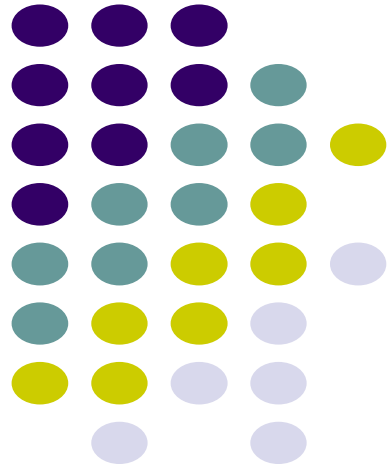
10. Citofluorimetria esempi di applicazioni

Prof. Gian Matteo Rigolin
Ematologia
Azienda Ospedaliero Universitaria
Arcispedale S. Anna Ferrara

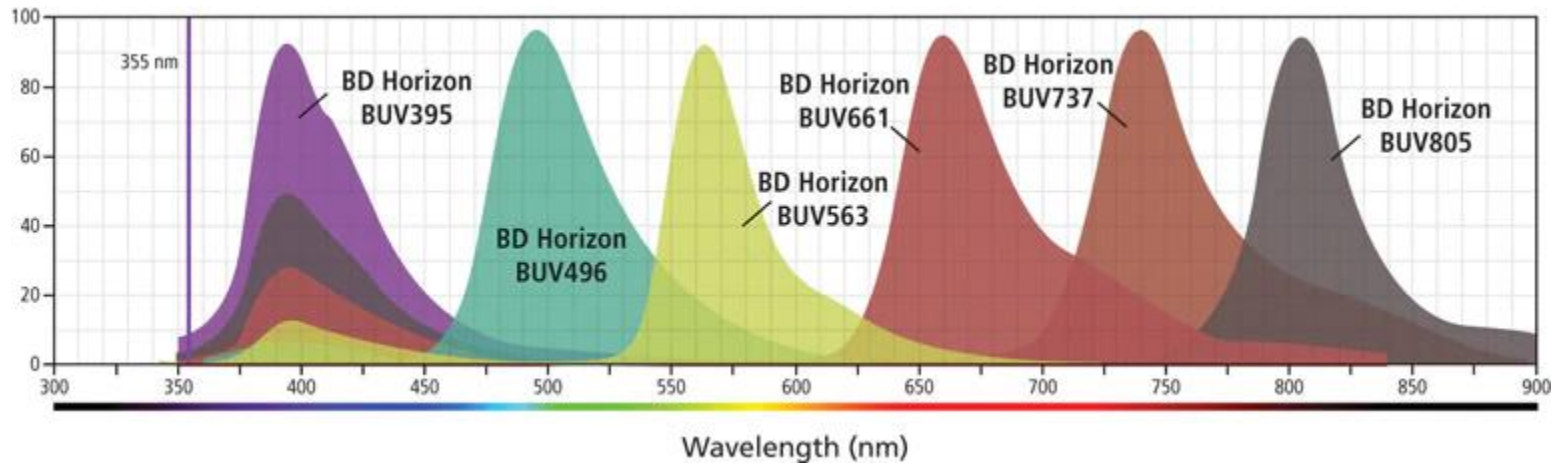


ESEMPI DI KIT
PANNELLI DI ANTICORPI MONOCLONALI

BD **OneFlow™ Setup Beads**

- The BD OneFlow Setup Beads **guarantee data reproducibility and allow for intra- and inter-laboratory instrument standardization by providing assay-specific target values** as per EuroFlow SOPs.4

- In association with BD FACSDiva™ CS&T IVD beads and Application Setting module, BD OneFlow Setup Beads deliver daily standardization control and instrument performance monitoring .


100
80
60
40
20
0
355 nm
BD Horizon BUV395
BD Horizon BUV496
BD Horizon BUV563
BD Horizon BUV661
BD Horizon BUV737
BD Horizon BUV805
300
350
400
450
500
550
600
650
700
750
800
850
900
Wavelength (nm)

Cytometer Setup Report

Cytometer:	BD FACSCanto II	Institution:	
Serial Number:	R33896202817	Director:	
Software:	BD FACSCanto v.3.1.5878.21241	Operator:	FACS
Date:	5/18/2018 3:05:21 PM	Overall Result:	PASS

Setup Beads

Bead Product: BD FACS 7-Color Setup Beads, Catalog Number: 335775
Lot Information: Lot ID 84770, Exp.: 2018-08-31

Detectors

Detector	Laser	FL Target	Voltage	ΔVoltage	Sensitivity	Spec	P/F*
FSC	Blue	457	305	7	NA	NA	PASS
SSC	Blue	545	403	1	NA	NA	PASS
FITC	Blue	454	451	5	49	15	PASS
PE	Blue	454	395	5	225	83	PASS
PerCP	Blue	465	553	3	19	9	PASS
PerCP-Cy5.5	Blue	441	544	7	53	25	PASS
PE-Cy7	Blue	470	602	21	216	114	PASS
APC	Red	506	607	0	105	40	PASS
APC-Cy7	Red	446	502	8	45	16	PASS

*ΔVoltage (change from previous setup): < 50 volts. Sensitivity: > Spec

Compensation

	Fluorophores (% spectral overlap)				PASS	spec: all values ≤ 100%	
Detector	FITC	PE	PerCP	PerCP-Cy5.5	PE-Cy7	APC	APC-Cy7
FITC	100.00	0.90	0.01	0.01	0.17	0.00	0.00
PE	17.99	100.00	0.03	0.03	1.20	0.00	0.00
PerCP	2.25	15.76	100.00	100.00	4.23	0.84	0.27
PerCP-Cy5.5	2.25	15.76	100.00	100.00	4.23	0.84	0.27
PE-Cy7	0.29	1.49	8.95	21.40	100.00	0.14	4.64
APC	0.01	0.15	5.68	3.85	0.01	100.00	16.83
APC-Cy7	0.00	0.02	0.69	2.97	2.84	2.66	100.00

Lasers

Laser	Power (mW)	Spec. (mW)	P/F	Current (A)
Blue	20.13	16.1-24.14	PASS	0.80
Red	17.25	14.4-21.6	PASS	NA

Fluidics

FACSFlow Pressure

Pressure	3.8 PSI
Spec	3.9 +/- 0.1 PSI
P/F	PASS

Sample Pressure (PSI)

High	Medium	Low
2.2	1.3	0.5

BD™ FC Beads - 8-color kit for BD OneFlow™ Assays

- With a simplified procedure for standardizing 8-color compensation, BD™ FC Beads drastically increase laboratory efficiency by minimizing training needs.
- Available as ready-to-use 3-µm polystyrene beads coupled to fluorochromes and dried in single-use 12 x 75-mm tubes, BD FC Beads eliminate the need for using single-vial reagents as well as label-specific compensation, **minimizing the process time for full 8-color compensation.**

BD OneFlow LST (Lymphoid Screening Tube)

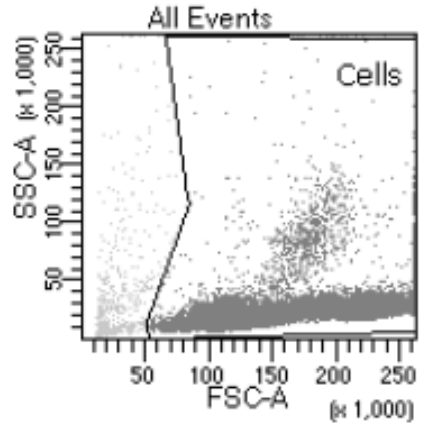
- **The BD OneFlow LST (Lymphoid Screening Tube) is a pre-configured single-dose, ready-to-use 8-color 12-antibodies reagent, that is provided as a single-test tube format.**

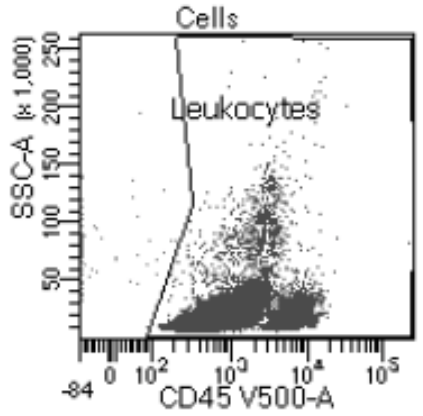
- **The BD OneFlow LST is intended for flow cytometric immunophenotyping of normal and aberrant mature lymphocyte populations of B, T and NK cell lineages in peripheral blood, bone marrow, and lymph nodes, as an aid in diagnosis of haematological disorders.**

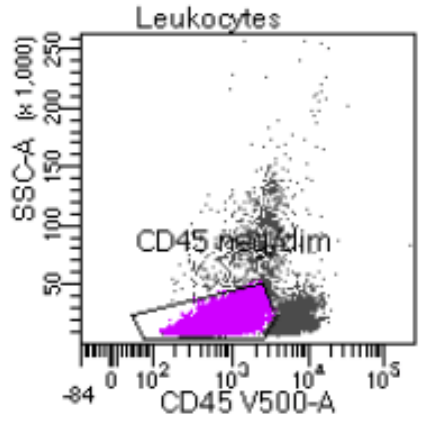
- As screening tube, the BD OneFlow LST can guide the need for further analysis in combination with panel(s) specifically designed for the classification of different form of malignancies (B, T or NK).
- The BD OneFlow LST is available in the 20 test/box size (4 pouches of 5 tubes each).

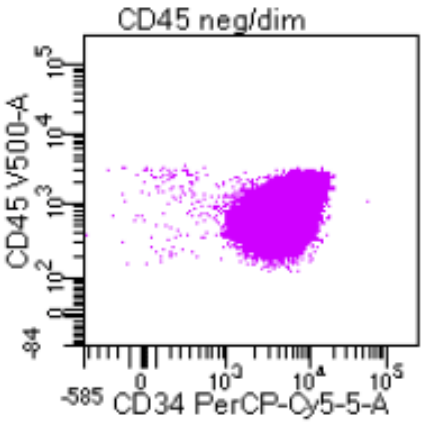
- Dark blue color-coded boxes, pouches and tubes allow for easy visual identification

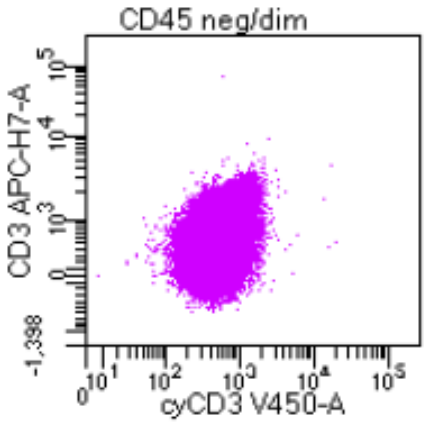
Antibody	Fluorochrome	Clone	Tube	Target Populations
MPO	FITC	MPO-7	C	Myeloid lineage marker
CD79a	PE	HM57	C	B-lineage marker
CD34	PerCP-Cy™5.5	8G12	S	Backbone marker (B-ALL and AML panels). Identification of immature cells
CD19	PE-Cy™7	SJ25-C1	S	Backbone marker (BCP-ALL panel). B-lineage marker
CD7	APC	M-T701	S	T-lineage marker
CD3	APC-H7	SK7	S	Backbone marker (T-ALL panel).
CD3	Horizon™ V450	UCHT-1	C	Backbone marker (T-ALL panel). Maturity marker for T-cells.
CD45	Horizon™ V500-C	2D1	S	Backbone marker. Identification of immature cells.

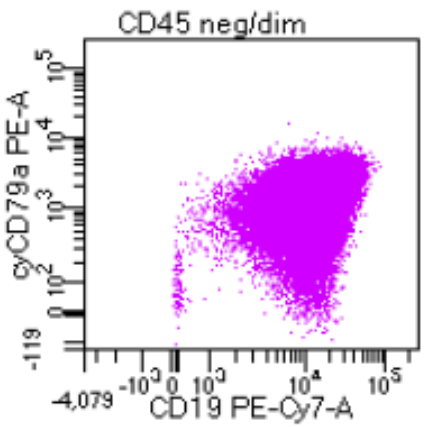


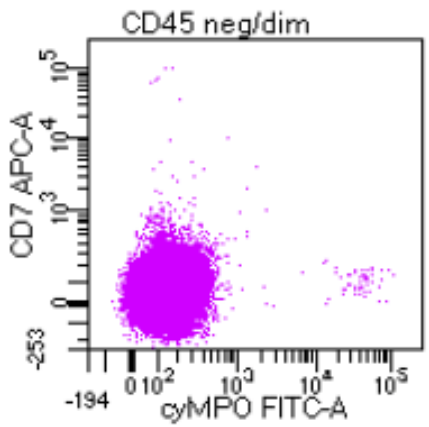












Specimen Name:	ALOT	Record Date:	14-Mar-2017 14:42:09
Tube Name:	ALOT	PATIENT ID:	

Population	#Events
All Events	100,000

Tube: ALOT

Population

- All Events
  - Cells
    - Leukocytes
      - CD45 neg/dim

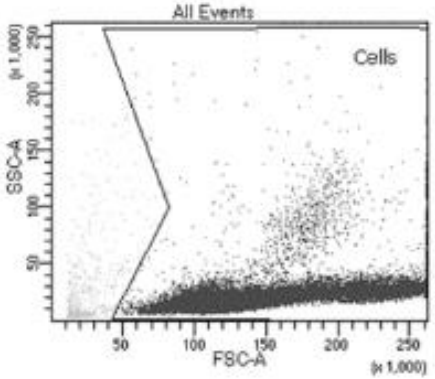
BD OneFlow™ ALOT Acquisition Template

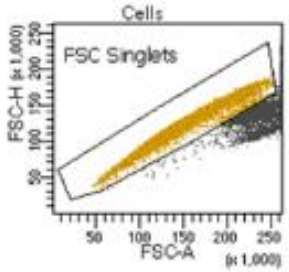
Specimen Name: ALOT
Tube Name: ALOT
Record Date: 14-Mar-2017 14:42:09
PATIENT ID:

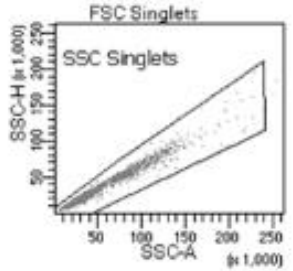
Population	#Events	%Parent	%Grand Parent
Leukocytes	94,375	99.9	99.9
cyCD3+	2,041	2.2	2.2
CD19+	90,527	95.9	95.9
Non-Lymphoid	1,028	55.5	1.1
CD45 neg/dim	90,335	95.7	95.7

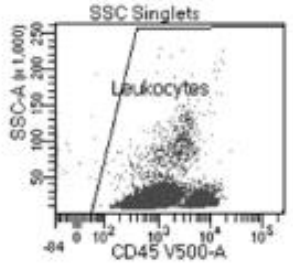
Tube: ALOT

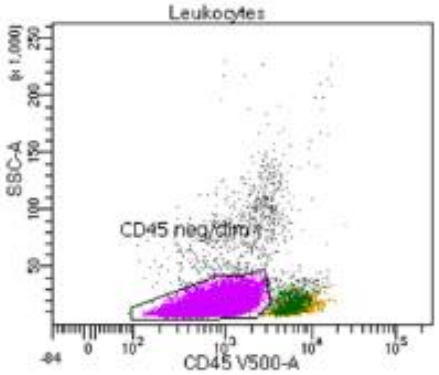
Population
- All Events
  - Cells
    - FSC Singlets
      - SSC Singlets
        - Leukocytes
          - cyCD3+
            - T cells
          - CD19+
            - B cells
          - cyCD3+ OR CD19+
          - NOT(cyCD3+ OR CD19+)
            - Non-Lymphoid
              - cyMPO+
          - CD45 neg/dim
            - CD34+

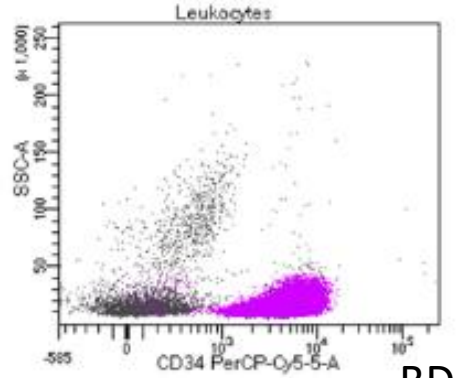








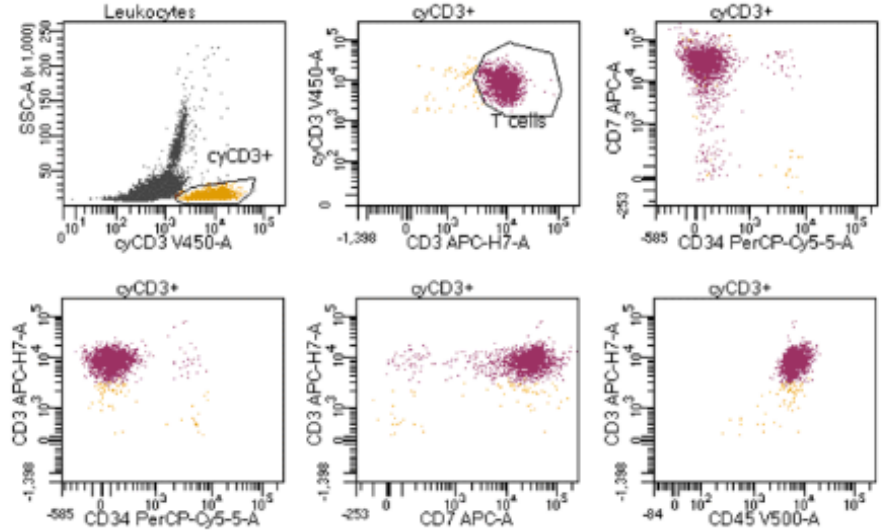




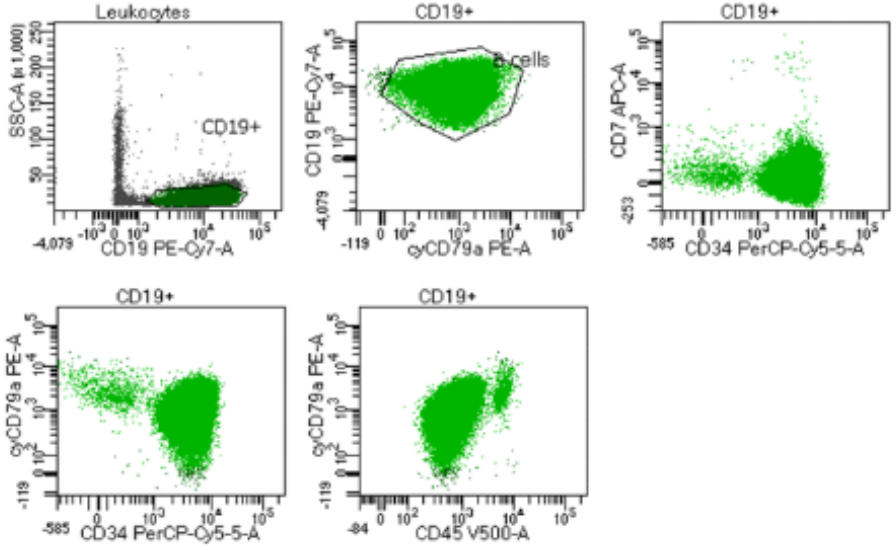
Specimen Name: ALOT
Tube Name: ALOT
Record Date: 14-Mar-2017 14:42:09
PATIENT ID:

Population	Parent Name	#Events	%Parent	%Grand Parent
T cells	cyCD3+	1,974	96.7	2.1
B cells	CD19+	90,368	99.8	95.8

T cells



B cells

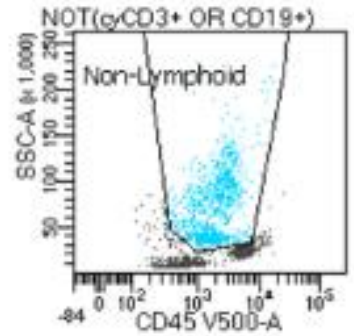


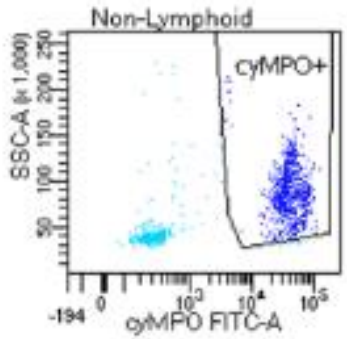
BD OneFlow™ ALOT Analysis Template

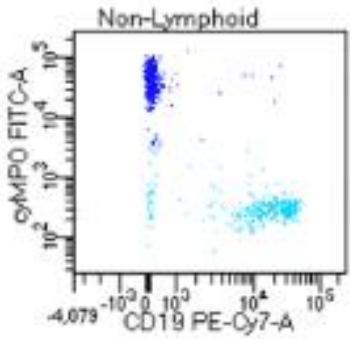
Specimen Name:	ALOT	Record Date:	14-Mar-2017 14:42:09
Tube Name:	ALOT	PATIENT ID:	

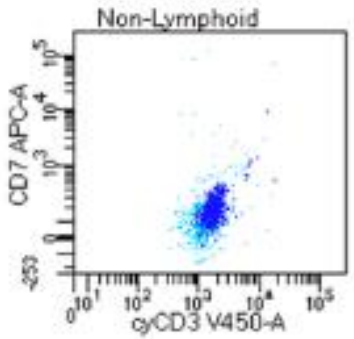
Population	Parent Name	#Events	%Parent	%Grand Parent
Non-Lymphoid	NOT(cyCD3+ O...	1,028	55.5	1.1
cyMPO+	Non-Lymphoid	651	63.3	35.1

