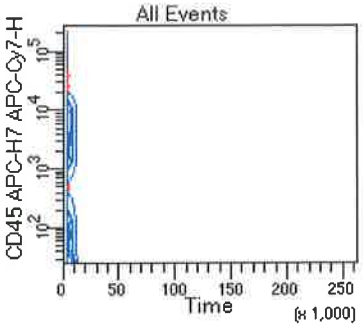

B cells
CD45 APC-H7 APC-Cy7-H
CD13+33 PE PE-H


B cells
CD45 APC-H7 APC-Cy7-H
ABNORMAL
CD34 PerCP-Cy55 PerCP-Cy55-H


B cells
CD45 APC-H7 APC-Cy7-H
CD10 APC APC-H

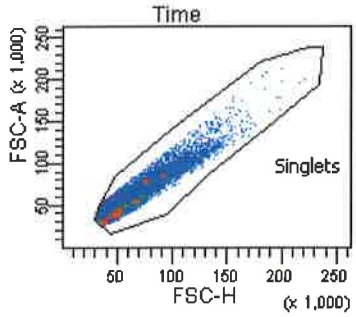

B cells
CD9 FITC FITC-H
CD13+33 PE PE-H

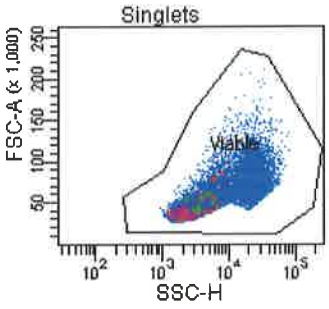


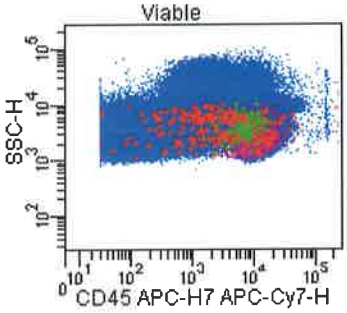


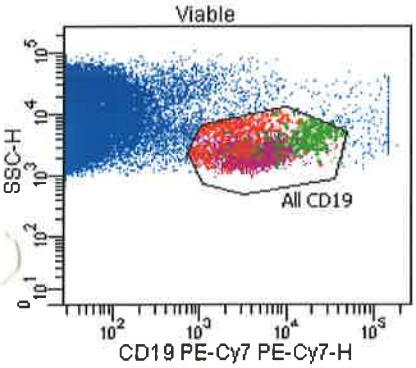
Tube: A-MRD

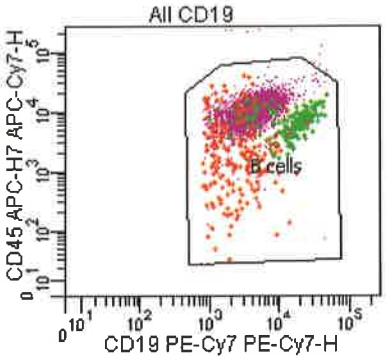
Population	#Events	%Parent	%Total
All Events	366,548	####	100.0
Time	366,548	100.0	100.0
Singlets	362,730	99.0	99.0
Viable	362,725	100.0	99.0
All CD19	4,174	1.2	1.1
B cells	4,157	99.6	1.1
P1	183	4.4	0.0
P2	194	4.7	0.1

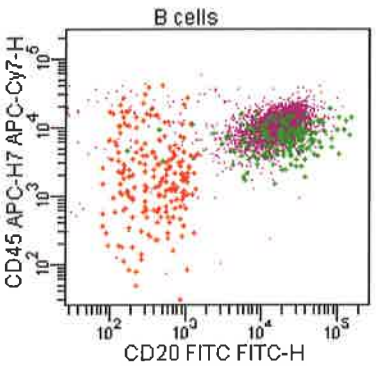


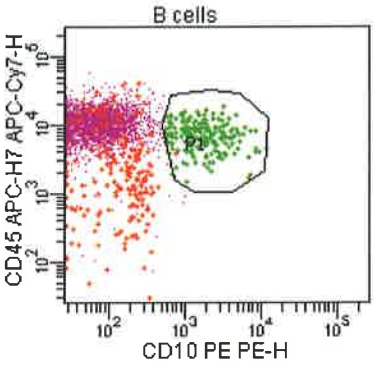


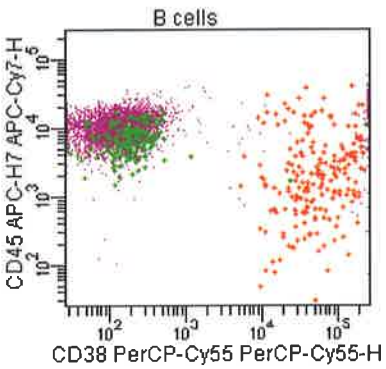


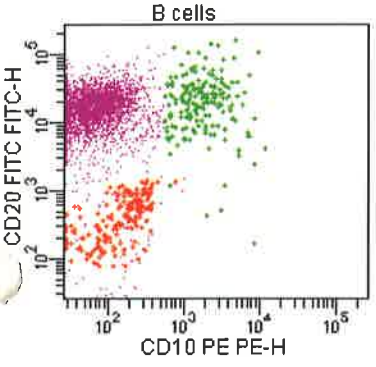


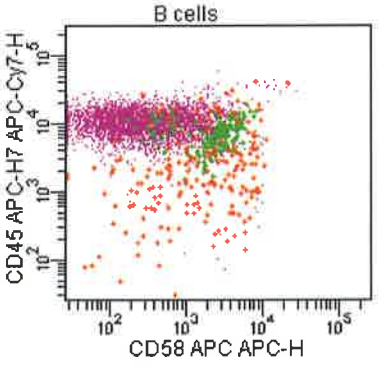


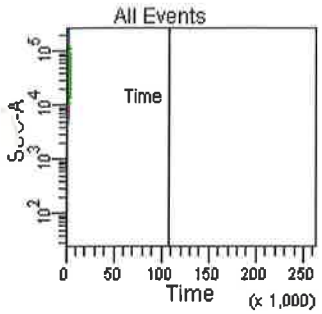


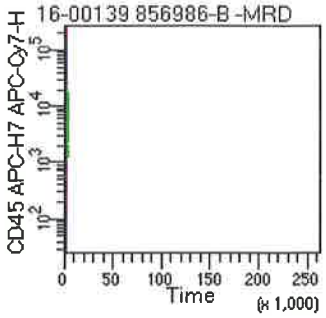


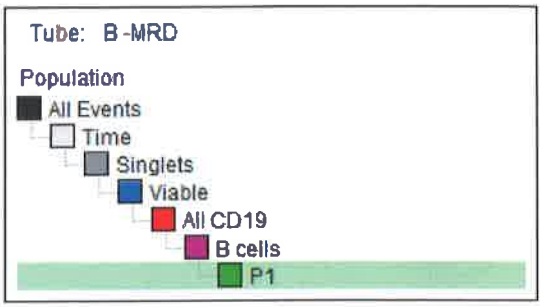


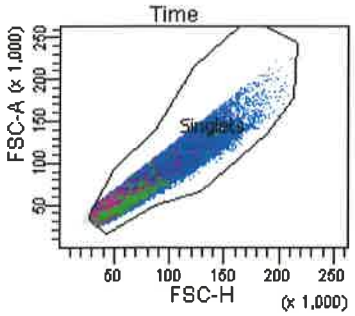


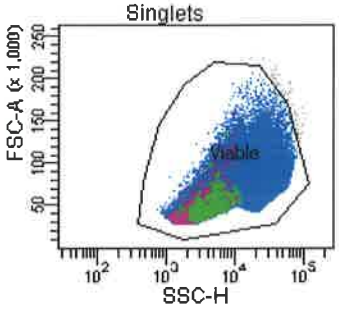


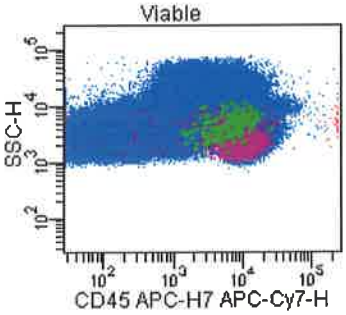

All Events
Time
Time
(x 1,000)

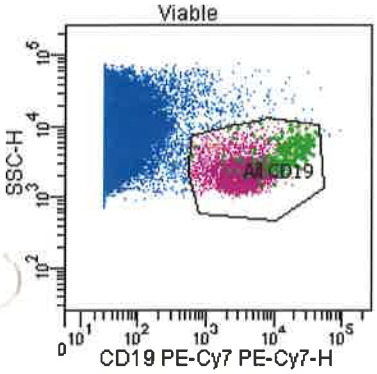

16-00139 856986-B -MRD
CD45 APC-H7 APC-Cy7-H
Time
(x 1,000)

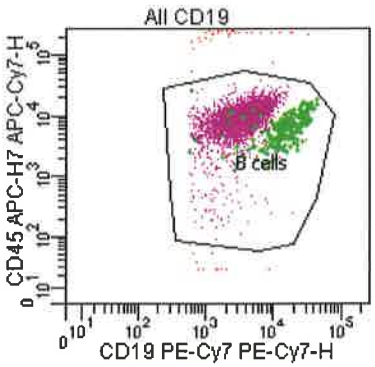

Tube: B -MRD
Population
All Events
Time
Singlets
Viable
All CD19
B cells
P1

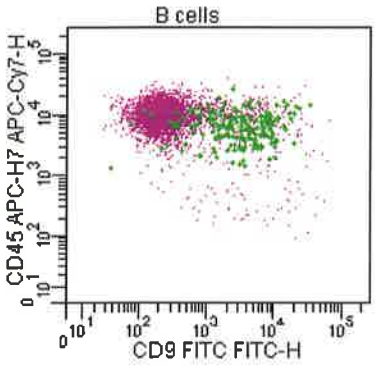

Time
FSC-A (x 1,000)
Singlets
FSC-H
(x 1,000)