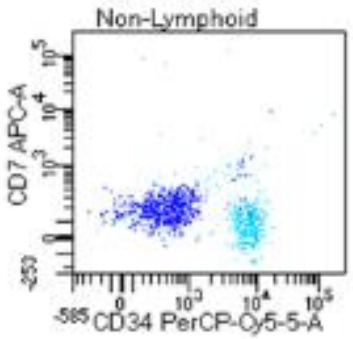
Non-Lymphoid cells

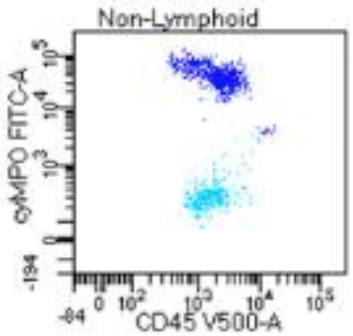


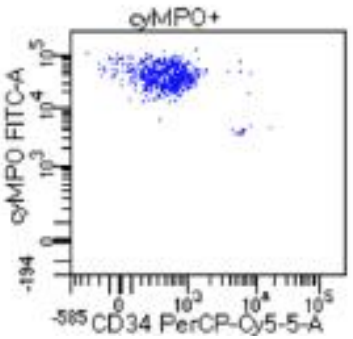










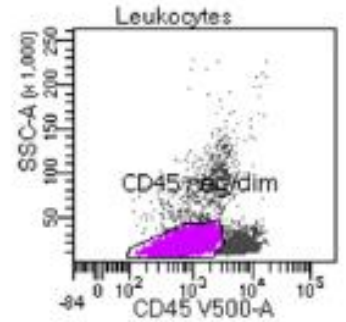


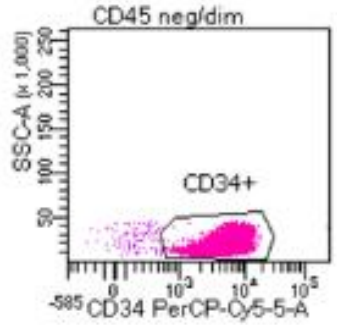
BD OneFlow™ ALOT Analysis Template

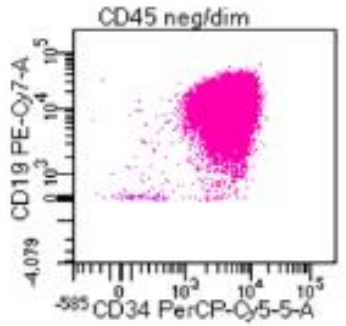
Specimen Name:	ALOT	Record Date:	14-Mar-2017 14:42:09
Tube Name:	ALOT	PATIENT ID:	

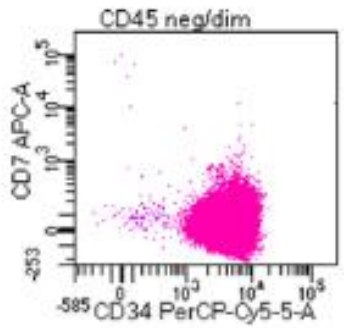
Population	Parent Name	#Events	%Parent	%Grand Parent
CD45 neg/dim	Leukocytes	90,335	95.7	95.7
CD34+	CD45 neg/dim	90,225	99.9	95.6

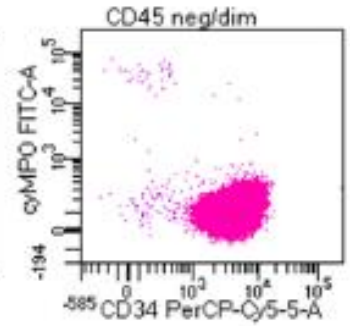
CD45 neg/dim cells

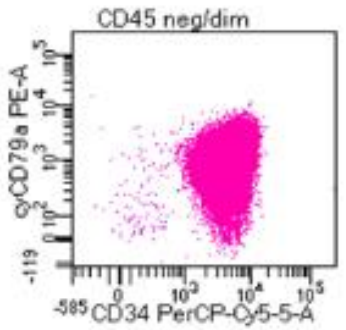


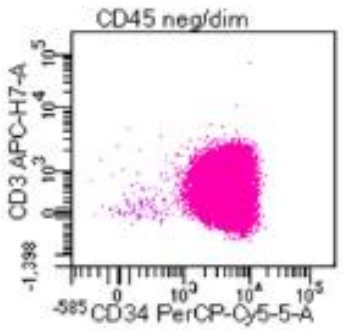


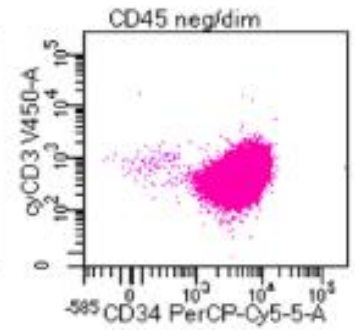












BD OneFlow LST (Lymphoid Screening Tube)

- **The BD OneFlow LST (Lymphoid Screening Tube) is a pre-configured single-dose, ready-to-use 8-color 12-antibodies reagent, that is provided as a single-test tube format.**

- **The BD OneFlow LST is intended for flow cytometric immunophenotyping of normal and aberrant mature lymphocyte populations of B, T and NK cell lineages in peripheral blood, bone marrow, and lymph nodes, as an aid in diagnosis of haematological disorders**.

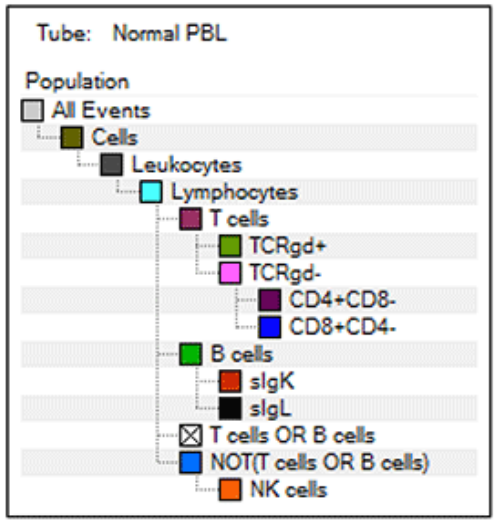
- As screening tube, the BD OneFlow LST can guide the need for further analysis in combination with panel(s) specifically designed for the classification of different form of malignancies (B, T or NK). The BD OneFlow LST is available in the 20 test/box size (4 pouches of 5 tubes each).

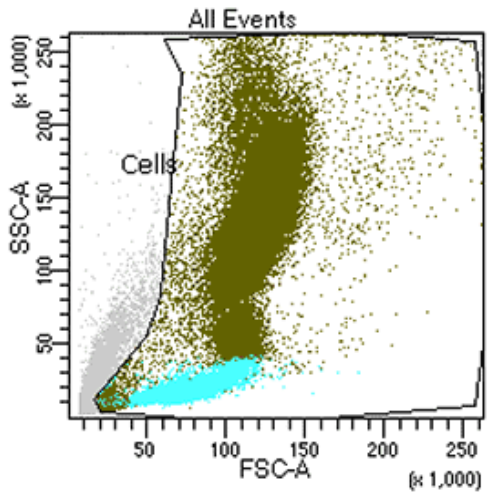
- Dark blue color-coded boxes, pouches and tubes allow for easy visual identification.

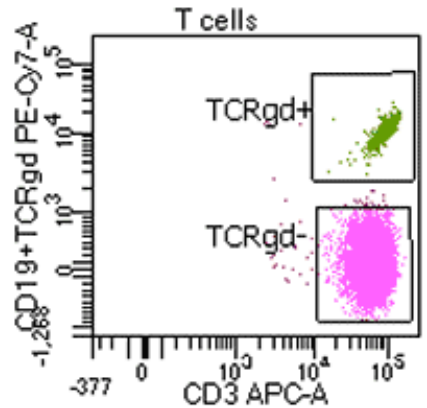
Antibody	Fluorochrome	Clone	Target Populations
CD45	BD Horizon™ V500-C	2D1 (anti-HLe-1)	Mature lymphocytes, B-cell precursor
CD19	PE-Cy™ 7	SJ25-C1	B cells, T- and NK-cells by exclusion
CD20	BD Horizon™ V450	L27	B cells, T- and NK-cells by exclusion
Anti-Lambda	FITC	1-155-2	Normal and clonally expanded B cells
Anti-Kappa	PE	TB28-2	Normal and clonally expanded B cells
CD38	APC-H7	HB7	Plasma cells and B-cell precursors, Lymphoid malignancies, NK cells
CD3	APC	SK7	T cells, B- and NK-cells by exclusion
CD4	BD Horizon™ V450	SK3 (Leu-3a)	T cell subpopulations
CD8	FITC	SK1 (Leu-2a)	T cell subpopulations
CD5	PerCP-Cy™ 5.5	L17F12	T cell subpopulations
Anti-TCRγδ	PE-Cy™ 7	11F2	T cell subpopulations
CD56	PE	MY31 (Leu-19)	NK cells

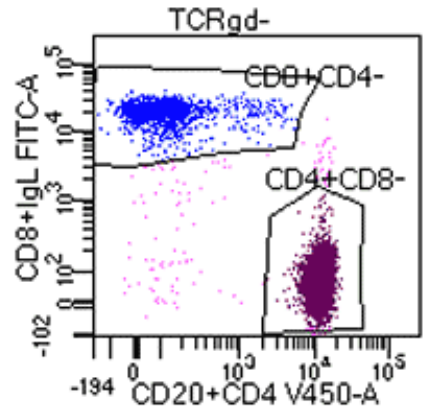
Specimen Name:	OneFlow LST	Record Date:	11-Nov-2014 14:00:51
Tube Name:	Normal PBL	PATIENT ID:	1810 t6

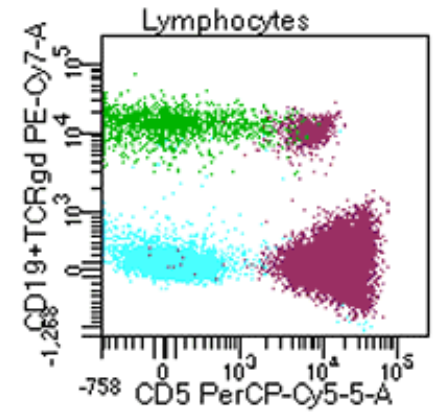
Population	#Events
All Events	100,000

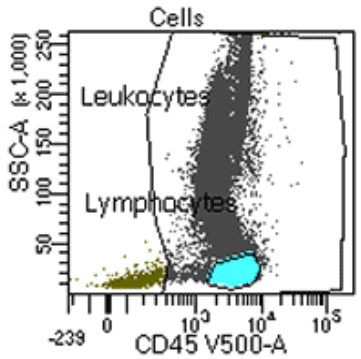


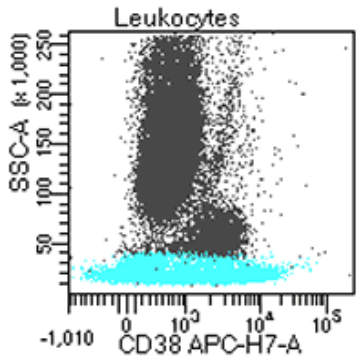


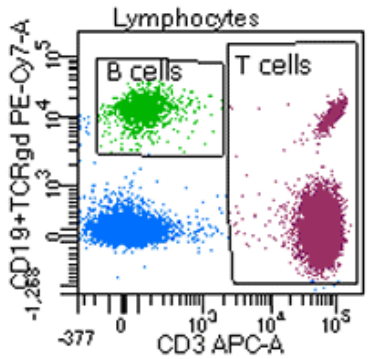


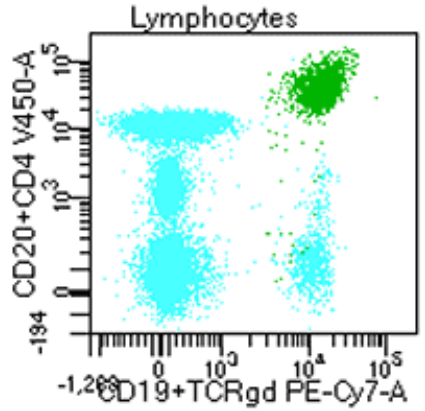


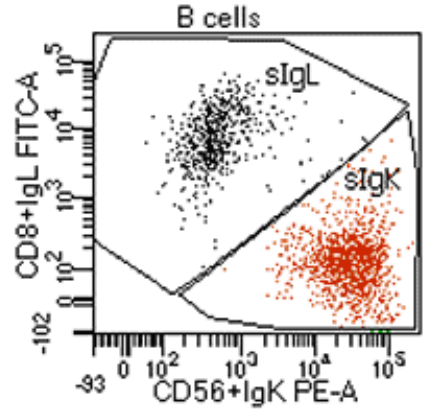


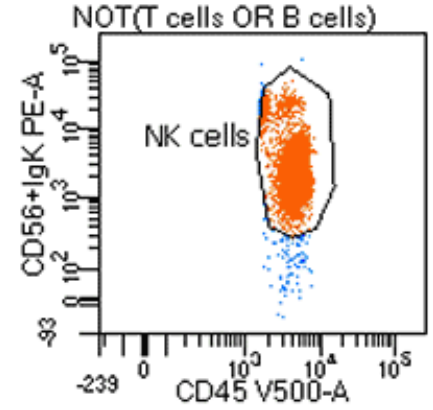








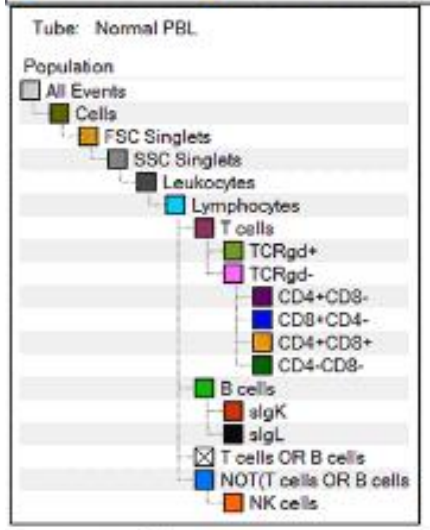


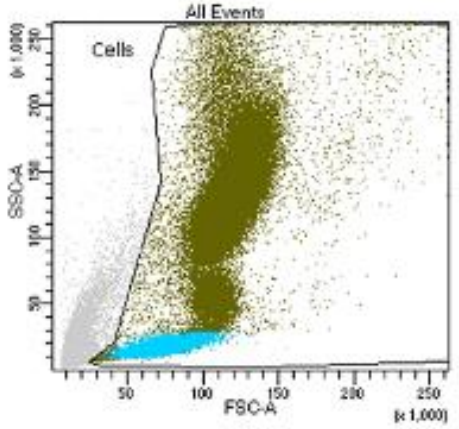


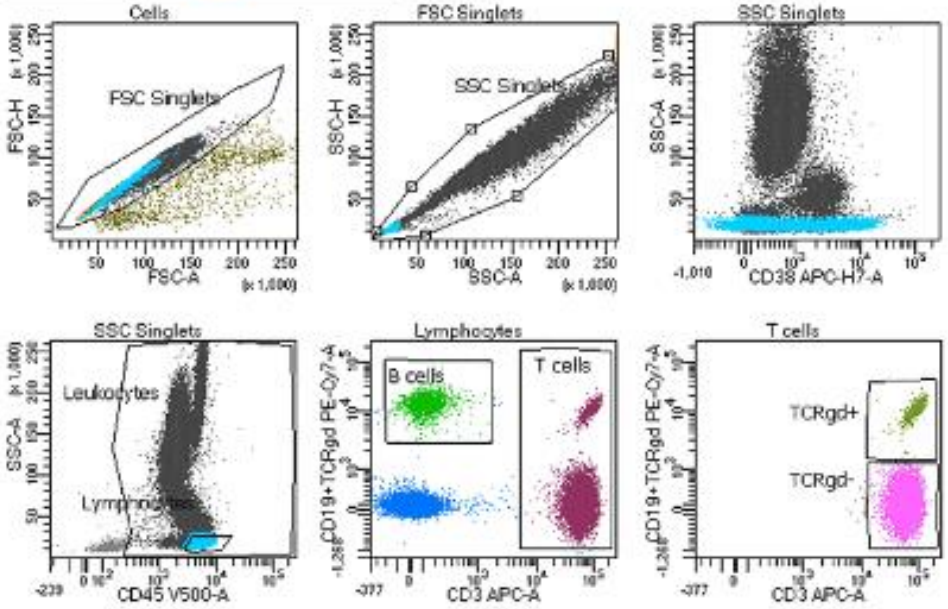
BD OneFlow LST Acquisition Template (v2.0)

Specimen Name:	OneFlow LST	Record Date:	11-Nov-2014 14:00:51
Tube Name:	Normal PBL	PATIENT ID:	1810 t6

Population	Parent Name	%Parent	%Grand Parent
Lymphocytes	Leukocytes	21.7	21.6
T cells	Lymphocytes	53.4	11.6
B cells	Lymphocytes	10.1	2.2
NK cells	NOT(T cells OR B cells)	98.9	36.1



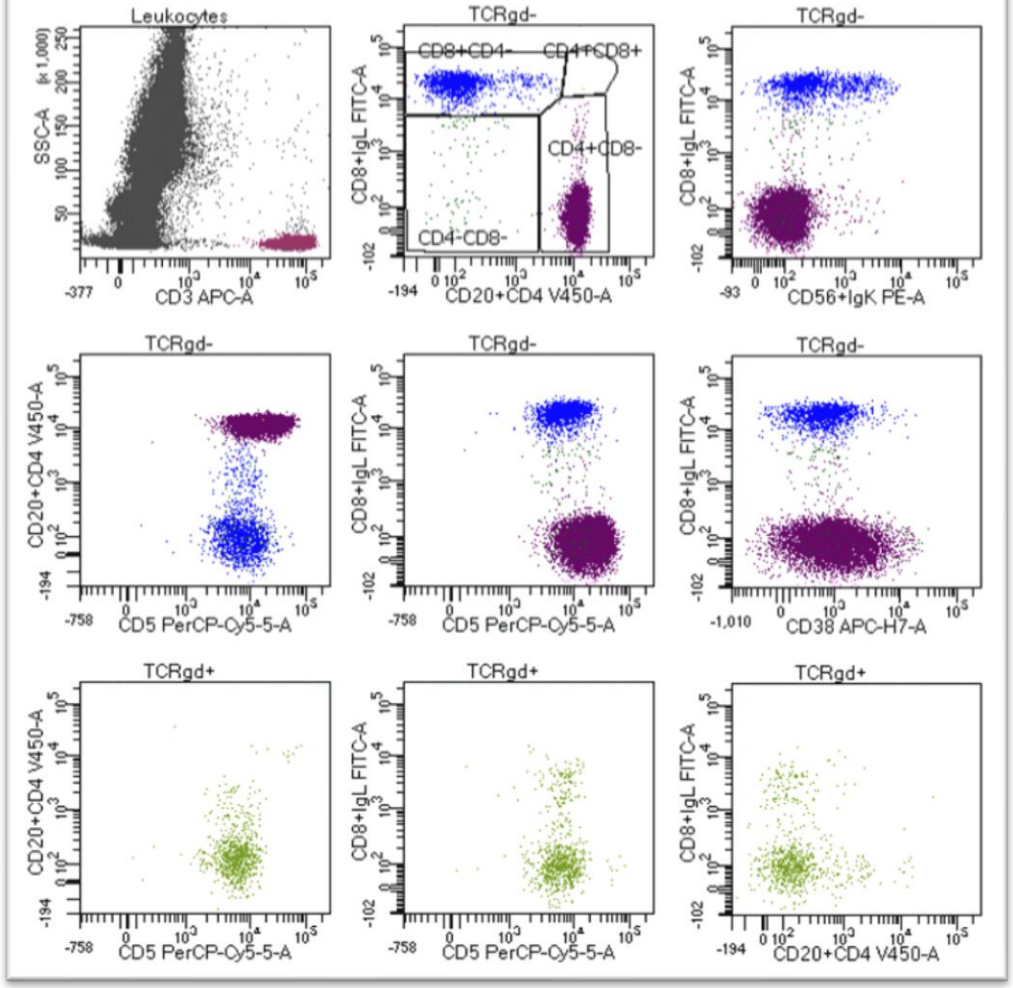




Specimen Name:	OneFlow LST	Record Date:	11-Nov-2014 14:00:51
Tube Name:	Normal PBL	PATIENT ID:	1810 t6

Population	Parent Name	%Parent	%Grand Parent
T cells	Lymphocytes	53.4	11.6
TCRgd+	T cells	8.5	4.5
TCRgd-	T cells	91.4	48.8
CD4+CD8-	TCRgd-	80.8	73.8
CD8+CD4-	TCRgd-	17.9	16.4
CD4+CD8+	TCRgd-	0.0	0.0
CD4-CD8-	TCRgd-	1.0	1.0

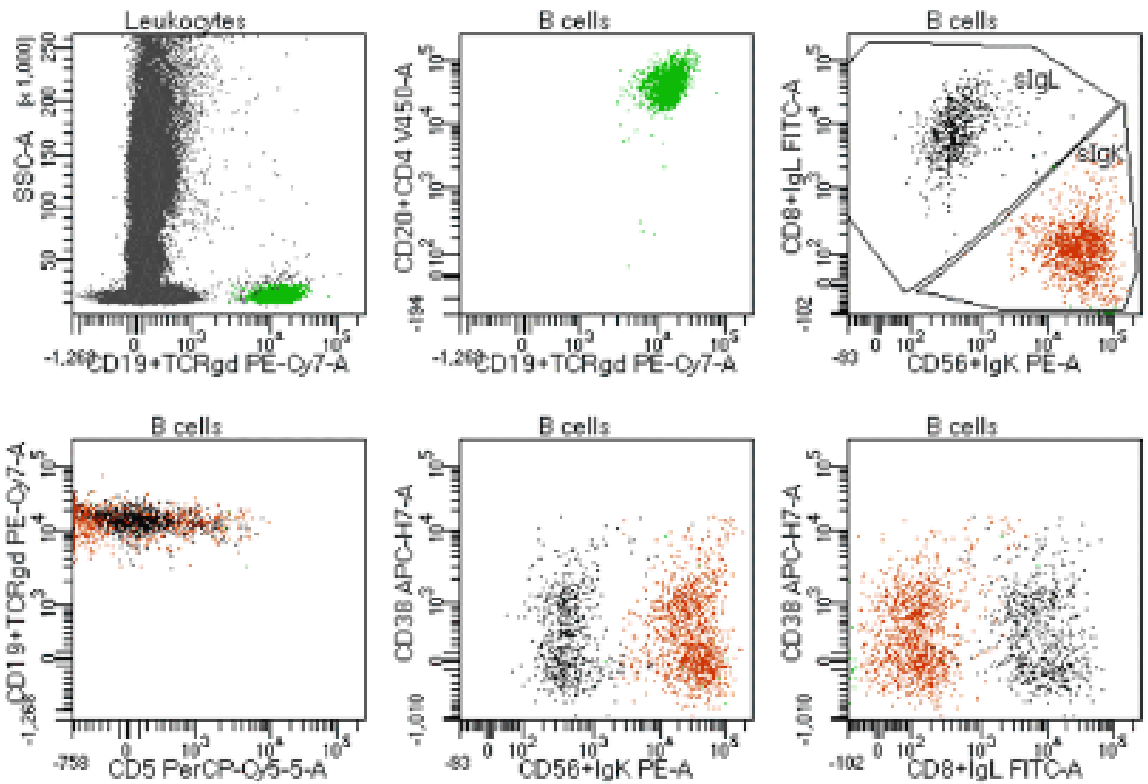
T cells



Specimen Name:	OneFlow LST	Record Date:	11-Nov-2014 14:00:51
Tube Name:	Normal PBL	PATIENT ID:	1810 t6

Population	Parent Name	%Parent	%Grand Parent
B cells	Lymphocytes	10.1	2.2
sIgK	B cells	64.6	6.5
sIgL	B cells	34.5	3.5

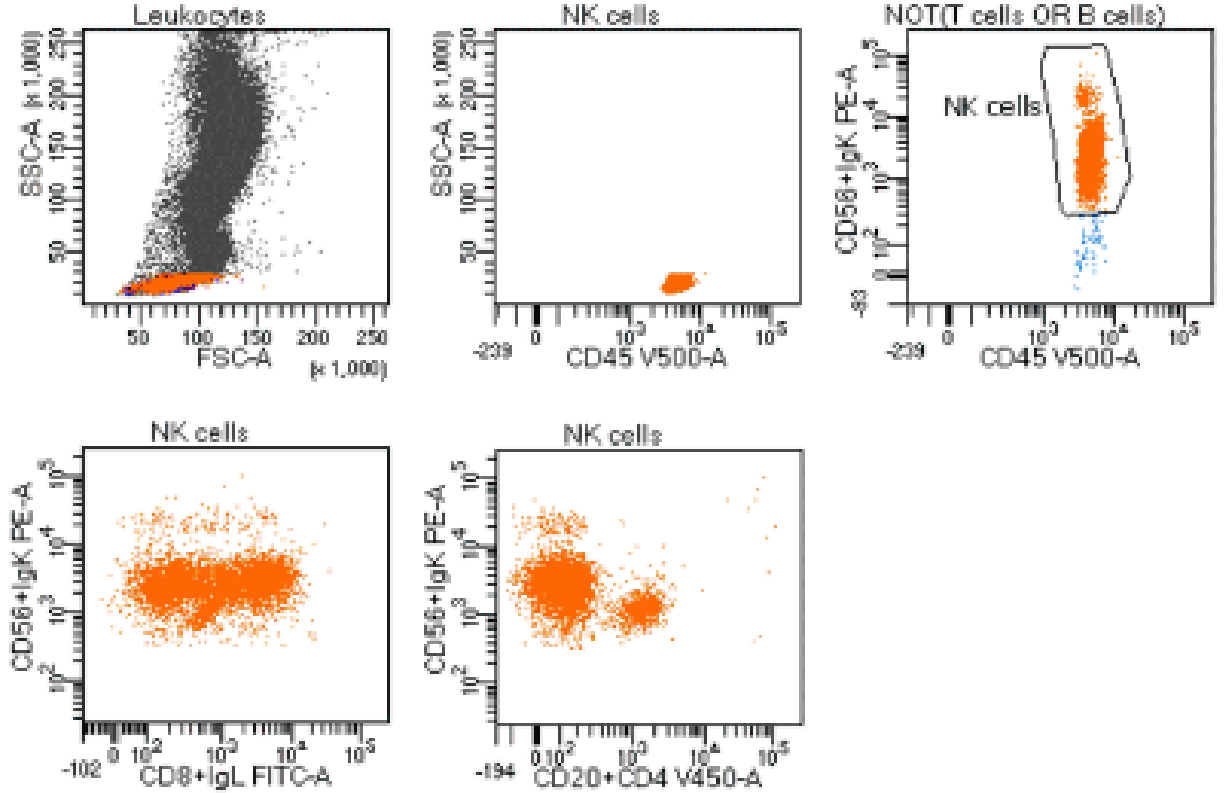
B cells



Specimen Name:	OneFlow LST	Record Date:	11-Nov-2014 14:00:51
Tube Name:	Normal PBL	PATIENT ID:	1810 t6

Population	Parent Name	%Parent	%Grand Parent
NK cells	NOT(T cells OR B cells)	98.9	36.1

NK cells



BD OneFlow LST Analysis Template (v2.0)

Experiment Name:	OneFlow LST_PCST_PCD_BCLPDT1
Plate Name:	
Specimen Name:	OneFlow LST
Tube Name:	Normal PBL
Record Date:	11-Nov-2014 14:00:51
CST SETUP STATUS:	SUCCESS
CST BEADS LOT ID:	44530
CYTOMETER CONFIG NAME:	3-laser, 8-color (4-2H-2V) (BD default)
CYTOMETER CONFIG CREATE DATE:	2007-01-02T12:00:00-08:00
CST SETUP DATE:	2014-11-11T13:17:58-08:00
CST BASELINE DATE:	2014-09-16T09:26:44-07:00
CST PERFORMANCE EXPIRED:	2014-11-12T13:17:58-08:00
CST REGULATORY STATUS:	CE-IVD Performance Check
CST BEADS EXPIRED:	false
SAMPLE ID:	1810
PATIENT ID:	1810 t6
CASE NUMBER:	55556
$OP:	Administrator
$INST:	BD Institute XY
GUID:	62b5621e-c397-4997-b953-47e4e1c069b9
$SYS:	Windows 7 6.1
$FIL:	OneFlow LST tst6_1810 tst6_001.fcs
CREATOR:	BD FACSDiva Software Version 8.0.1
SETTINGS:	20141031091347
PRODUCT ID:	658619,8888888,2015-10-27,765432
TEMPLATE VERSION ID:	LSTv1.0

Population	Parent Name	#Events	%Parent	%Grand Parent	%Total
All Events	####	100,000	####	####	100.0
Cells	All Events	83,877	83.9	####	83.9
FSC Singlets	Cells	82,568	98.4	82.6	82.6
SSC Singlets	FSC Singl...	82,412	99.8	98.3	82.4
Leukocytes	SSC Singl...	81,776	99.2	99.0	81.8
Lymphocytes	Leukocytes	17,774	21.7	21.6	17.8
T cells	Lymphocy...	9,488	53.4	11.6	9.5
TCRgd+	T cells	802	8.5	4.5	0.8
TCRgd-	T cells	8,672	91.4	48.8	8.7
CD4+CD8-	TCRgd-	7,005	80.8	73.8	7.0
CD8+CD4-	TCRgd-	1,556	17.9	16.4	1.6
CD4+CD8+	TCRgd-	1	0.0	0.0	0.0
CD4-CD8-	TCRgd-	91	1.0	1.0	0.1
B cells	Lymphocy...	1,798	10.1	2.2	1.8
sIgK	B cells	1,162	64.6	6.5	1.2
sIgL	B cells	620	34.5	3.5	0.6
NOT(T cells OR B cells)	Lymphocy...	6,488	36.5	7.9	6.5
NK cells	NOT(T ce...	6,414	98.9	36.1	6.4

BD OneFlow LST Analysis Template (v2.0)

BD OneFlow™ B-CLPD T1 (B-cell Chronic Lymphoproliferative Diseases Tube 1)

- **The BD OneFlow™ B-CLPD T1 (B-cell Chronic Lymphoproliferative Diseases Tube 1) is a pre-configured single-dose, ready-to-use 8-color reagent that is provided as a single-test tube format.**

- **The BD OneFlow B-CLPD T1 tube is a classification tube that is used for specimens with B-lineage populations needing further investigation in combination with the BD OneFlow LST (Lymphoid Screening Tube). The BD OneFlow B-CLPD T1 is intended for flow-cytometric immunophenotyping of B cells in peripheral blood and bone marrow as an aid in the diagnosis of chronic lymphocytic leukemia (CLL) and other B-cell chronic lymphoproliferative diseases.**

- It is available in the 20 test/box size (4 pouches of 5 tubes each).

- Boxes, pouches and tubes are color coded with a lighter blue color than the one identifying BD OneFlow LST, allowing for reagent visual identification.

- The blue color (dark and light) identifies the BD OneFlow B-cell Chronic Lymphoproliferative Disease Panel.

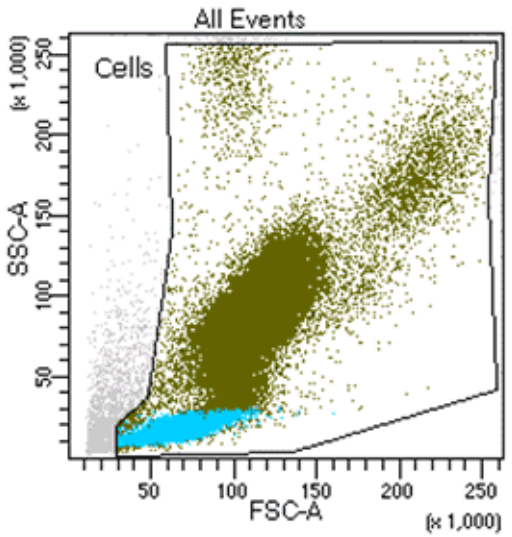
Antibody	Fluorochrome	Clone	Target Populations
CD23	FITC	EBVCS-5	Contributes to the classification of CLL or all other mature B-cell diseases
CD10	PE	MEM-78	Contributes to the classification of CLL or all other mature B-cell diseases
CD79b	PerCP-Cy™5.5	SN8	Contributes to the classification of CLL or all other mature B-cell diseases
CD19	PE-CY™7	SJ25-C1	Backbone marker. In common with BD OneFlow LST
CD200	APC	MRC OX-104	Contributes to the classification of CLL or all other mature B-cell diseases
CD43	APC-H7	1G10	Contributes to the classification of CLL or all other mature B-cell diseases
CD20	BD Horizon™ V450	L27	Backbone marker. In common with BD OneFlow LST
CD45	BD Horizon™ V500-C	2D1	Backbone marker. In common with BD OneFlow

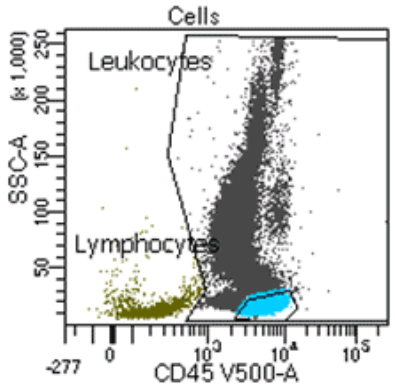
Specimen Name:	OneFlow B-CLPD-T1	Record Date:	12-Nov-2014 15:15:31
Tube Name:	Normal PBL	PATIENT ID:	7210 t8

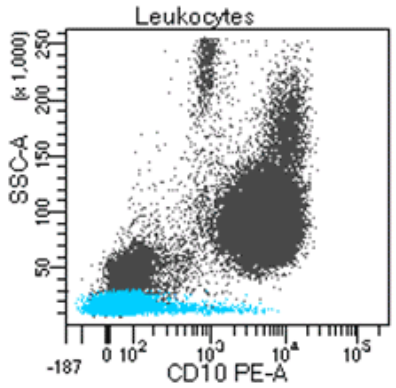
Population	#Events
All Events	100,000

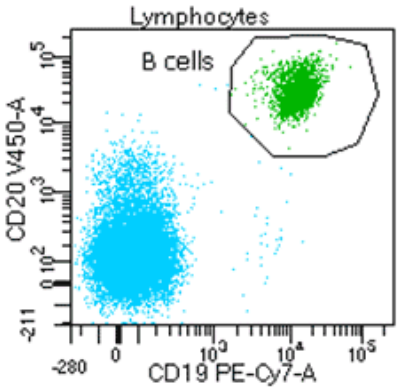
Tube: Normal PBL

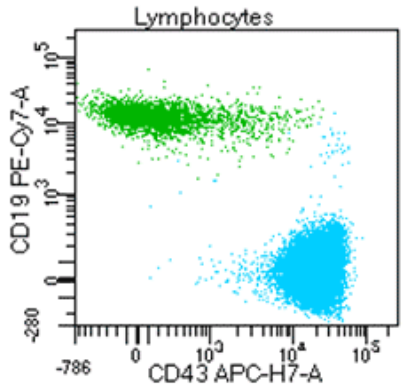
Population
- All Events
  - Cells
    - Leukocytes
      - Lymphocytes
        - B cells

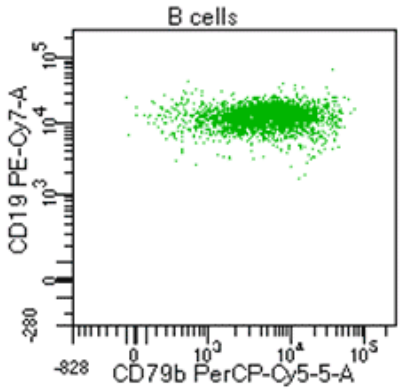


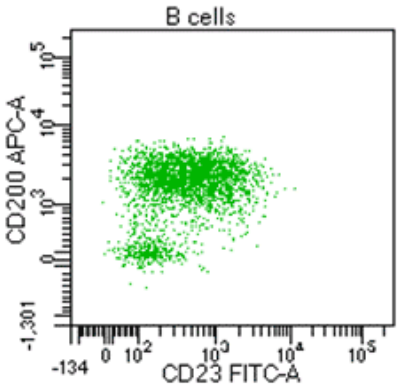












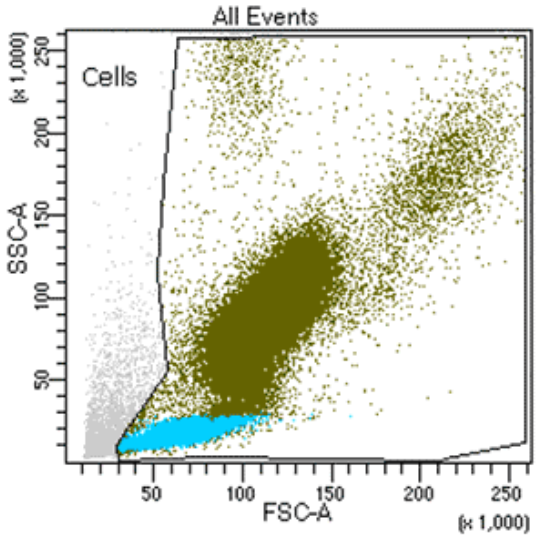
BD OneFlow B-CLPD T1 Acquisition Template

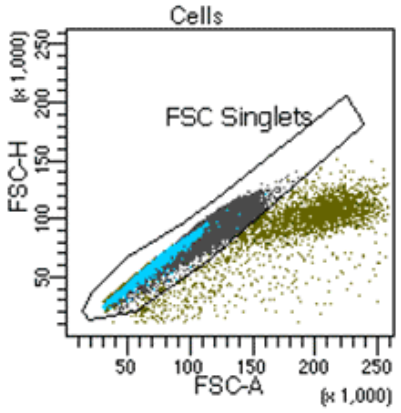
Specimen Name:	OneFlow B-CLPD-T1	Record Date:	12-Nov-2014 15:15:31
Tube Name:	Normal PBL	PATIENT ID:	7210 t8

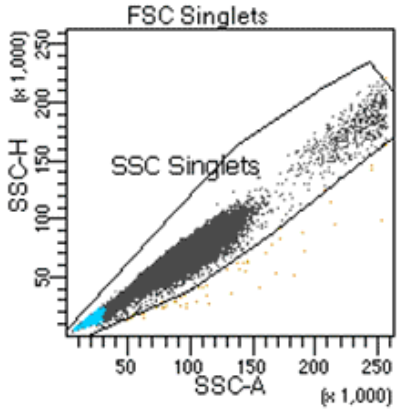
Population	Parent Name	#Events	%Parent
Lymphocytes	Leukocytes	19,053	22.8

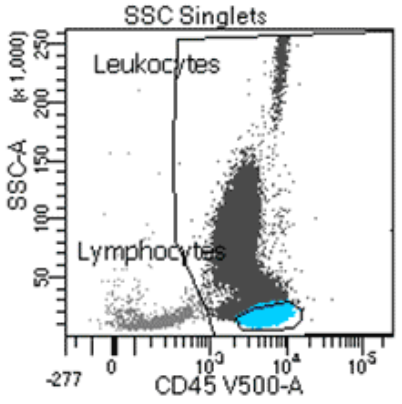
Tube: Normal PBL

Population
- All Events
  - Cells
    - FSC Singlets
      - SSC Singlets
        - Leukocytes
          - Lymphocytes
            - B cells







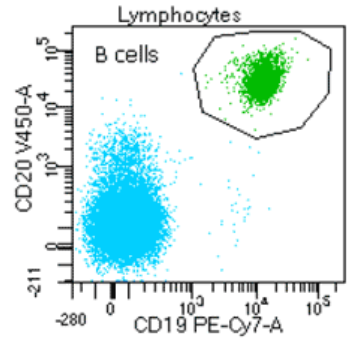


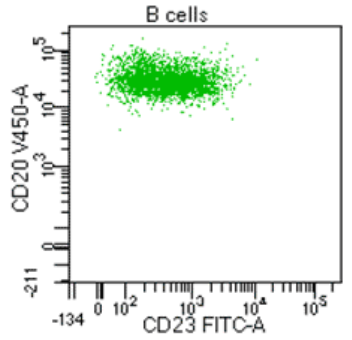
BD OneFlow B-CLPD T1 Analysis Template

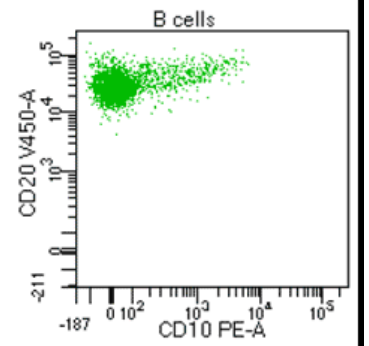
Specimen Name:	OneFlow B-CLPD-T1	Record Date:	12-Nov-2014 15:15:31
Tube Name:	Normal PBL	PATIENT ID:	7210 t8

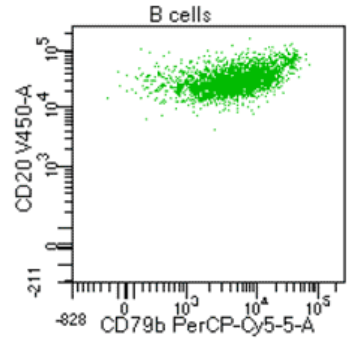
Population	Parent Name	%Parent	%Grand Parent
B cells	Lymphocytes	16.2	3.7

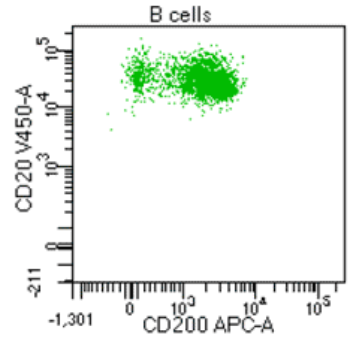
B cells

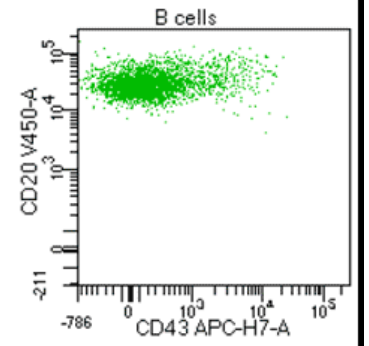


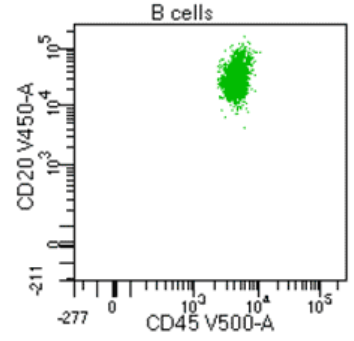












Experiment Name:	OneFlow LST_PCST_PCD_BCLPDT1
Specimen Name:	OneFlow B-CLPD-T1
Tube Name:	Normal PBL
Record Date:	12-Nov-2014 15:15:31
CST SETUP STATUS:	SUCCESS
CST BEADS LOT ID:	44530
CYTOMETER CONFIG NAME:	3-laser, 8-color (4-2H-2V) (BD default)
CYTOMETER CONFIG CREATE DATE:	2007-01-02T12:00:00-08:00
CST SETUP DATE:	2014-11-12T09:27:13-08:00
CST BASELINE DATE:	2014-09-16T09:26:44-07:00
CST PERFORMANCE EXPIRED:	2014-11-13T09:27:13-08:00
CST REGULATORY STATUS:	CE-IVD Performance Check
CST BEADS EXPIRED:	False
SAMPLE ID:	7210
PATIENT ID:	7210 t8
CASE NUMBER:	7777
$OP:	Administrator
$INST:	BD Institute XY
GUID:	130c03d2-e583-4862-b061-b5c62b77c269
$SYS:	Windows 7 6.1
$FIL:	OneFlow B-CLPD-T1_d7210_001.fcs
CREATOR:	BD FACSDiva Software Version 8.0.1
SETTINGS:	20141031091347
PREF GW NAME:	
TEMPLATE VERSION ID:	BCLPDT1v1.0
SPECIMEN TYPE:	Blood EDTA
DOCTOR:	mm
PRODUCT ID:	659293;5556666;2015-10-30;333444

Population	Parent Name	#Events	%Parent	%Grand Parent	%Total
All Events	####	100,000	####	####	100.0
Cells	All Events	88,108	88.1	####	88.1
FSC Singlets	Cells	85,156	96.6	85.2	85.2
SSC Singlets	FSC Singlets	85,093	99.9	96.6	85.1
Leukocytes	SSC Singlets	83,592	98.2	98.2	83.6
Lymphocytes	Leukocytes	19,053	22.8	22.4	19.1
B cells	Lymphocytes	3,080	16.2	3.7	3.1

BD OneFlow B-CLPD T1 Analysis Template

BD OneFlow™ PCST (Plasma Cell Screening Tube)

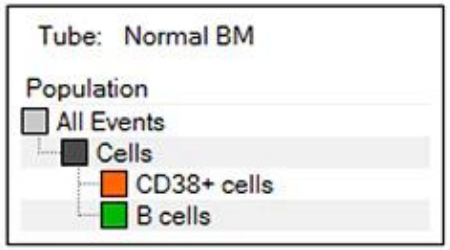
- The BD OneFlow™ PCST (Plasma Cell Screening Tube) is a pre-configured single-dose 8-color reagent, made of two tubes: one containing the cytoplasmic markers (C tube) and one containing the surface markers (S tube).