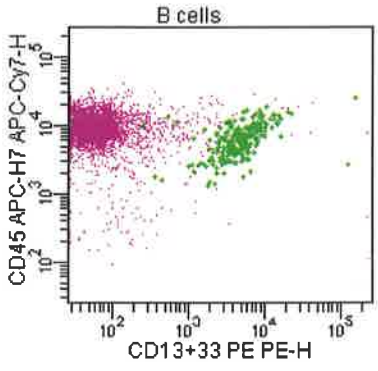

Singlets
FSC-A (x 1,000)
Viable
SSC-H

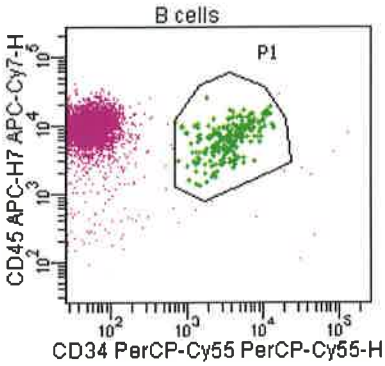

Viable
SSC-H
CD45 APC-H7 APC-Cy7-H

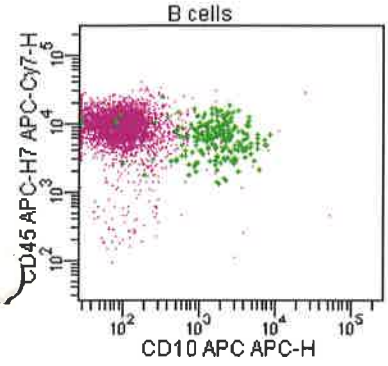

Viable
SSC-H
All CD19
CD19 PE-Cy7 PE-Cy7-H

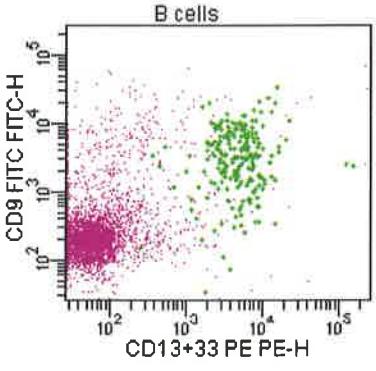

All CD19
CD45 APC-H7 APC-Cy7-H
B cells
CD19 PE-Cy7 PE-Cy7-H

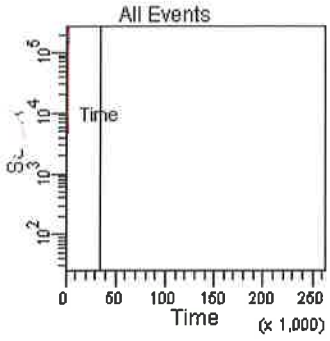

B cells
CD45 APC-H7 APC-Cy7-H
CD9 FITC FITC-H

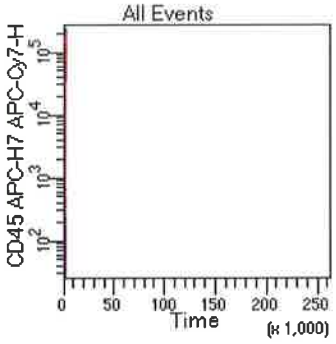

B cells
CD45 APC-H7 APC-Cy7-H
CD13+33 PE PE-H

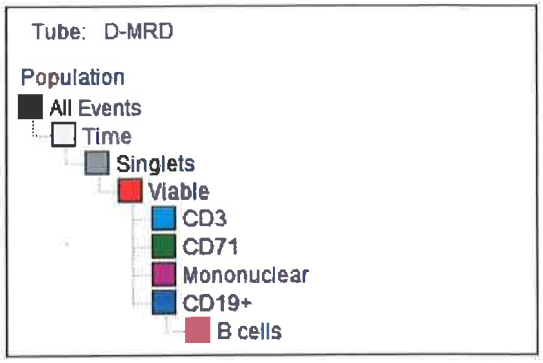

B cells
P1
CD45 APC-H7 APC-Cy7-H
CD34 PerCP-Cy55 PerCP-Cy55-H

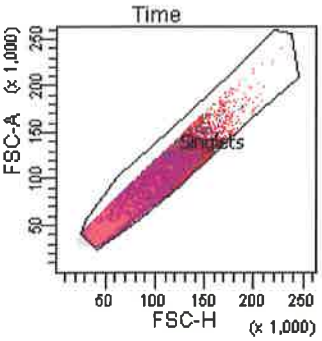

B cells
CD45 APC-H7 APC-Cy7-H
CD10 APC APC-H

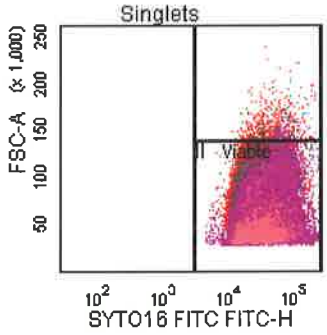

B cells
CD9 FITC FITC-H
CD13+33 PE PE-H

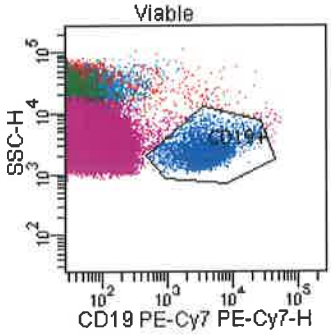