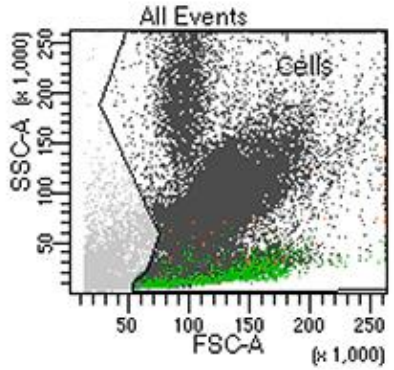
- The BD OneFlow PCST is intended for flow-cytometric immunophenotyping of normal polyclonal and aberrant plasma cell populations in bone marrow as an aid in the diagnosis of hematological disorders.

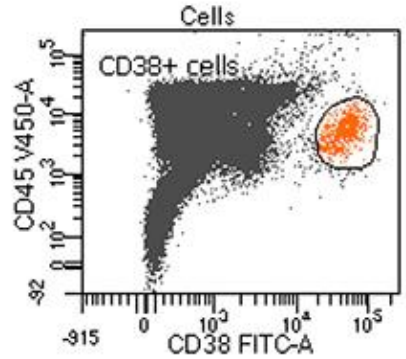
- It is available in the 10 test/box size (4 pouches of 5 tubes each: 2 pouches of S tubes and 2 pouches of C tubes).

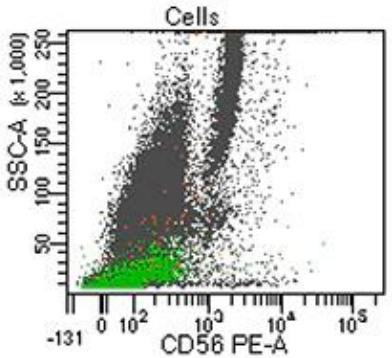
- Dark green color-coded boxes, pouches and tubes allow for easy visual identification.

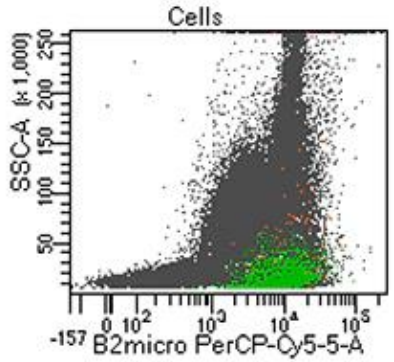
Antibody	Fluorochrome	Clone	Tube	Target Populations
CD38	FITC	HB7	S	Backbone marker. Identification of normal and aberrant plasma cells
CD56	PE	MY31	S	Identification of normal and aberrant plasma cells
β2-Microglobulin	PerCP-Cy™5.5	TÜ99	S	Prognostic marker
CD19	PE-Cy™7	SJ25-C1	S	Backbone marker. Identification of normal and aberrant plasma cells
Anti-Kappa	APC	TB28-2	C	Plasma cells clonality
Anti-Lambda	APC-H7	1-155-2	C	Plasma cells clonality
CD45	Horizon™ V450	2D1	S	Backbone marker. Identification of normal and aberrant plasma cells
CD138	Horizon™ V500-C	MI15	S	Backbone marker. Identification of plasma cells

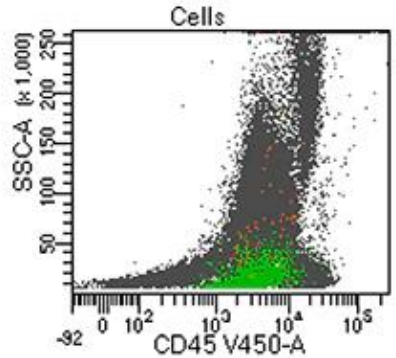


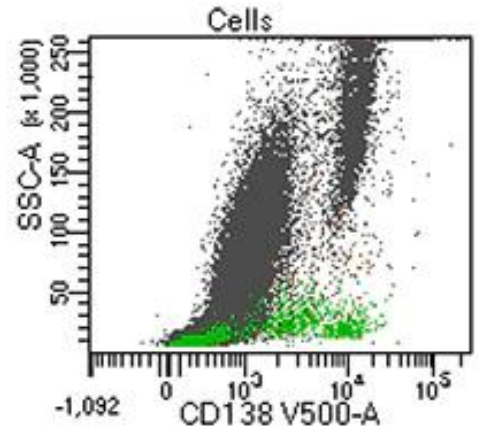


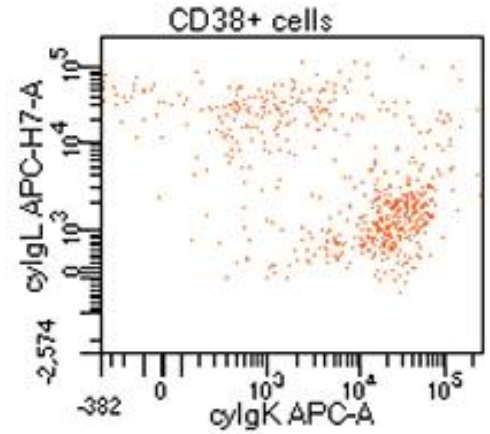


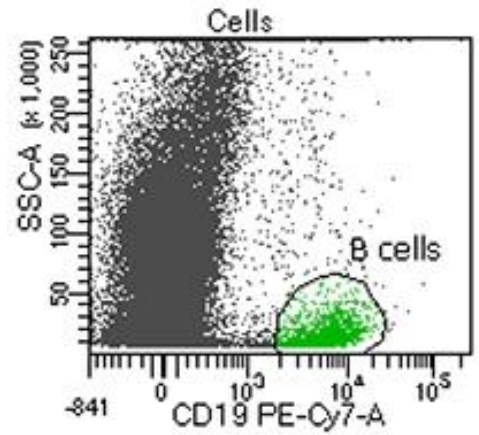


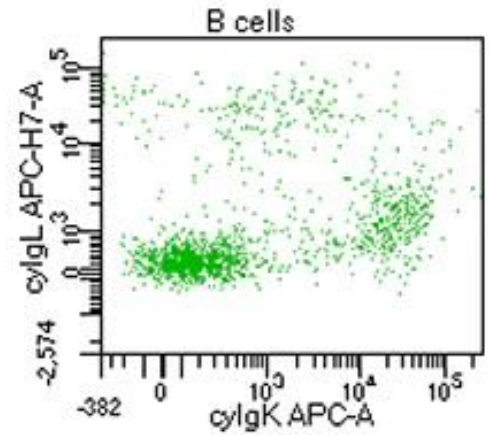








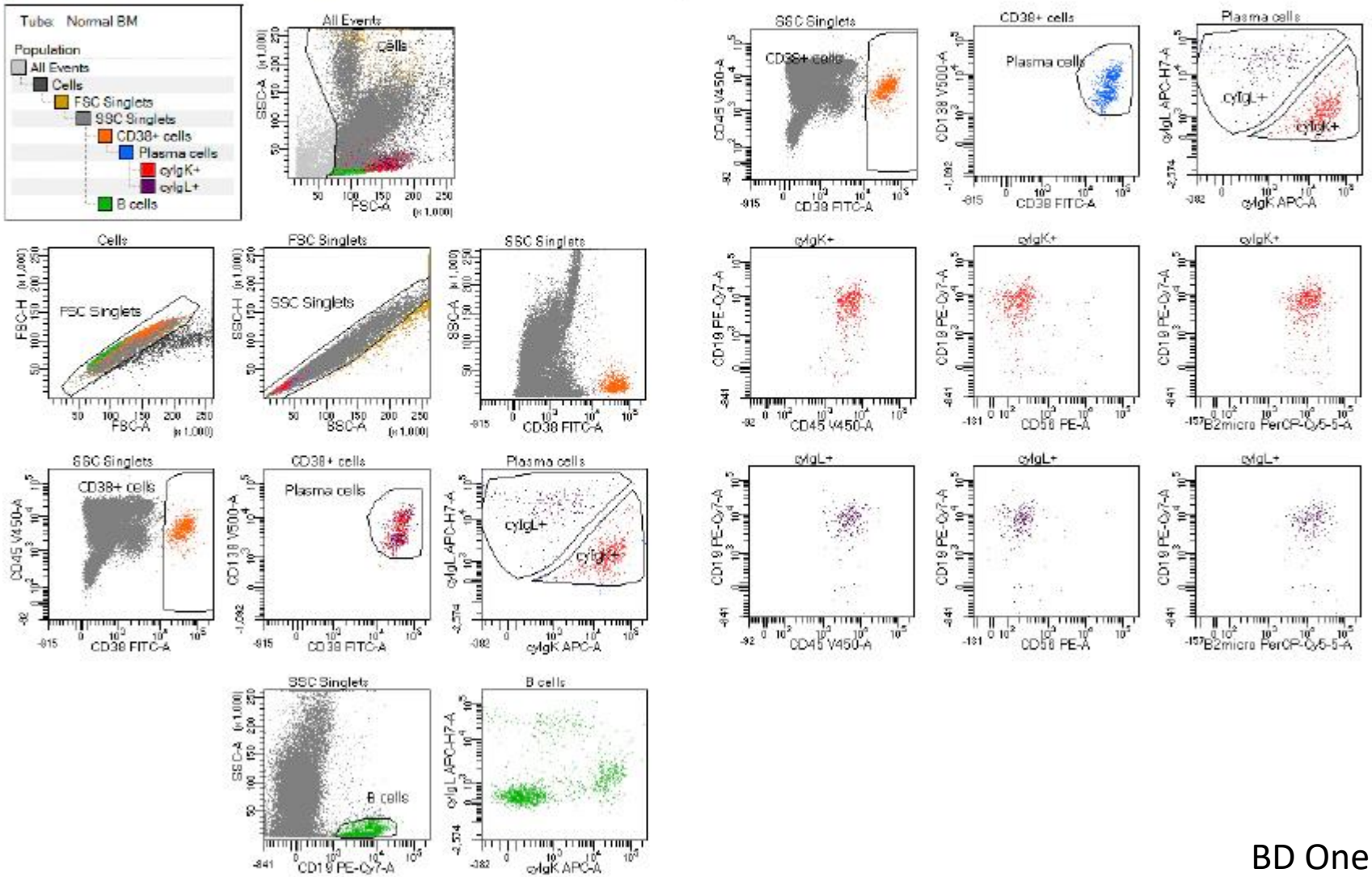




BD OneFlow™ PCST Acquisition Template

Specimen Name: OneFlow PCST
Tube Name: Normal BM

Population	Parent Name	#Events	%Parent	%Grand Parent	%Total
All Events	####	100,000	####	####	100.0
Plasma cells	CD38+ cells	643	98.3	0.9	0.6
cyIgK+	Plasma cells	410	63.8	62.7	0.4
cyIgL+	Plasma cells	200	31.1	30.6	0.2
Cells	All Events	78,233	78.2	####	78.2
FSC Singlets	Cells	76,662	98.0	76.7	76.7
SSC Singlets	FSC Singlets	75,287	98.2	96.2	75.3
CD38+ cells	SSC Singlets	654	0.9	0.9	0.7
B cells	SSC Singlets	1,404	1.9	1.8	1.4



BD OneFlow™ PCST Analysis Template

BD OneFlow™ PCD (Plasma Cell Dyscrasia)

- The BD OneFlow™ PCD (Plasma Cell Dyscrasia) tube is a pre-configured single-dose, ready-to-use 8-color reagent.

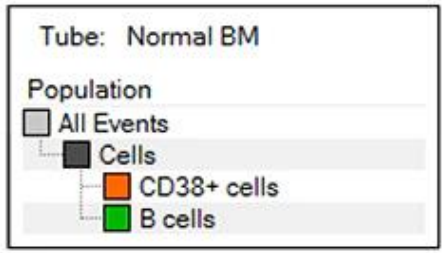
- The BD OneFlow™ PCD tube is a classification tube that shall be used for specimens with plasma cell populations needing further investigation as determined by the BD OneFlow™ PCST (Plasma Cell Screening Tube). The BD OneFlow PCD tube is intended for flow-cytometric immunophenotyping of normal and aberrant plasma cells in bone marrow as an aid in the diagnosis of multiple myeloma or other plasma cell disorders.

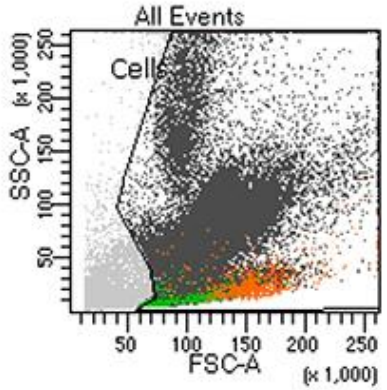
- It is available in the 10 test/box size (4 pouches of 5 tubes each).

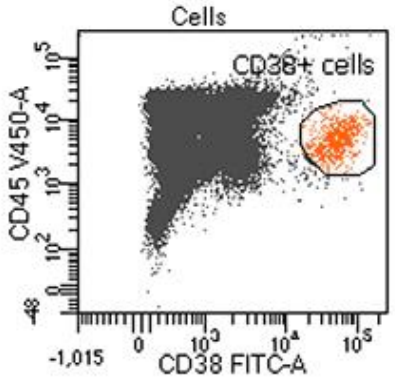
- Boxes, pouches and tubes are color coded with a lighter green color than the one identifying BD OneFlow PCST, allowing for reagent visual identification.

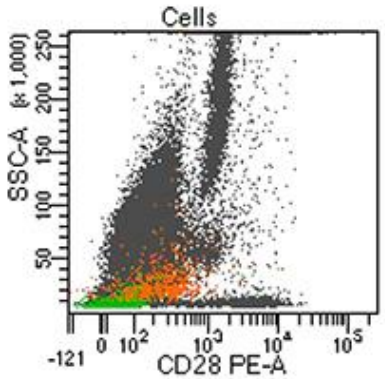
- The green color (dark and light) identifies the BD OneFlow Plasma Cell Disorder (PCD) Panel.

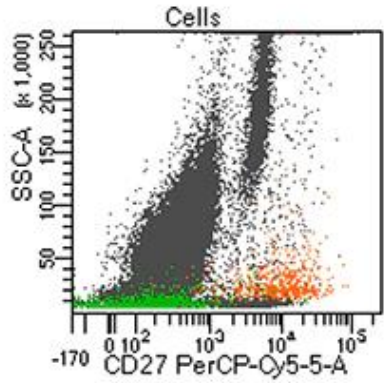
Antibody	Fluorochrome	Clone	Target Populations
CD38	FITC	HB7	Backbone marker. Identification of normal and aberrant plasma cells
CD28	PE	L293	Aberrant plasma cells
CD27	PerCP-Cy™5.5	L128	Aberrant plasma cells
CD19	PE-Cy™7	SJ25-C1	Backbone marker. Identification of normal and aberrant plasma cells
CD117	APC	104D2	Aberrant plasma cells
CD81	APC-H7	JS81	Aberrant plasma cells
CD45	Horizon™ V450	2D1	Backbone marker. Identification of normal and aberrant plasma cells
CD138	Horizon™ V500-C	MI15	Backbone marker. Identification of plasma cells

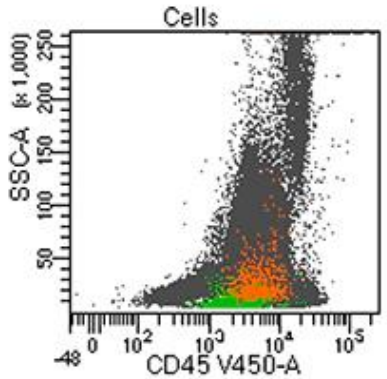


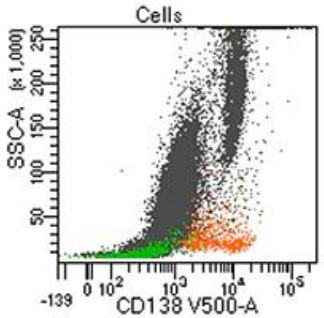


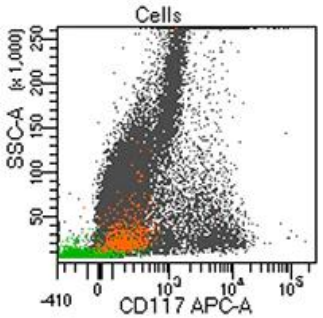


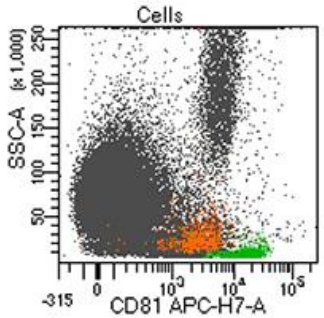


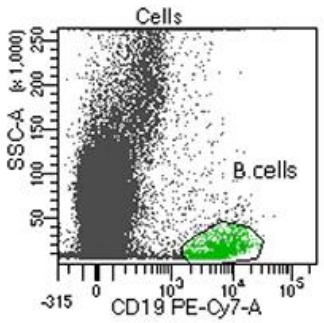












BD OneFlow™ PCD Acquisition Template

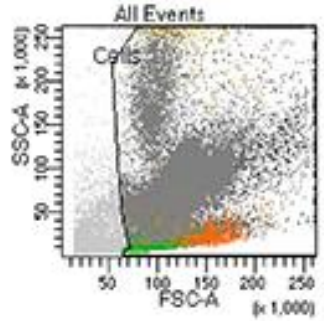
BD OneFlow™ PCD Analysis Template

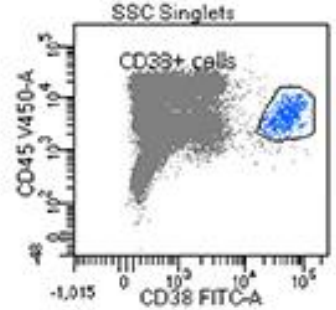
Specimen Name: OneFlow PCD
Tube Name: Normal BM

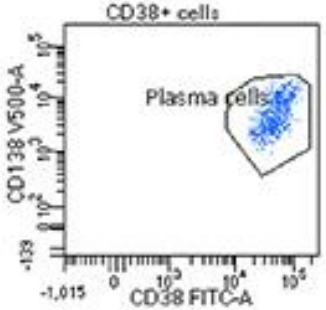
Population	Parent Name	#Events	%Parent	%Grand Parent	%Total
All Events	#####	100,000	#####	#####	100.0
Plasma cells	CD38+ cells	588	99.8	0.7	0.6
Cells	All Events	81,355	81.4	#####	81.4
FSC Singlets	Cells	79,796	98.1	79.8	79.8
SSC Singlets	FSC Singlets	78,810	98.8	96.9	78.8
CD38+ cells	SSC Singlets	589	0.7	0.7	0.6
B cells	SSC Singlets	1,644	2.1	2.1	1.6

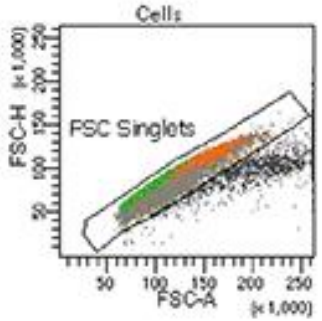
Tube: Normal BM

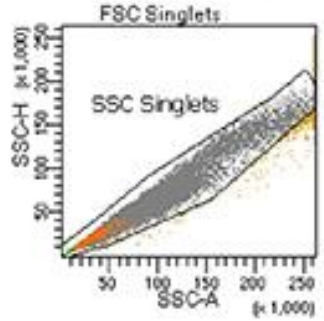
Population
- All Events
  - Cells
    - FSC Singlets
      - SSC Singlets
        - CD38+ cells
          - Plasma cells
        - B cells

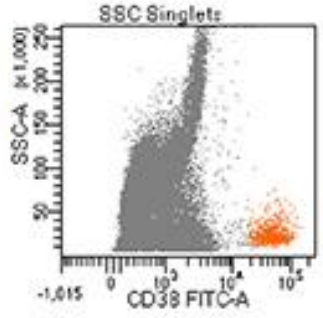


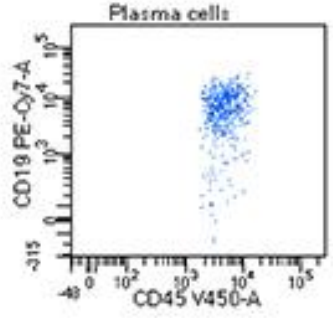


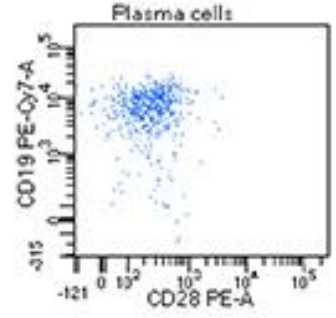


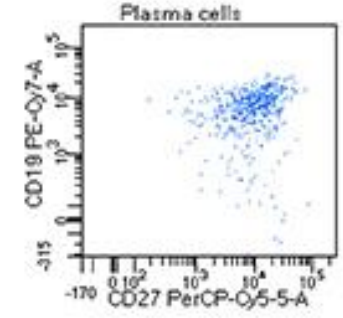


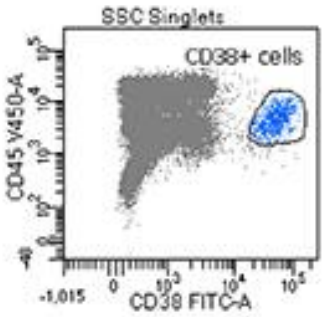


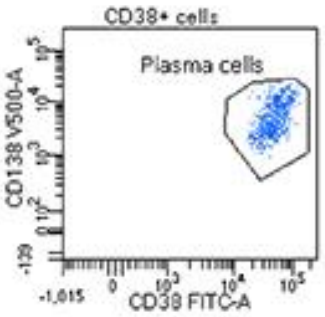


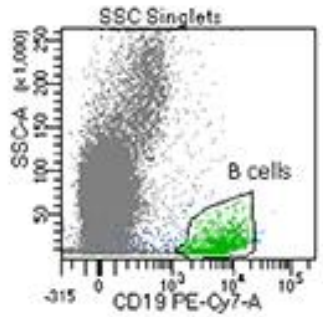




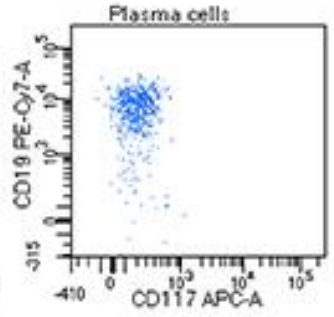


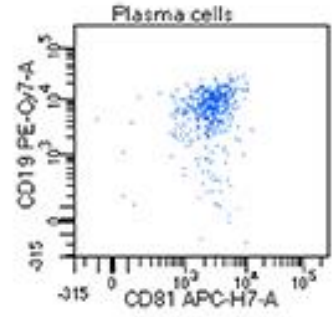




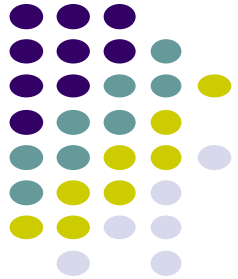


by: Admini
5 10:48:12





Validation of Assays and Quality Assurance



- Tutti gli strumenti devono seguire i controlli di qualità giornalieri secondo le raccomandazioni dei produttori.
- La partecipazione a un programma adeguato di controllo della qualità esterno (EQA) dovrebbe essere intrapresa.
- Esistono molti programmi di test di competenza che operano a livello locale, nazionale o internazionale.

Application Setup Report
BD Stem Cell

Cytometer:	BD FACSCanto II	Institution:	
Serial Number:	R33896202817	Director:	
Software:	BD FACSCanto v.3.1.5878.21241	Operator:	FACS
Date:	5/18/2018 3:19:11 PM		

Cytometer Setup

Cytometer Setup Report: 5/18/2018 3:05:21 PM, Overall Result: PASS
Bead Product: BD FACS 7-Color Setup Beads, Catalog Number: 335775
Lot Information: Lot ID 84770, Exp.: 2018-08-31

Detectors

Detector	Laser	Voltage
FSC	Blue	305
SSC	Blue	403
FITC	Blue	395
PE	Blue	364
7AAD	Blue	490
Trucount beads	Red	569

Compensation

	Fluorophores (%spectral overlap)			
Detector	FITC	PE	7AAD	Trucount beads
FITC	100.00	0.61	0.01	0.00
PE	26.46	100.00	4.50	0.00
7AAD	2.77	13.20	100.00	0.62
Trucount beads	0.02	0.17	7.75	100.00

Threshold (Operator: And)

FITC	400

CONTROLLO INTERNO

BD Stem Cell Control Kit

$CD34^+$ Whole Blood Process Control

Assay Values & Expected Ranges

Lot Number **BC0518**
Expiration Date **2018-06-02**

Levels	Lot Number	Total WBC/µL *	$CD34^+$/µL (Range)	$CD34^+$ as % of CD45 (Range)
$CD34^+$ Low	BC0518L	5,935	12.6 (8.6 – 16.6)	0.213 (0.147 – 0.279)
$CD34^+$ High	BC0518H	5,926	36.0 (26.6 – 45.4)	0.608 (0.450 – 0.766)

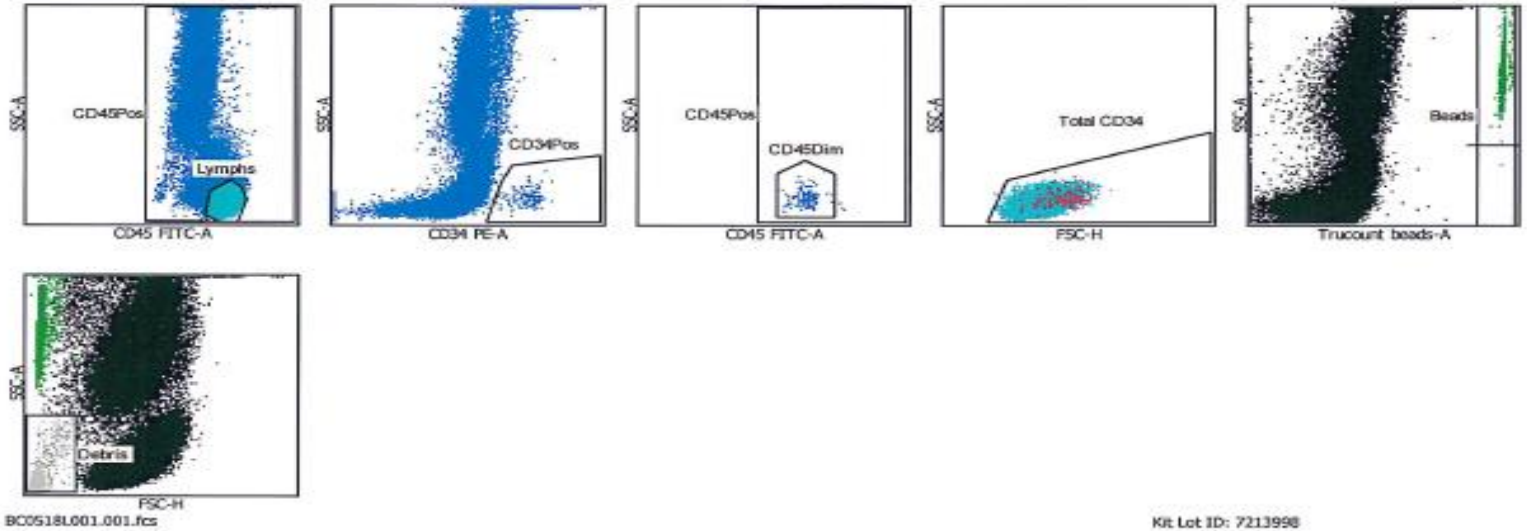
*For use with flow cytometry dual-platform method.

Control			
BC0518L	**Low**		
Director:		Panel:	BD Stem Cell
		Acquired:	5/18/2018 3:26:44 PM
		Analyzed:	5/18/2018 3:43:29 PM
		TruC Lot ID:	17066
		Bead/Pellet:	49850
		Status:	OK
		Operator:	FACS
		Reviewer:	
		Results:	BC0518L.csv

Column #1: Column #2: Column #3:

BD FACSCanto II R33896202817 BD FACSCanto v.3.1.5878.21241

BD Stem Cell Total Events: 89723



BC0518L001.001.fcs

Kit Lot ID: 7213998

CD34+ Abs Cnt (cells/µl)	12.57
CD45+ Abs Cnt (cells/µl)	6098.50
CD34+ Events	154
CD45+ Events	74699
Bead Events	6106
CD34+ % CD45+	0.21
CD34+ CV (%)	8.06

QC Messages

Manual Gate is in effect.
Inspect all dot plots.

Comments

Control

BC0518H | **High**

Director:

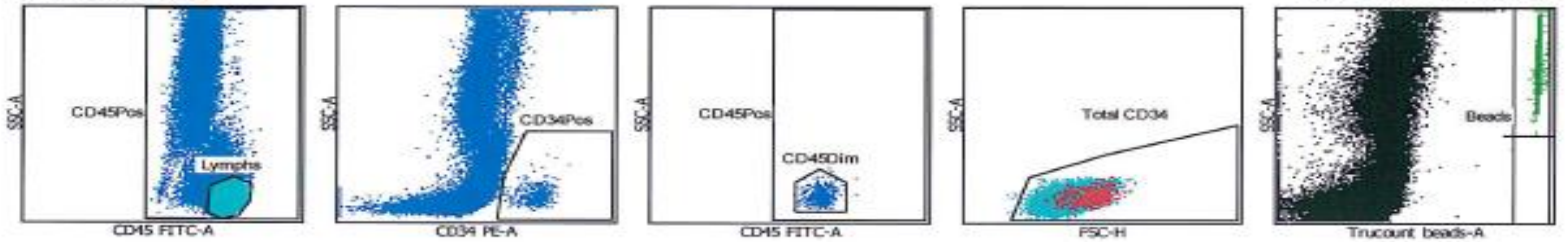
Panel:	BD Stem Cell
Acquired:	5/18/2018 3:31:21 PM
Analyzed:	5/18/2018 3:45:15 PM
TruC Lot ID:	17066
Bead/Pellet:	49850
Status:	OK
Operator:	FACS
Reviewer:	
Results:	BC0518H.csv

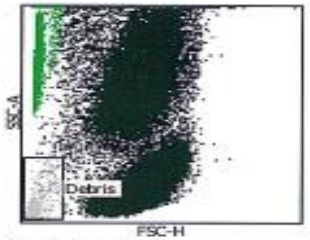
Column #1: | Column #2: | Column #3:

BD FACSCanto II R33896202817 | BD FACSCanto v.3.1.5878.21241

BD Stem Cell

Total Events: 89018





BC0518H002.001.fcs

Kit Lot ID: 7213998

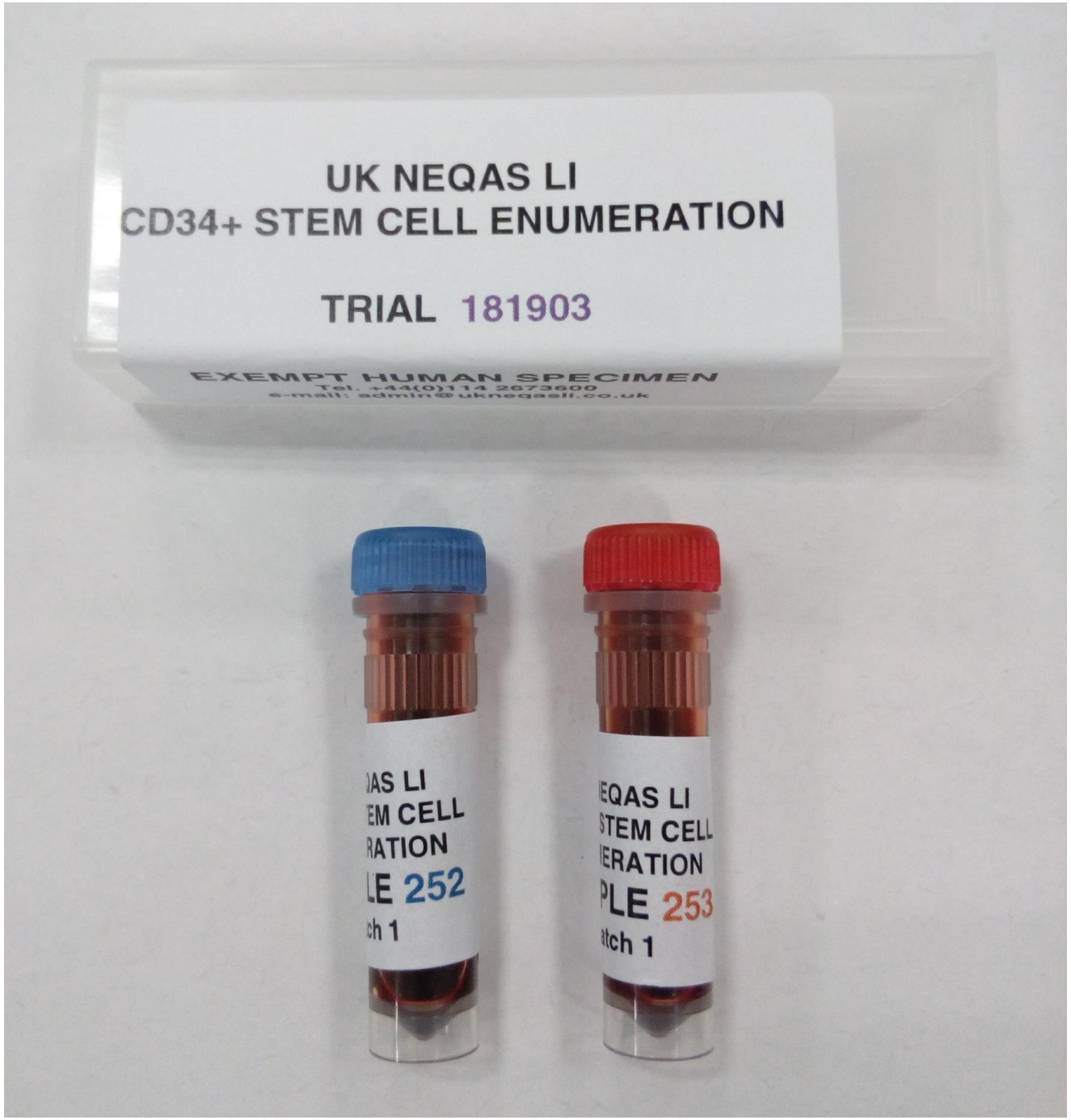
CD34+ Abs Cnt (cells/µl)	36.18
CD45+ Abs Cnt (cells/µl)	6043.46
CD34+ Events	448
CD45+ Events	74825
Bead Events	6172
CD34+ % CD45+	0.60
CD34+ CV (%)	4.72

QC Messages

Manual Gate is in effect.
Inspect all dot plots.

Comments

CONTROLLO ESTERNO DI QUALITA’



UK NEQAS
Leucocyte Immunophenotyping

Sheffield Teaching Hospitals NHS
NHS Foundation Trust

CD34+ Stem Cell Enumeration Programme

All Participant Report

Distribution - 181902 | Sample - 250 | Participant ID - 43031

Date Issued - 18 June 2018 | Closing Date - 06 July 2018 | Machine Used - Facscanto II

Trial Comments

This trial was issued to 335 participants

Sample Comments

The sample was manufactured by UK NEQAS using stabilised CD34+ samples and stabilised leucodepleted blood

Absolute Values Results and Performance

Please note: Performance monitoring for this programme is on absolute values only. Percentage results are shown for information purposes only.

Cell Population	Your Results (cells/µL)	Robust Mean (cells/µL)	Robust SD (cells/µL)
CD34 Absolute Values	57.71	55.95	4.84

Cell Population	z Score*	Performance Status for this Sample	Performance Status Classification Over 12 Sample Period		
			Satisfactory	Action	Critical
CD34 Absolute Values	0.36	Satisfactory	12	0	0

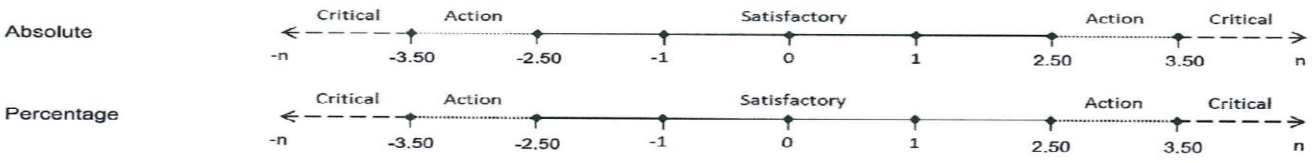
Percentage Values Results and Performance

Cell Population	Your Results %	Robust Mean %	Robust SD %
CD34 Percentage Values	0.64	0.63	0.04

Cell Population	z Score*	Performance Status for this Sample	Performance Status Classification Over 12 Sample Period		
			Satisfactory	Action	Critical
CD34 Percentage Values	0.25	Satisfactory	11	1	0

***z Score Limits Definitions**

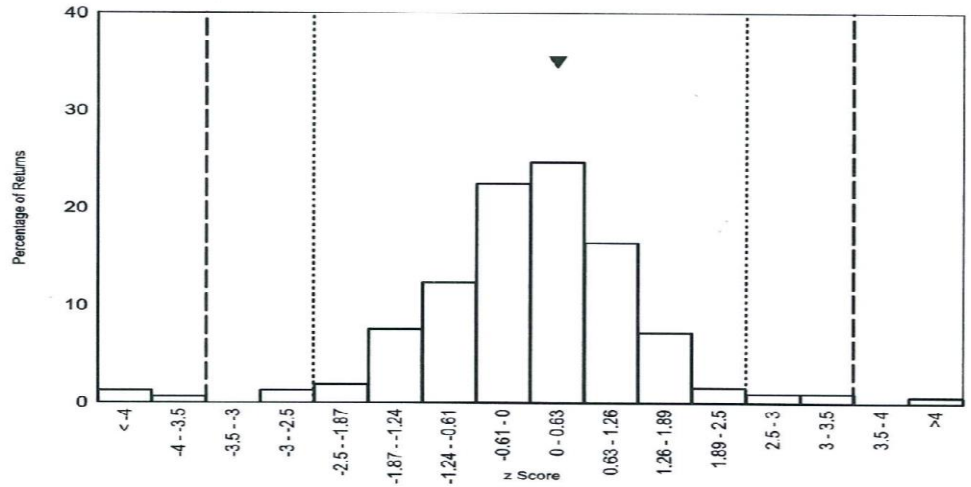
Please note the scale below is applicable to the tables above and to the z score histograms and Shewhart control charts that follow. It is <u>not</u> applicable to the Cusum control charts.



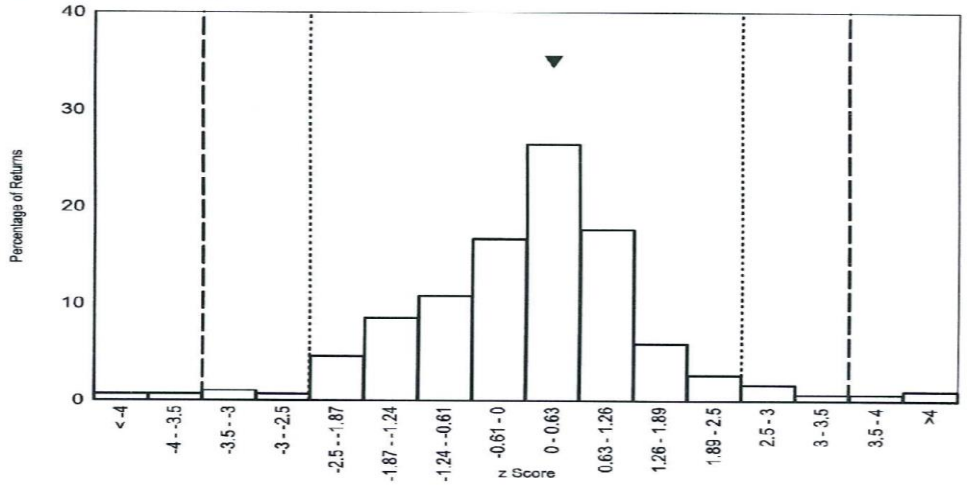
CD34+ Stem Cell Enumeration Programme

Histograms of Participant z Scores

Absolute Values (cells/µL)
Please note ▼ denotes your result



Percentage Values (%)
Please note ▼ denotes your result

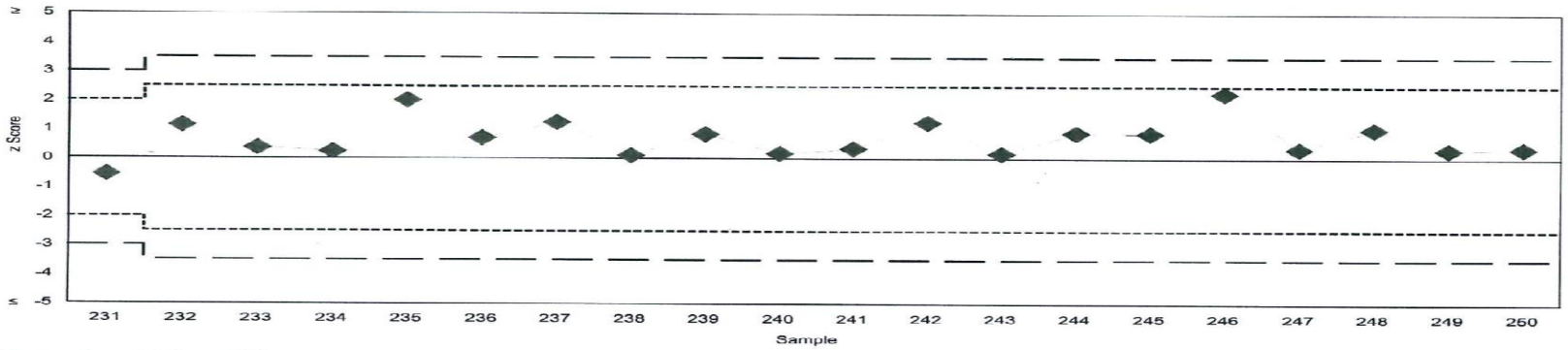


CD34+ Stem Cell Enumeration Programme

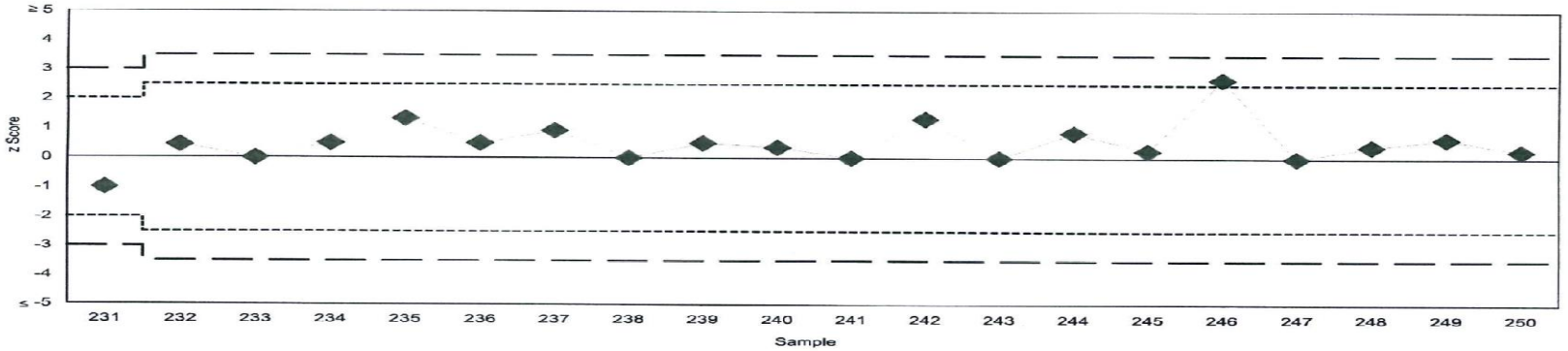
Shewhart Control Charts

(Please note each data point represents a single sample)

Absolute Values (cells/µL)



Percentage Values (%)