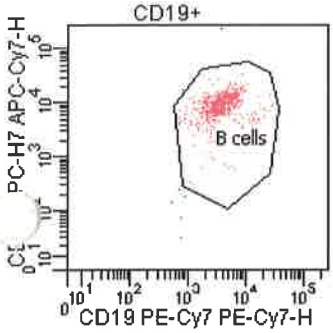


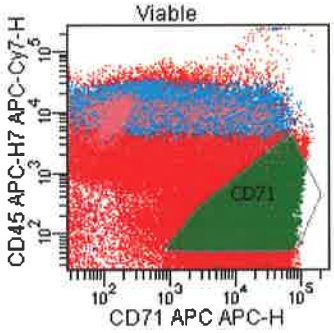


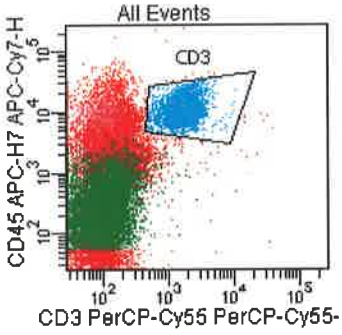


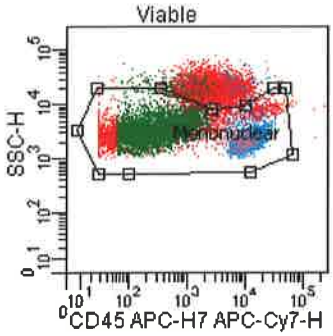


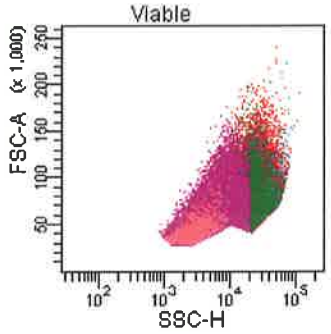






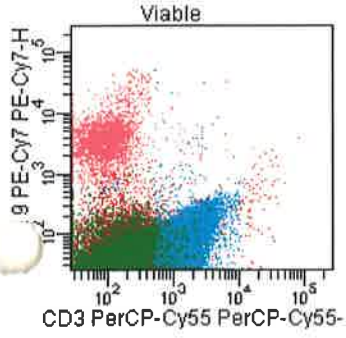


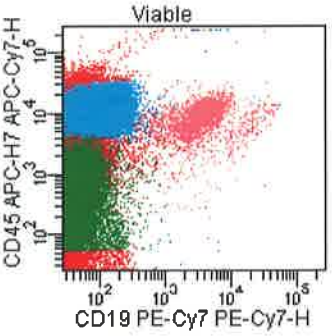




Experiment Name:	BALL-A 2019 CASE 2
Specimen Name:	16-00139 856986
Tube Name:	D-MRD
Record Date:	Jan 5, 2016 2:11:54 PM
$OP:	HPDevelopmental
GUID:	f6fe2cd1-6998-4346-81b2-2fe02fe...
Serial Number:	

Population	#Events	%Parent	%Grand Par...	%Total
All Events	303,939	####	####	100.0
Time	303,939	100.0	####	100.0
Singlets	282,827	93.1	93.1	93.1
Viable	282,827	100.0	93.1	93.1
CD3	37,845	13.4	13.4	12.5
CD71	86,889	30.7	30.7	28.6
Mononuclear	174,267	61.6	61.6	57.3
CD19+	3,420	1.2	1.2	1.1
B cells	3,379	98.8	1.2	1.1