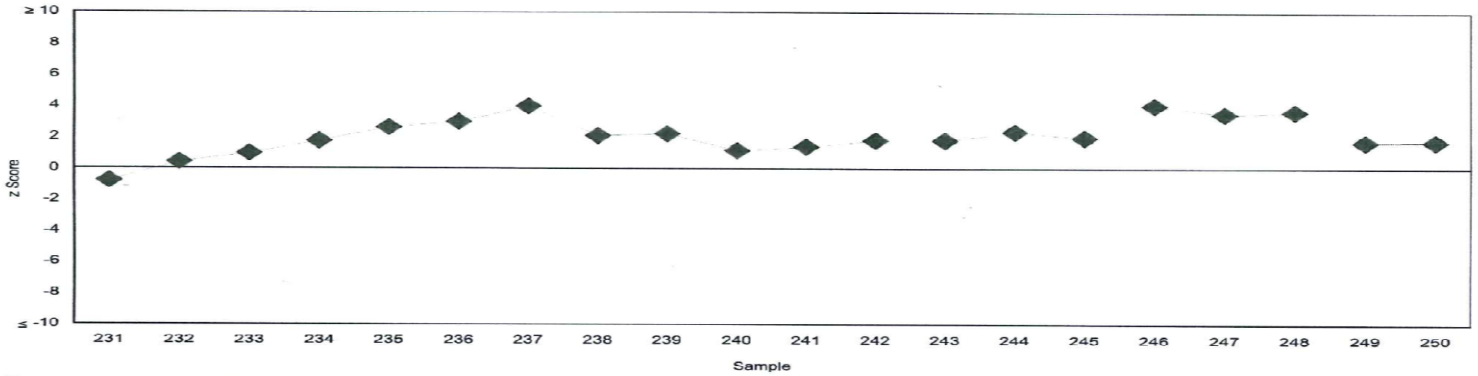


CD34+ Stem Cell Enumeration Programme

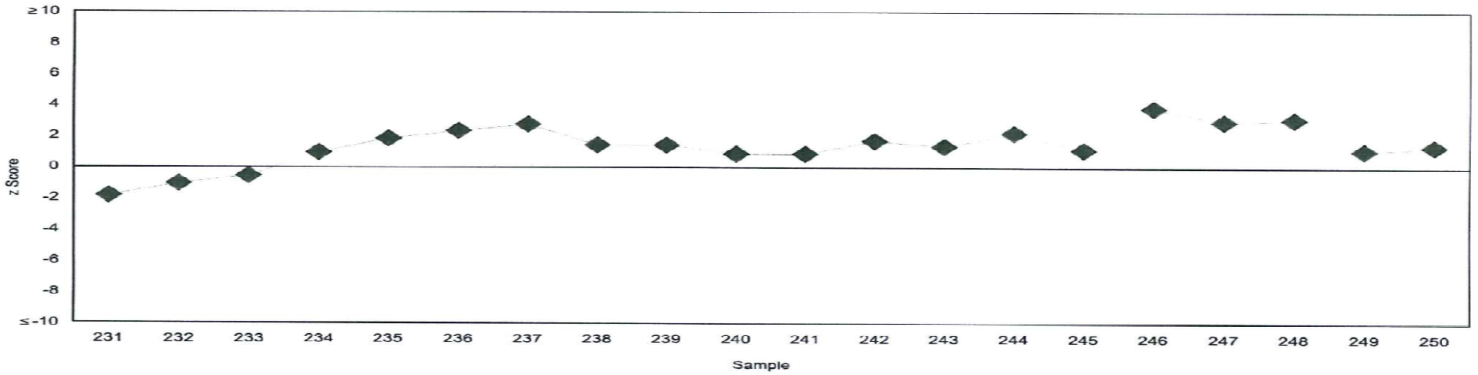
Cusum Control Charts

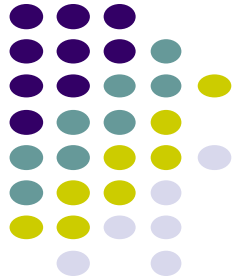
(Please note each data point represents the sum of the z scores of the current sample and the two previous samples)

Absolute Values (cells/µL)



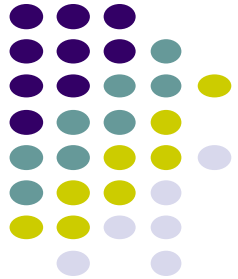
Percentage Values (%)





ESEMPI

Identificazione di cellule linfoidi B mature anomale



- Le cellule linfoidi B mature neoplastiche possono essere distinte dalle cellule normali mediante l'identificazione di 2 principali tipi di anomalie fenotipiche:

- restrizione di classe della catena leggera delle immunoglobuline

- espressione dell'antigene aberrante.

restrizione di classe della catena leggera delle immunoglobuline

- Contrariamente alla maggior parte delle popolazioni normali e reattive, le neoplasie delle cellule B mature di solito rappresentano un singolo clone di cellule che esprimono solo una classe di catene leggere Ig (cioè, kappa o lambda).

- Non si deve presumere che la limitazione della classe di catene leggere Ig sia sinonimo di monoclonalità o sia di per sé diagnostica della neoplasia.

- I risultati dell'immunofenotipizzazione FC devono essere interpretati insieme ad altri dati clinici, morfologici e talvolta genotipici.

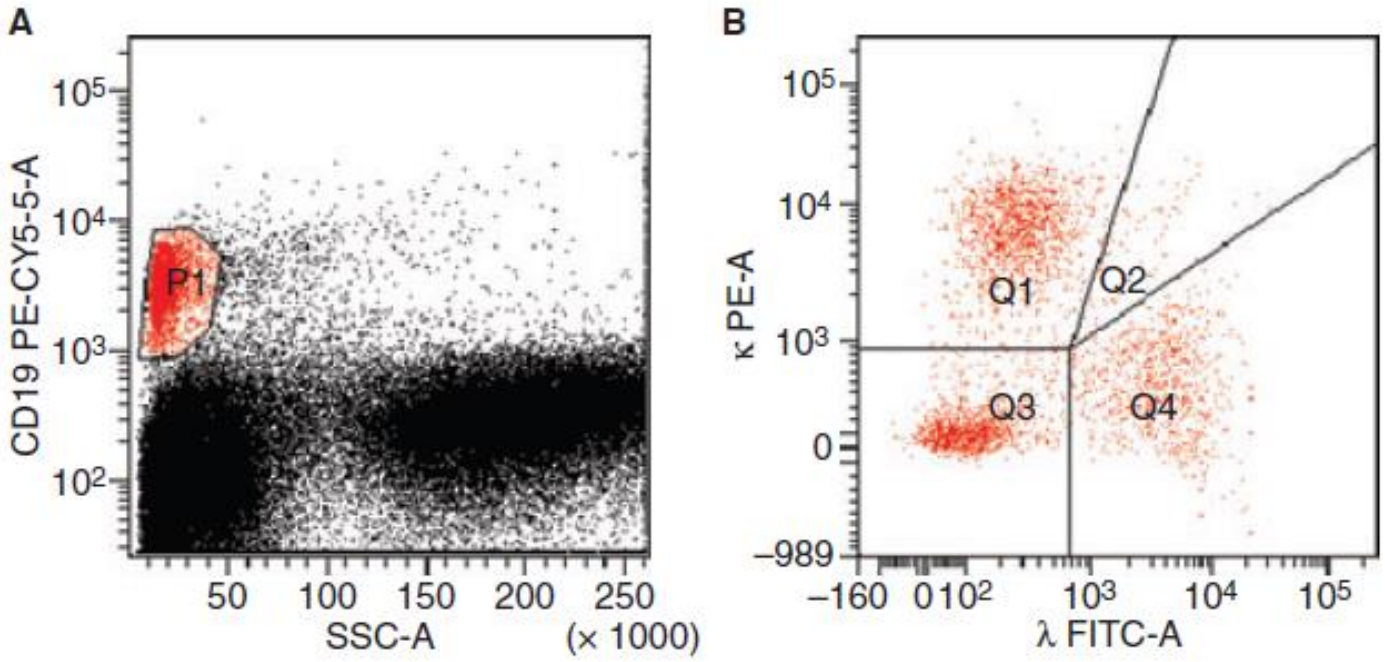
Disordini linfoproliferativi B-Cellulari

- Le cellule B normali/reattive sono policlonali
  - con rapporto κ/λ di 1,5 (range 0,9-3).

- Le neoplasie delle cellule B sono espansioni clonali di cellule B che esprimono solo un tipo di catena leggera Ig (k o λ).

- L'analisi dell'espressione della catena leggera nella popolazione totale di cellule B e nelle cellule positive CD5/CD19 o CD10/CD19 costituisce la base per la diagnosi del linfoma a cellule B.

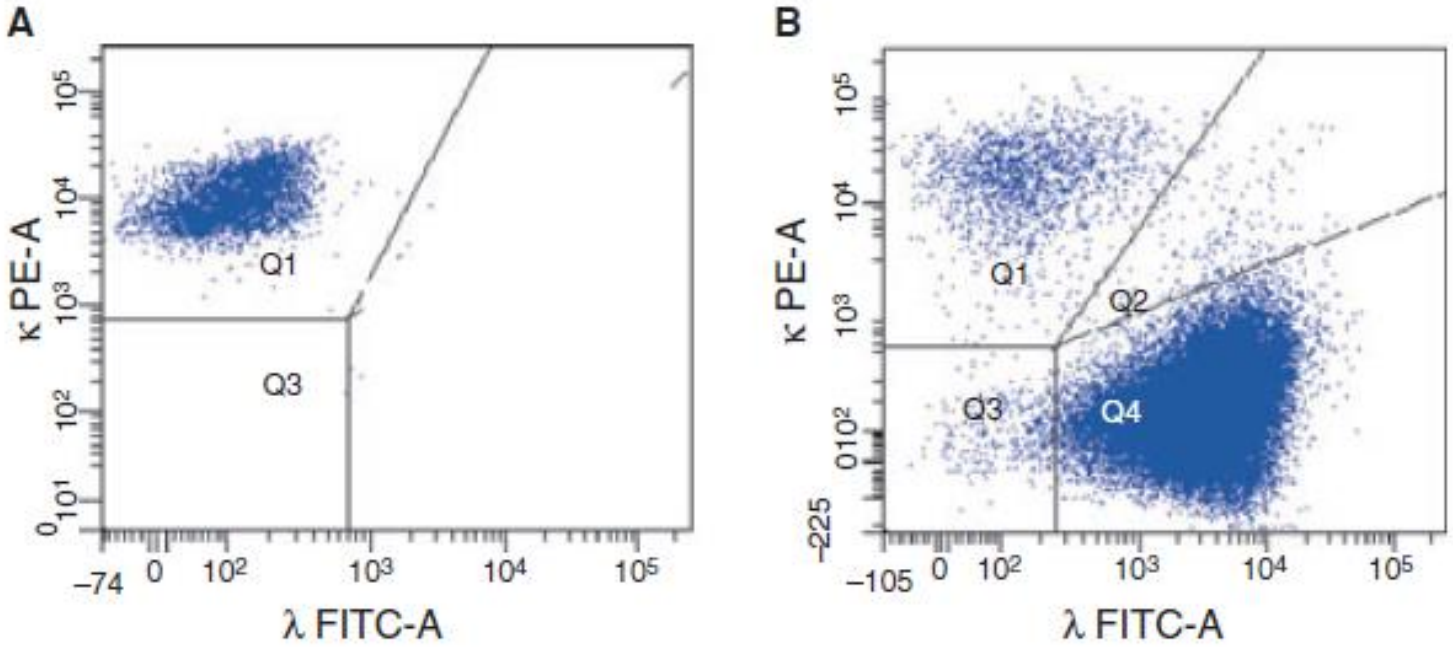
B-cells identified by gating the CD19+ events



CD19 is expressed at all stages of B-cell development from progenitor to plasma cell. Plot B shows that the gated cells do indeed consist of a mixture of Kappa positive and Lambda positive mature B-cells and surface immunoglobulin negative B-cell progenitors.

De Tute RM. Histopathology 2011, 58, 90–105

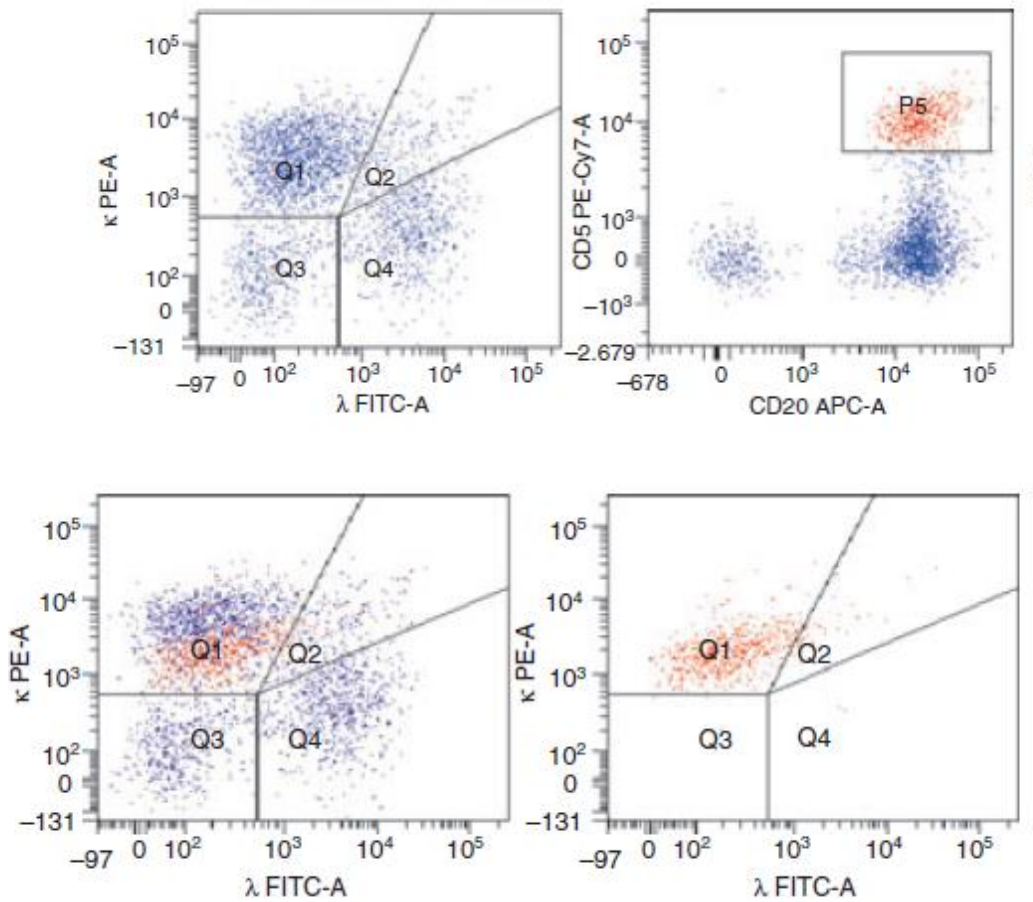
Monoclonal populations in samples of lymphomas



Plot A: a kappa positive neoplasm and no normal B-cells remain.
Plot B: although there are some normal polyclonal cells still present, there is still an obvious lambda positive population.

De Tute RM. Histopathology 2011, 58, 90–105

Small monoclonal populations can be hidden in a normal polyclonal background.



De Tute RM. Histopathology 2011, 58, 90–105

restrizione di classe della catena leggera delle immunoglobuline

- L'interpretazione della colorazione per catene leggere kappa e lambda Ig può essere resa più difficile dalla presenza di un legame non specifico.
  - Il legame non specifico (citofilo) degli anticorpi può verificarsi attraverso l'associazione con i recettori Fc e l'adesione dell'anticorpo alle cellule "appiccicose", comprese le cellule danneggiate o morenti.

- Il legame degli anticorpi alle cellule non B può essere escluso valutando solo le cellule che esprimono uno o più antigeni associati alla linea B:
  - ad esempio, eseguendo il gate su celle CD19 o CD20.

- Il legame non specifico può anche essere minimizzata mediante incubazione di cellule con un reagente bloccante come sieri immunitari prima della incubazione con anticorpi anti-catena leggera.

Examples of immunophenotypes for some of the most commonly seen mature B cell neoplasms

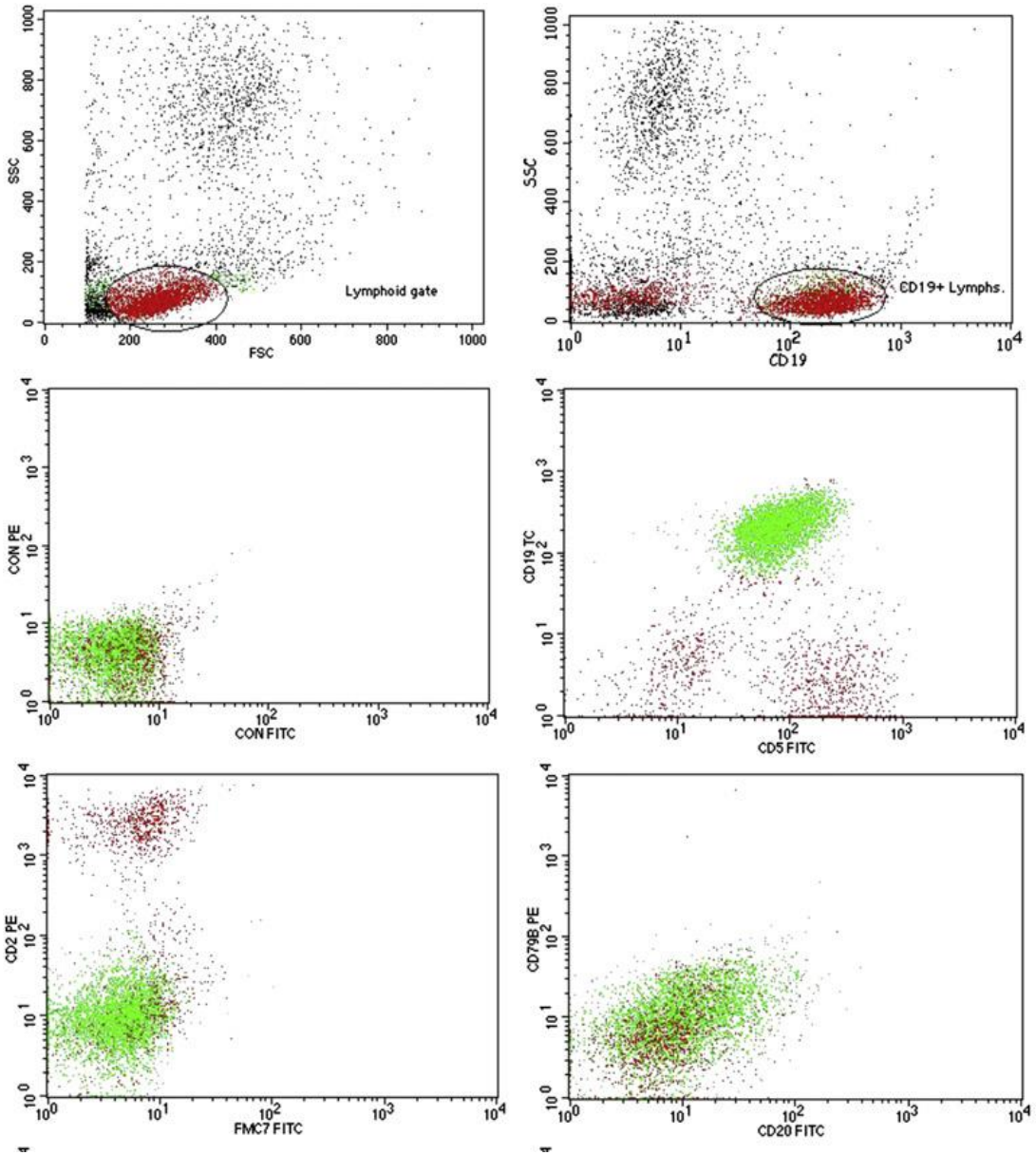
Disease entity	Typical phenotype	Atypical expression
Chronic lymphocytic leukaemia (CLL)	$CD19^{+}$, $CD20^{+}$ (weak), $CD5^{+}$, $CD81^{+}$ (weak), $CD79b^{-}$ (weak), $CD43^{++}$, $CD23^{+}$, $CD200^{+}$, $CD52^{++}$, $CD10^{-}$, $CD38^{variable}$, weak surface immunoglobulins such as kappa/lambda, IgM and IgD	Atypical cases can show weak or absent CD5 expression, lack of CD23, strong CD20 or combinations of the aforementioned
Hairy cell leukaemia (HCL)	$CD19^{++}$, $CD20^{++}$, very strong surface immunoglobulin, $CD22^{++}$, $CD103^{+}$, $CD25^{+}$, $CD11c^{+}$, $CD10^{-}$, $CD5^{-}$	Atypical cases can lack CD25 expression and are classified as variant HCL (vHCL). CD10 positivity can be seen in a significant number of individuals, with reported frequencies ranging from 10% to 26% of cases[18,51,52]
Mantle cell lymphoma (MCL)	$CD19^{+}$, $CD20^{+}$, $CD5^{+}$, $CD23^{-}$, $CD200^{-}$, $CD52^{++}$, $CD10^{-}$	Atypical cases can be CD5 negative and instances with CD23 and/or CD200 expression are not uncommon[20]
Follicular lymphoma (FL)	$CD19^{+}$ (weak), $CD20^{+}$, $CD10^{+}$, $CD38^{+}$, $CD43^{-}$	Atypical cases, reported as approximately 50% of samples,[10] can have weak or absent CD10 expression and the majority of these cases are high-grade[53]

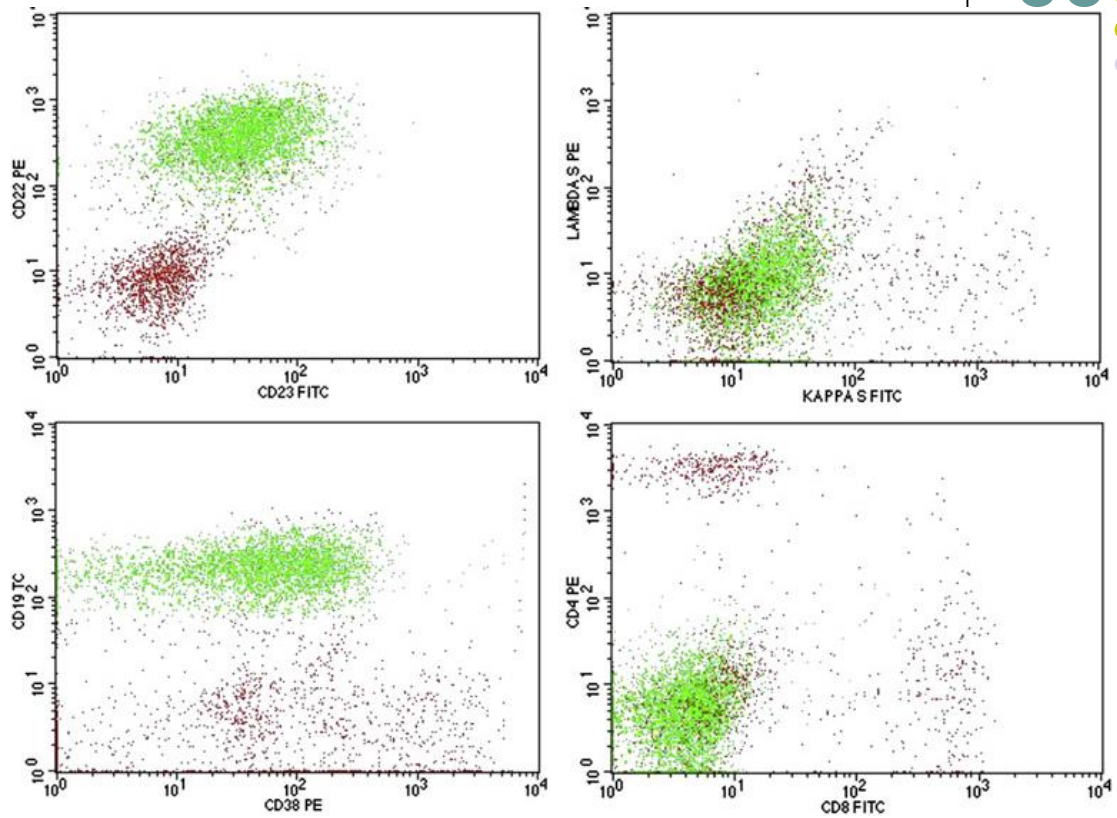
De Tute RM. Histopathology 2011, 58, 90–105

Leucemia linfatica cronica (CLL)

- L'immunofenotipo caratteristico della CLL comprende
  - positività per CD19, CD5, CD23 e CD200,
  - espressione debole di catene leggere CD20 e Ig e spesso espressione di IgM con o senza IgD.
  - FMC7 è negativo o solo parzialmente espresso nella maggior parte dei casi;
  - CD79b e CD22 sono assenti o debolmente espressi nella membrana cellulare.
  - CD11c, CD25 e altri marcatori che riconoscono le molecole di adesione sono variamente positivi in CLL.

FCM in CLL





FCM dot plots from a CLL gating on the CD19+ cell population. The majority of CD19þ cells are CD5+, CD23+, CD22+ and dim CD20, weak kappa+, and are negative with FMC7, CD79b and T-cell markers (CD2, CD4 and CD8). CD38 is strongly expressed in the CLL cells.

Matutes E, et al Best Practice & Research Clinical Haematology 23 (2010) 3–20

Immunophenotypic score (Score Matutes)

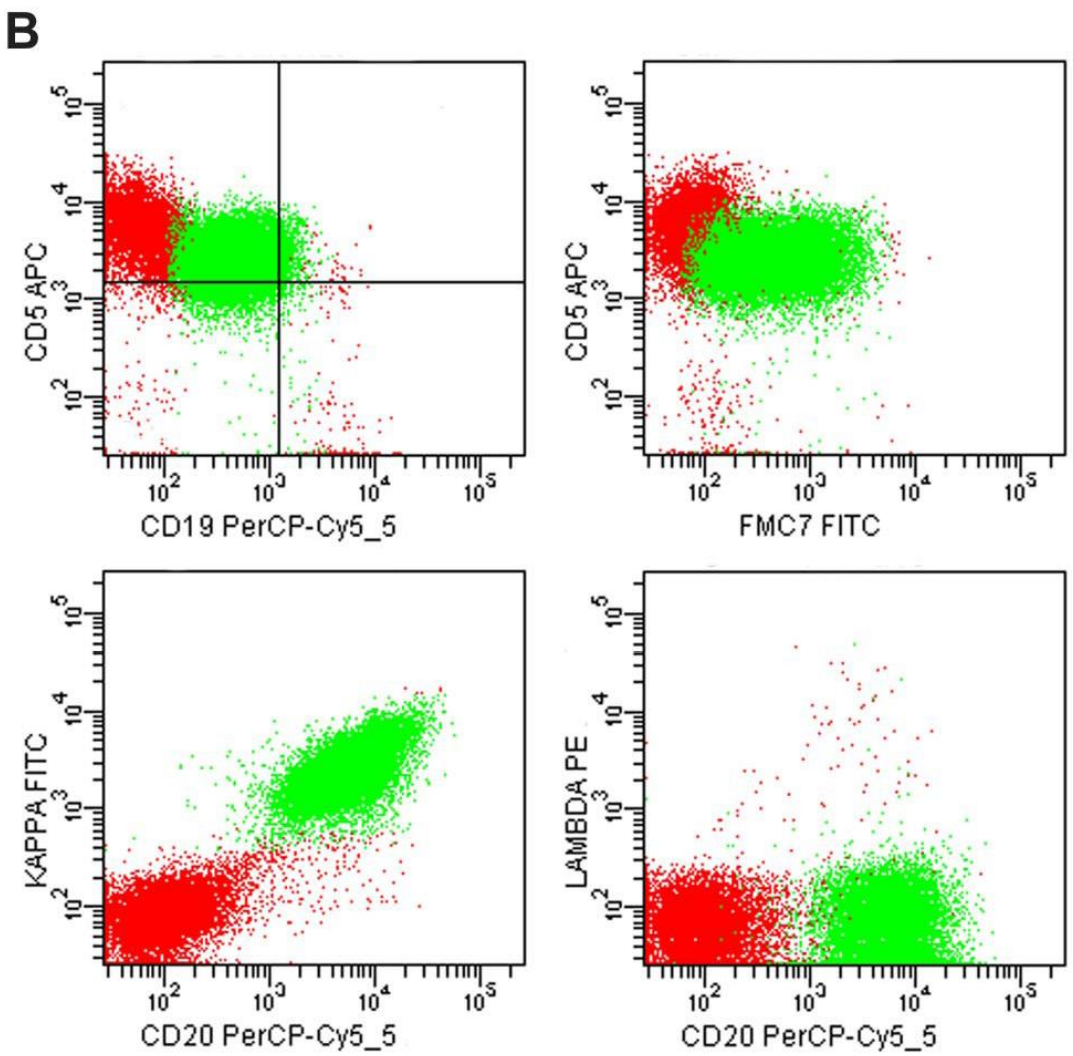
marker	Points	
	1	0
CD5	Positive	Negative
CD23	Positive	Negative
FMC7	Negative	Positive
sIg	Weak	Moderate/strong
CD22/CD79b	Weak/negative	Moderate/strong

Scores in CLL range from 3 to 5 while in the other B-cell disorders are 0-2
87% of CLL scored 5 and 4 and only 0.4% scored 0 or 1, whereas 89% of other B-cell leukemias and 72% of lymphomas scored 0 or 1; only one case (0.3%) scored 4 and none scored 5.

Matutes et al, 1994; Moreau et al, 1997

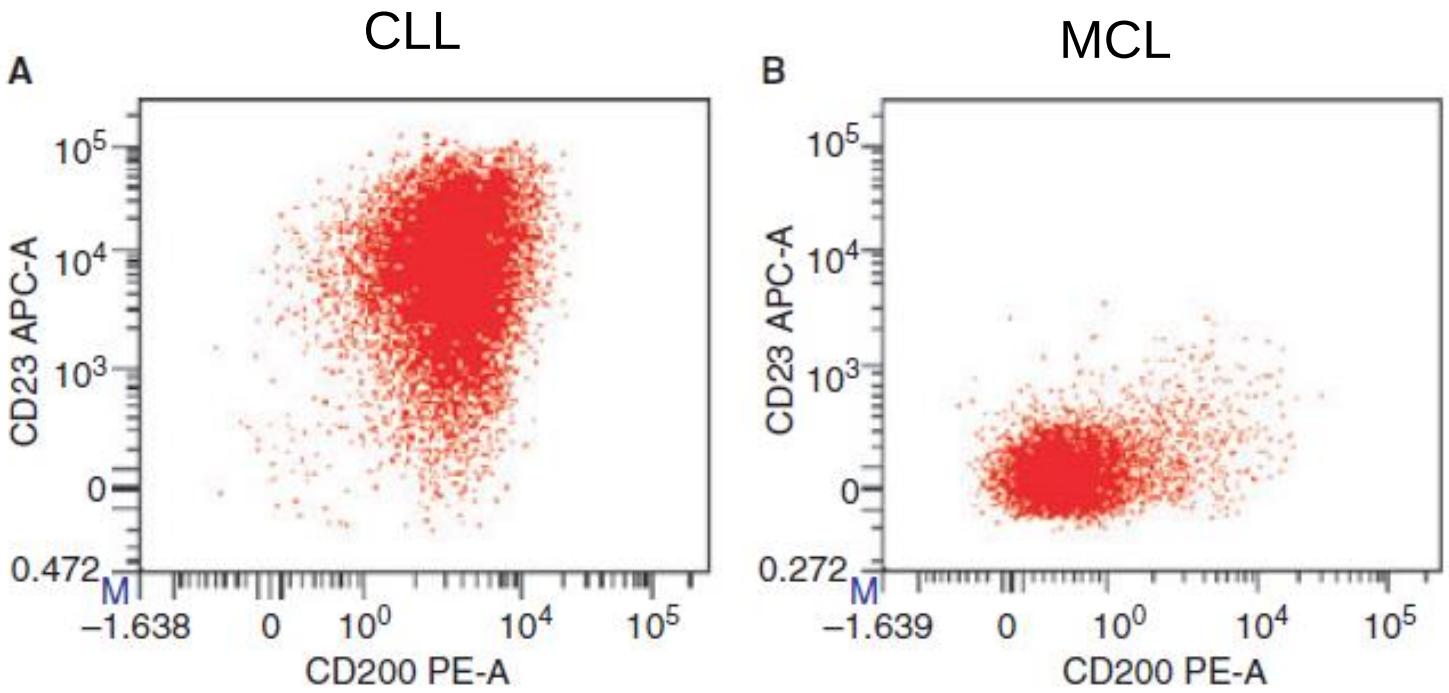
Mantle cell lymphoma.

Representative FC dot plots with population of interest highlighted in green: CD19 versus CD5 demonstrates CD5 B-cell population with weak intensity staining for CD19;
FMC-7 versus CD5 demonstrates positivity for FMC-7;
CD20 versus kappa and CD20 versus lambda demonstrate moderate intensity staining for CD20 and kappa immunoglobulin light chain restriction.
In addition, B cells were CD10- and CD23-.



Craig FE Foon KA. Blood 2008;111:3941-3967

Use of CD200 to discriminate CLL from MCL



Plot A shows gated B-cells from a BMA sample involved with CLL. The cells demonstrate expression of CD23 and CD200. Plot B displays B-cells from a patient with MCL. The cells have a typical MCL phenotype and are negative for both markers. CD200 is extremely useful in cases of MCL which exhibit atypical CD23 expression.

De Tute RM. Histopathology 2011, 58, 90–105

“CLL flow score” (simplified)

CLLflow score = %CD200^{+} + %CD5^{+}/CD23^{+} - %CD79b^{+} - %FMC7^{+}

- **If the CLL flow score is >0, a diagnosis of CLL is likely.**

- The CLLflow score showed
  - comparable sensitivity vs Matutes score.
  - markedly increased specificity (P < 0001).

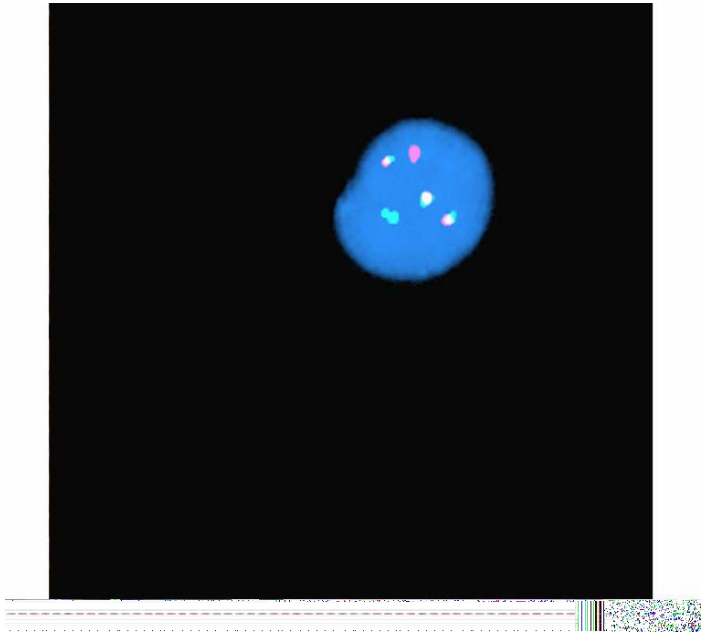
Matutes Score	non-CLL cases	CLL cases
0–2	**21 (53·8%)**	3 (1·4%)
3	12 (30·8%)	**12 (5·8%)**
4–5	6 (15·4%)	**193 (92·8%)**
	Specificity (53·8%)	**Sensitivity (98·6%)**

CLLflow Score	non-CLL cases	CLL cases
≤0	**34 (87·2%)**	6 (2·9%)
>0	5 (12·8%)	**202 (97·1%)**

Kohnke et al. BJH. 2017;179:480

Mantle cell lymphoma.

FISH demonstrating the *IGH/CCND1* [t(11,14)(q13;q32)] rearrangement. Hybridization with the LSI IGH/CCND1-XT dual color, dual fusion DNA probe demonstrates
- one green signal from the unrearranged chrom. 14q32,
- one red signal from the unrearranged 11q13,
- 3 fusion signals:
    - one from the derivative chrom 11,
    - one from the derivative chrom 14, and
    - an extra signal suggesting the presence of an additional copy of all or part of one of the derivative chromosomes involved in the *IGH/CCND1* rearrangement.

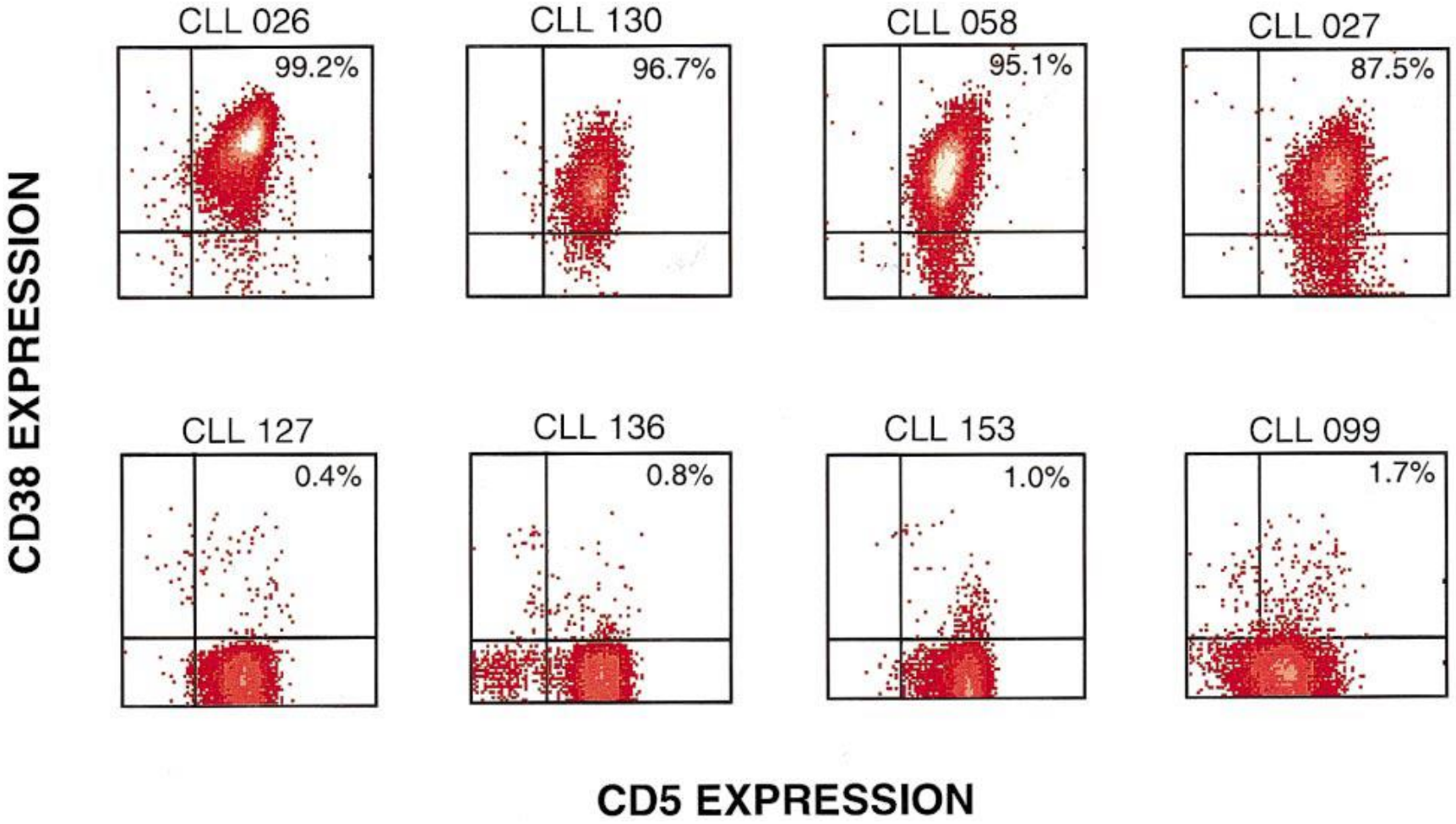


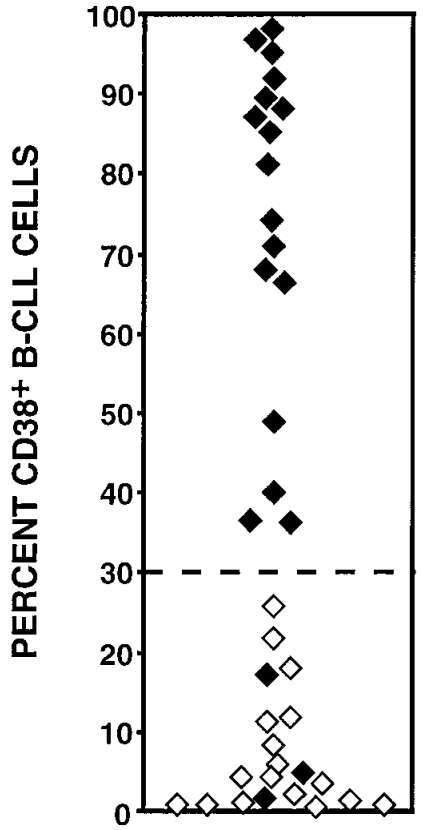
Craig FE Foon KA. Blood 2008;111:3941-3967

CD38 in CLL

- CD38 expression is an independent marker of a poor prognosis in CLL/SLL.
- Most studies use 30% as cut-off for positivity (in some studies 20%)
- The following factors can make determination of the percentage of CD38 cells difficult:
  - a spectrum of intensity for CD38 staining without clear distinction between positive and negative populations,
  - differences in intensity that derive from the fluorochrome,
  - bimodal staining with the presence of positive and negative cells in the same sample,
  - differences in staining between tissue sites such as PB and BM,
  - changes in CD38 expression during the course of the disease and with therapy.

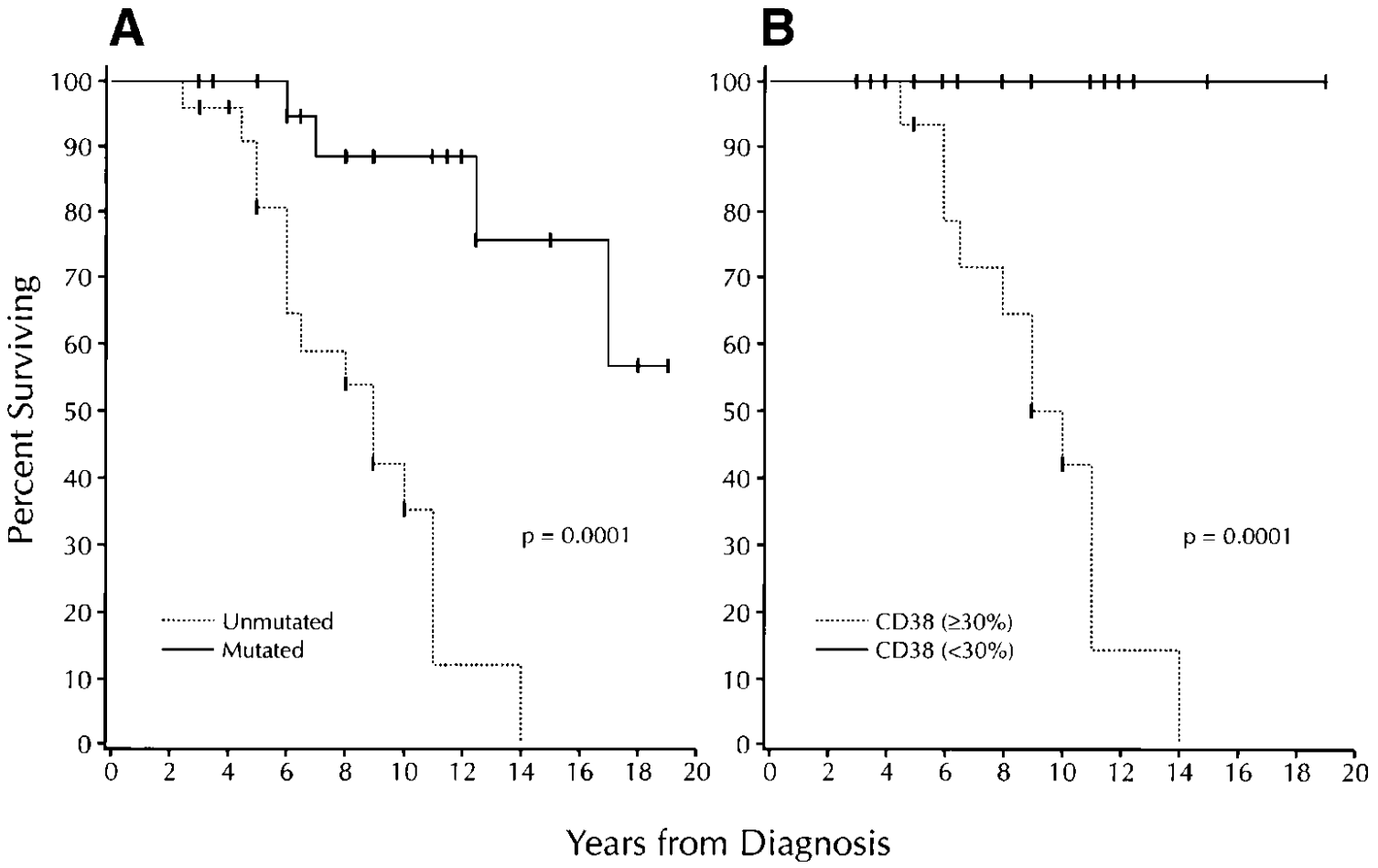
CLL and CD38





Damle RN et al, Blood 1999:94:1840

CLL: Survival according to CD38 and IGHV status



Damle RN et al, Blood 1999:94:1840

Minimal residual disease

MRD diagnostic tools in CLL: advantages and disadvantages

Method	Description	Advantages	Disadvantages
Flow Cytometry			
4-Color flow cytometry	Originally described by Rawstron et al[4]; uses standardized isolation, antibody combinations, and analysis[1,2,4,12]; of 50 antibody combinations tested, 3 were ultimately identified to have both low false-detection rates and interlaboratory variation (CD5/CD19 with CD20/CD38, CD81/CD22, CD79b/CD43)[4]	Commonly used, available; more rapid than consensus PCR[4]; does not require individual sequencing for primer creation[4]; 95% concordance with RQ-ASO IgH PCR at 10^{-4} detection level[4]	Less sensitive than PCR; interinstitutional differences in FLC approach may limit applicability[4]
Other FLC assays	6-Color FLC[13]; European Research Initiative on CLL 8-color FLC[14]; additional 8- and 10-color flow assays.[15,16]; FLC using CD160 surface antigen[17]	Improved sensitivity, efficiency; 6-color FLC shown to have 100% concordance with standardized 4-color assay at a level of 10^{-4}, but requires half the number of tubes[13]; 8-color ERIC FLC found to have detection level $<10^{-4}$ and acceptable correlation with the ISA standard ($R^2 = 0.99$)[18]	Less widely available

Thompson M et al. JAMA Oncol. 2018;4:394-400.