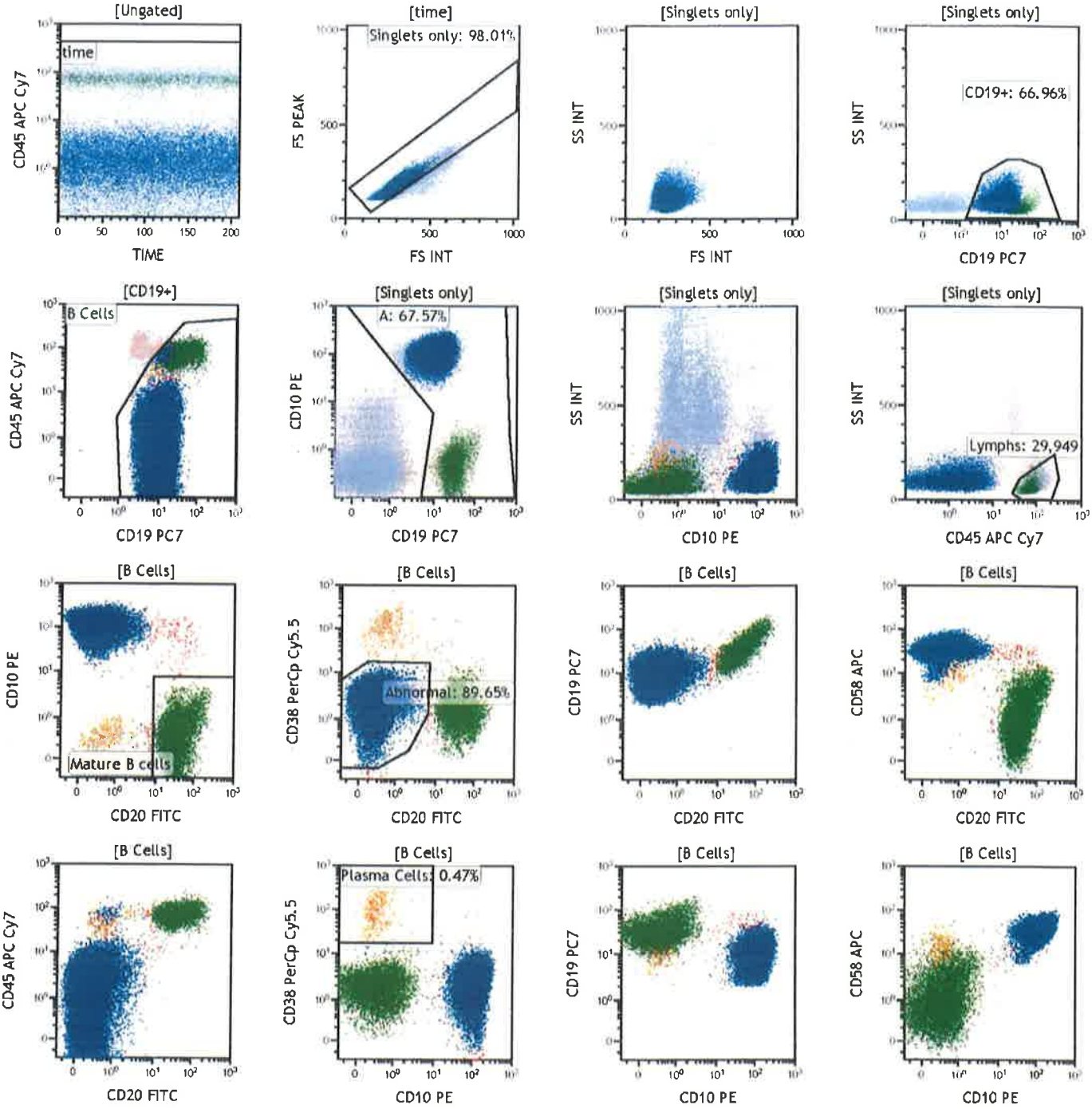


COLLEGE of AMERICAN PATHOLOGISTS

FLOW CYTOMETRY B-ALL MINIMAL RESIDUAL DISEASE

BALL-A 2019 (BALL-03)

Diagnostic Phenotype at Onset of Disease
Tube 1



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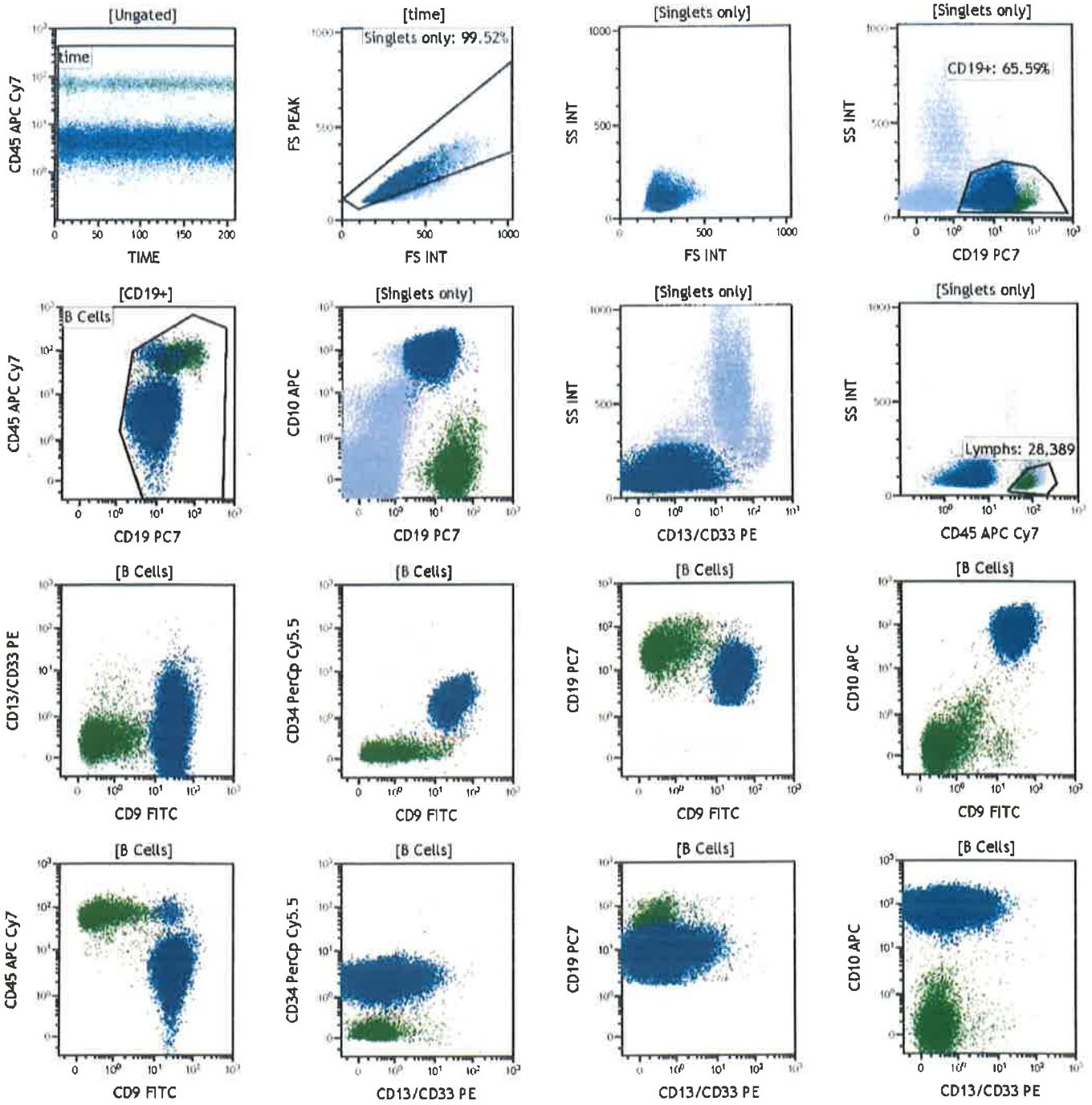
FLOW CYTOMETRY B-ALL MINIMAL RESIDUAL DISEASE

BALL-A 2019 (BALL-03)

Diagnostic Phenotype at Onset of Disease
Tube 2



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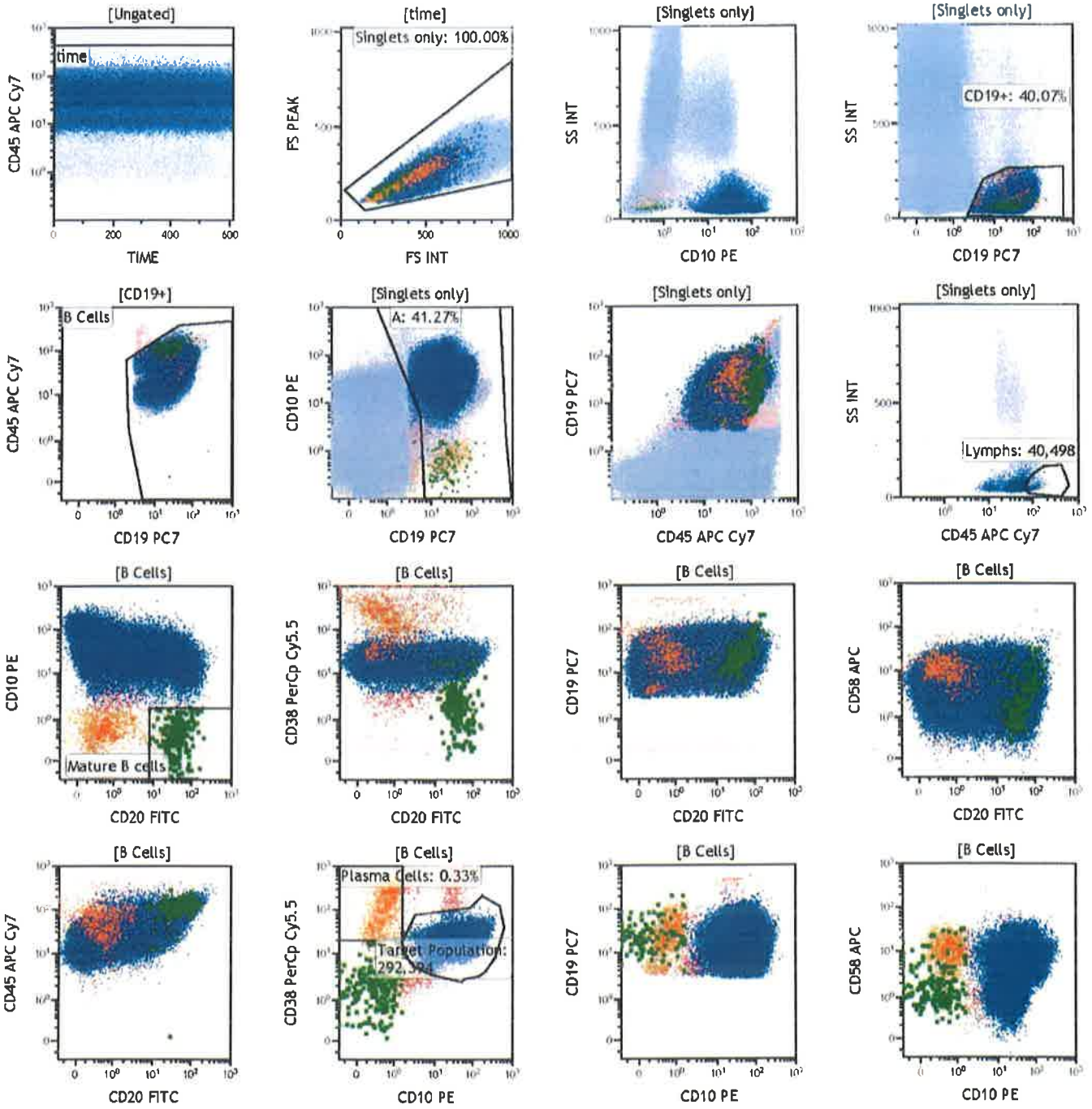
FLOW CYTOMETRY B-ALL MINIMAL RESIDUAL DISEASE

BALL-A 2019 (BALL-03)

Day 29 - Tube 1



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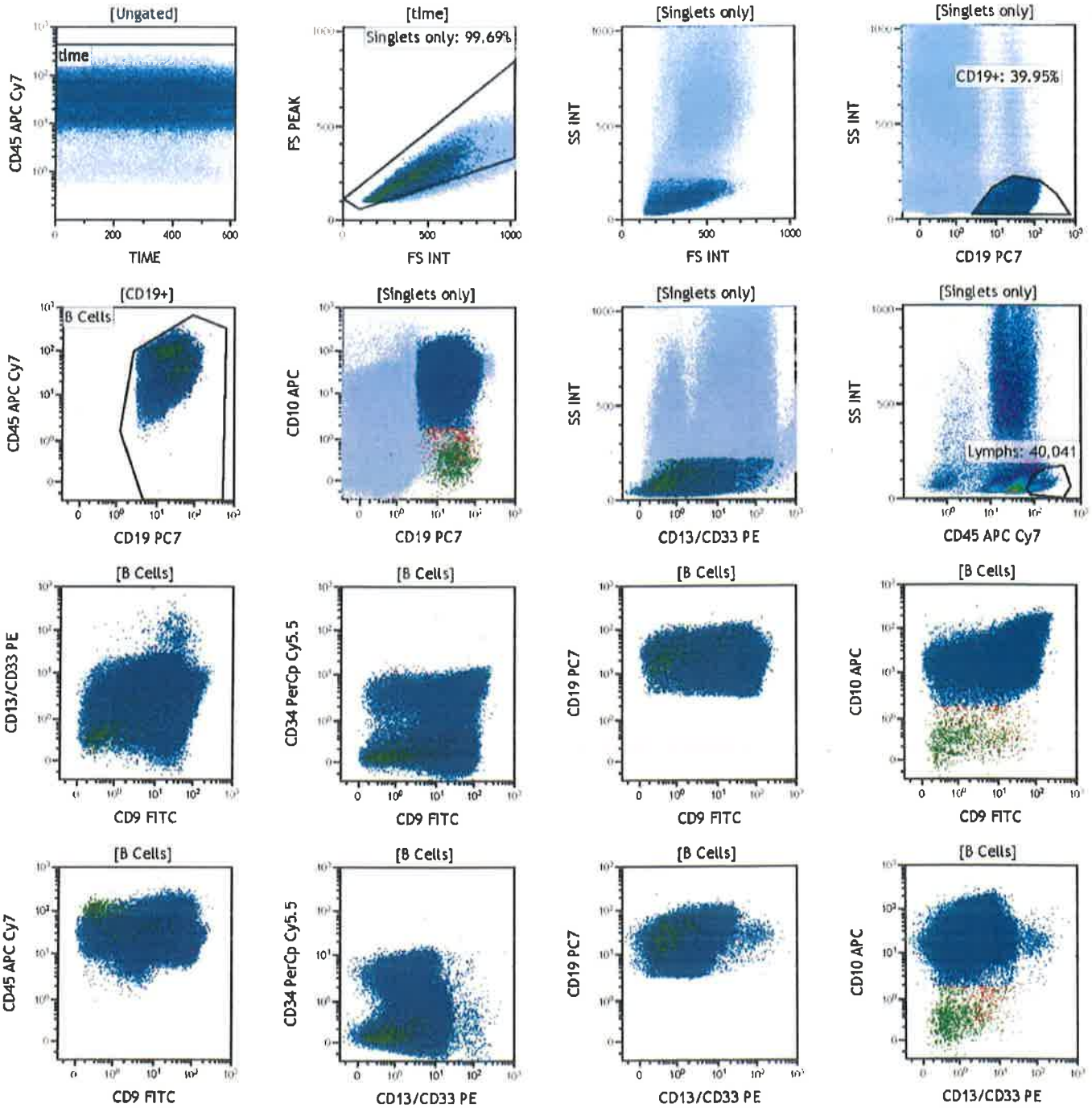
FLOW CYTOMETRY B-ALL MINIMAL RESIDUAL DISEASE

BALL-A 2019 (BALL-03)

Day 29 - Tube 2



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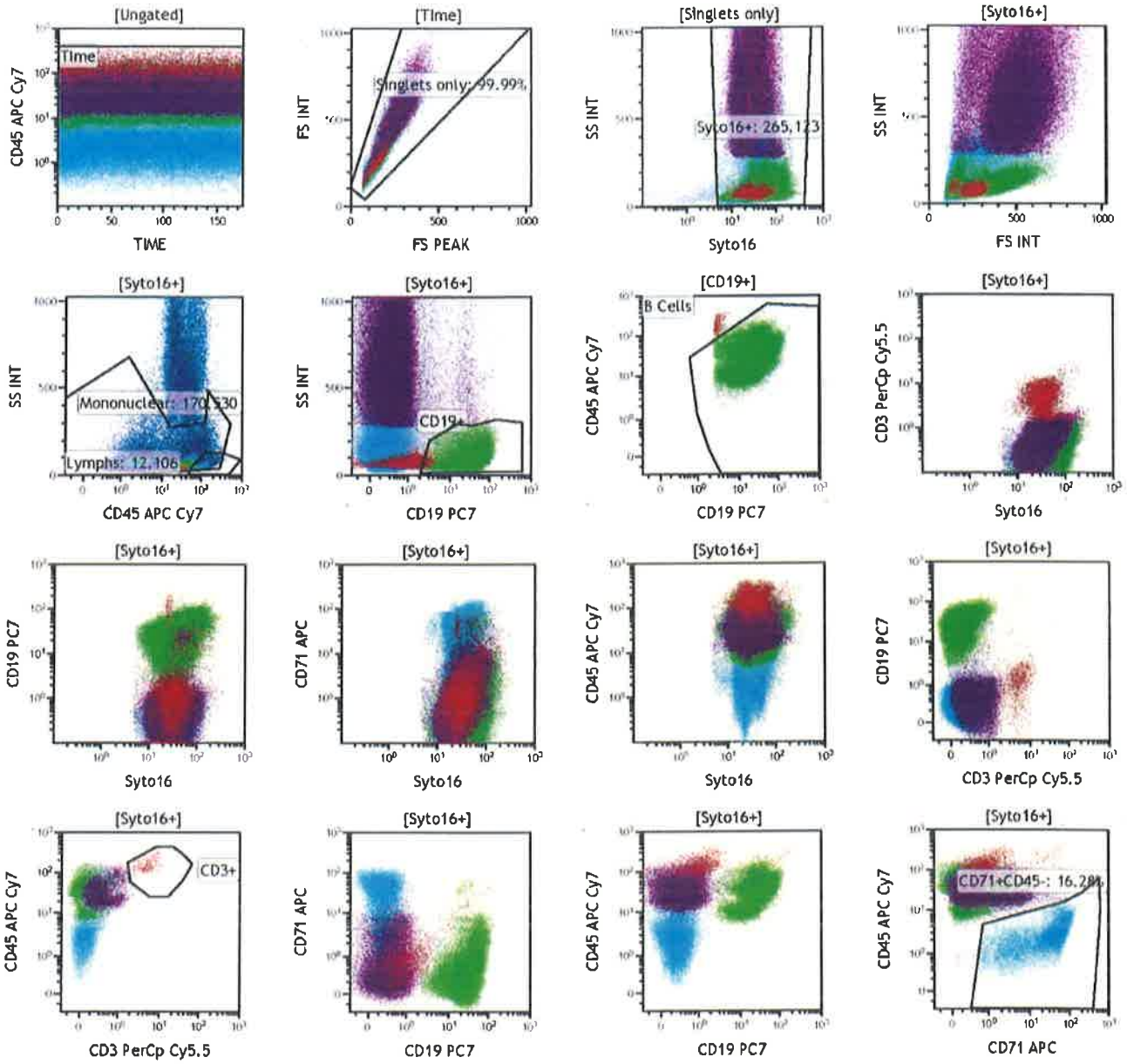
FLOW CYTOMETRY B-ALL MINIMAL RESIDUAL DISEASE

BALL-A 2019 (BALL-03)

Day 29 - Tube 3



Total #	268,201
[B Cells] Number	95,386
[Lymphs] Number	12,106
[Mononuclear] Number	170,530
[CD3+] Number	6,287