MRD diagnostic tools: advantages and disadvantages

Method	Description	Advantages	Disadvantages
PCR			
Consensus PCR	Uses clone-specific hypervariable complementary determining region 3 of IgH variable region[19]	Simple, rapid[19]	Limited sensitivity; results are not quantitative[19]
Nested clone-specific PCR[19]	Combines consensus IgH PCR and allele-specific primers to detect CLL cells	High sensitivity (10^{-6})[19]	Requires individual VH gene sequencing; results are not quantitative[19]
ASO *IGHV* PCR	Uses patient-specific primers[1]	Sensitive (10^{-5})[1]; Quantitative results	Time and labor intensive given need for patient-specific primers; decreased sensitivity compared with nested ASO PCR[1,19]
High-throughput sequencing	Current area of exploration in CLL research[14,20]; uses degenerate (not patient-specific) consensus primers followed by high-throughput sequencing to quantify MRD	Very sensitive level of (10^{-6})[21]; less time and labor intensive[20]	Less widely used[20]

ASO: allele specific oligonucleotide

Thompson M et al. JAMA Oncol. 2018;4:394-400.

Comparison of FLC techniques for MRD analysis	4 Color	≥6 Color
Percentage of patients applicable	>95%	>99%
Lower limit of quantification (LLOQ)	Confirmed 0.01% (10-4)	Reported 0.001% (10-5)
Approximate number of cells	500,000 events in 5 tubes ≥ 5 million cells	2 million events per tube ≥ 3 million cells
Lower limit of detection (sensitivity) (LOD)	Reported 0.005% (2 × 10-5)	Reported 0.001% (10-5)
Is the assay the same for every applicable patient?	YES	YES
Pre-treatment evaluation	Preferable	Preferable
Does the assay require fresh material	YES—samples must be <48h old and processed immediately	
Directly quantitative	YES—CLL cells are reported as a percentage of leukocytes	
Additional check for sample quality	NOT REQUIRED—identification of hematopoietic elements evaluated within the assay	
Harmonization	YES (ERIC)	
Independent prognostic factor for outcome in prospective clinical trial	PFS and OS	

Ghia P. Leukemia (2018) 32:1307–1316

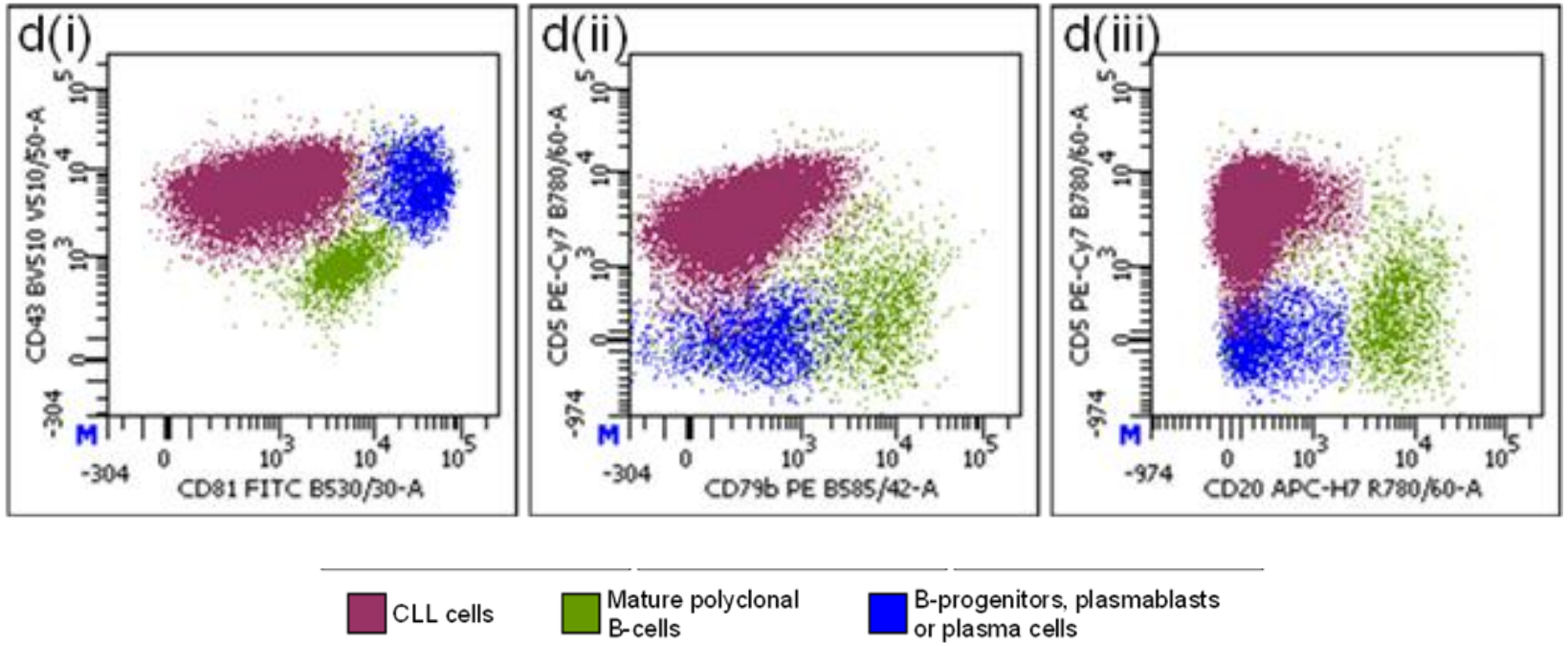
A complementary role of multiparameter FLC and high-throughput sequencing for MRD detection in CLL: an ERIC study

- The **primary aim** was to identify and validate in multiple centers a single-tube assay fulfilling the following conditions:
  1. reliable for MRD detection at the levels required by the IW on CLL guidelines.
  2. independent of instrument/reagent characteristics
  3. flexible enough to incorporate and validate new, additional markers in the future.

- The **secondary aim** was to explore the relative merits of the FLC assay and HTS to detect MRD.

Rawstron AC et al. Leukemia. 2016;30:929–936.

Panel definition: redundancy

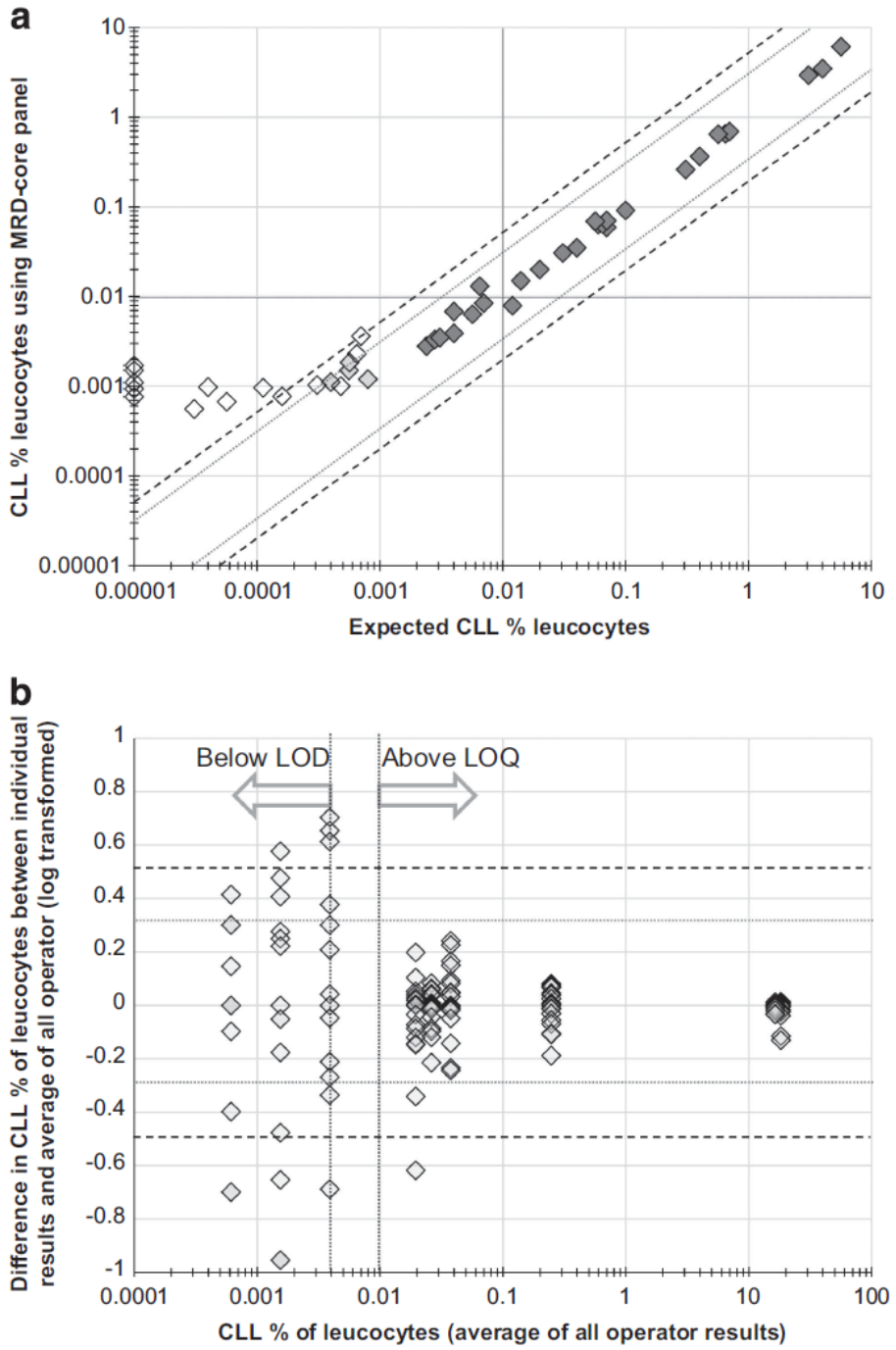


- **A core panel comprising six markers (CD19, CD20, CD5, CD43, CD79b and CD81) was defined as the most reliable and convenient.**
  - the inclusion of both CD20 and CD22 is redundant in cases with typical expression of ⩾2 markers CD5, CD79b, CD43 and CD81.
  - CD3 is not required in all cases

Rawstron AC et al. Leukemia. 2016;30:929–936.

Validation of the 6-marker core panel

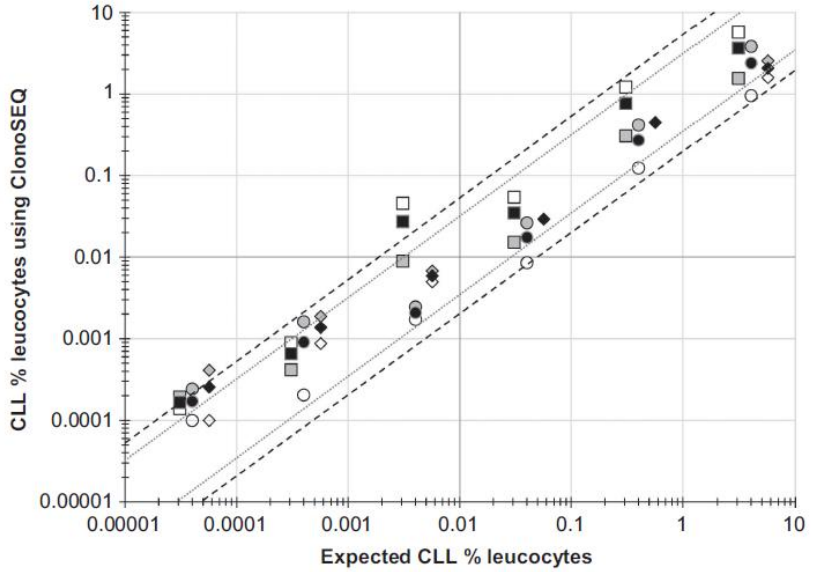
- **Good concordance between observed and expected CLL cell levels**
  - a limit of detection of 10^{-5}
  - a limit of quantification of 2.5×10^{-5}
- **Comparison with the 4-tube 4-color ERIC-harmonized panel**
  - Improved detection and quantification capabilities
  - Reduced acquisition time and amount of reagents
- **Acceptable interoperator variability.**

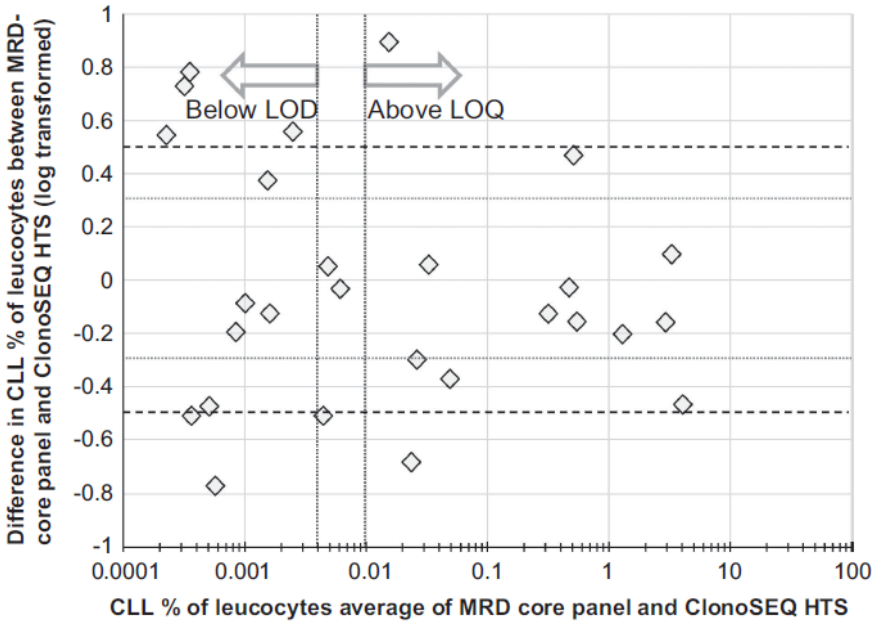


Rawstron AC et al. Leukemia. 2016;30:929–936.

Comparison between the 6-marker core panel and HTS

- **Good linearity to the 10^{-6} level.**
  - HTS detected CLL IGHV-D-J sequences in 22% samples with no detectable CLL cells by FLC.
- **There was acceptable (>90%) concordance at the 0.010% threshold.**
- **HTS demonstrated clear superiority in the limit of detection,**
  - there was a relatively high limit of agreement between the 2 techniques for data within the quantitative range (down to 0.010%/10−4).





Rawstron AC et al. Leukemia. 2016;30:929–936.

A complementary role of multiparameter FLC and HTS for MRD detection in CLL: an ERIC study

- The combination of both technologies would
  - permit a **highly sensitive approach to MRD** detection
  - provide a **reproducible and broadly accessible method** to quantify MRD and optimize treatment.

Rawstron AC et al. Leukemia. 2016;30:929–936.

2018 Recommendations regarding the response assessment in CLL

Diagnostic test	General practice	Clinical trial
History, physical examination	Always	Always
CBC and differential count	Always	Always
Marrow aspirate and biopsy	At cytopenia of uncertain cause	At CR or cytopenia of uncertain cause
Assessment for minimal residual disease	NGI	Desirable
Ultrasound of the abdomen*	Possible, if previously abnormal	NGI
CT scans of chest, abdomen, and pelvis	NGI	Recommended if previously abnormal and otherwise with a CR and PR

For a detailed description of these parameters, see section 5. General practice is defined as the use of accepted treatment options for a CLL patient not enrolled on a clinical trial.

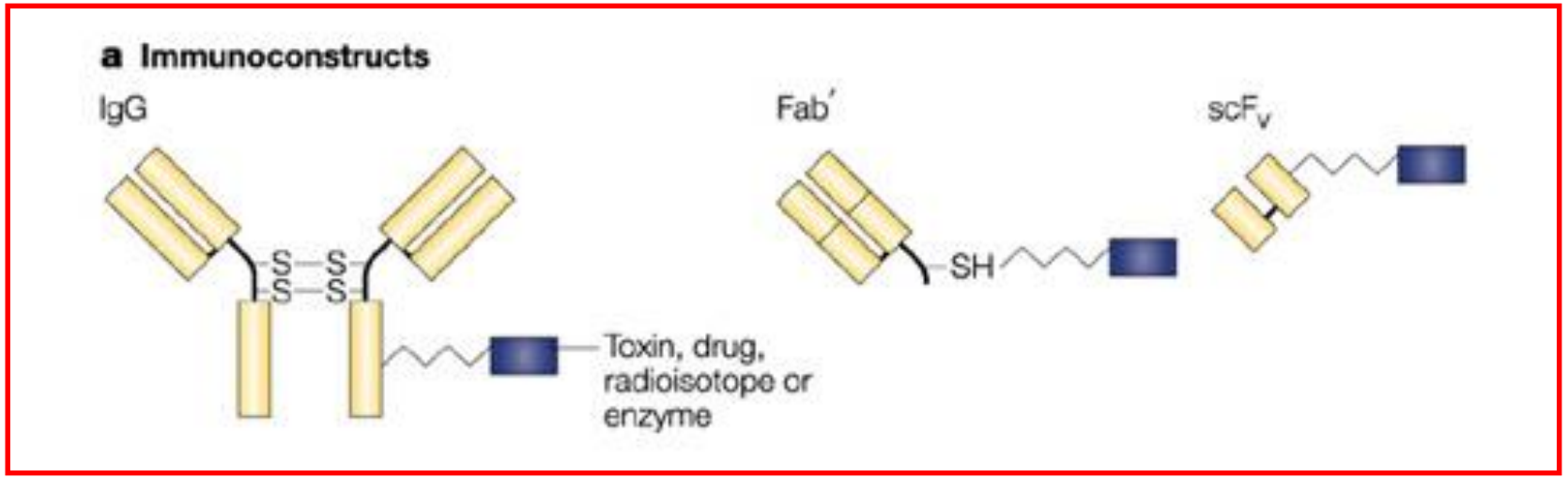
*Used in some countries to monitor lymphadenopathy and organomegaly.

Hallek et al. Blood. 2018;131(25):2745-2760)

TARGET TERAPEUTICO

Gemtuzumab Ozogamicin (GO): Mylotarg

- Anticorpo monoclonale umanizzato anti-CD33 legato covalentemente con la caliceamicina
- Caliceamicina: un derivato semisintetico di un potente antibiotico antitumorale che si inserisce nella struttura del DNA causando rotture nella struttura a doppia elica e determinando così la morte cellulare



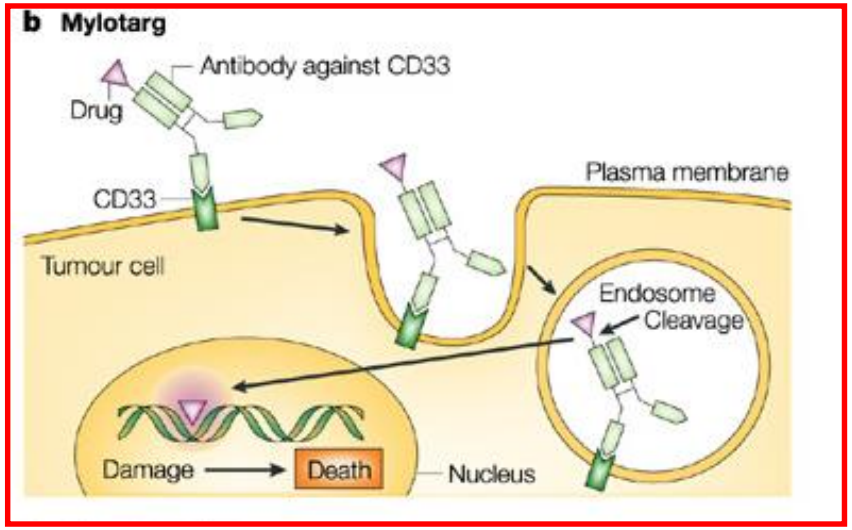
GO: Target

Target: CD33

- L’antigene CD33 è una proteina glicosilata transmembranaria (funzione sconosciuta) espressa:
  - sulle cellule mieloidi mature ed immature
  - sulle cellule eritroidi e megacariocitarie
  - sulla maggior parte delle cellule staminali emopoietiche ma non su quelle più immature
  - è poco espresso al di fuori il sistema emopoietico
- L’antigene CD33 è espresso in più del 90% delle LAM e delle sindromi mielodisplastiche

GO: modalità di azione

- Dopo il legame con l'antigene, GO è internalizzato mediante endocitosi.
- Il legame tra l'AtcMo e la caliceamicina viene scisso all'interno dei lisosomi dalle idrolasi acide, con conseguente rilascio della caliceamicina
- La caliceamicina liberata esercita la propria azione a livello del DNA con attivazione della apoptosi mdiata dalla p53



Applications

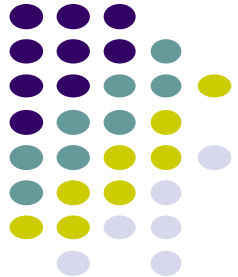
Cell Sorting

- Some flow cytometers are capable of physically separating the cells (fluorescence activated cell sorter, FACS) based on differences in any measurable parameters.

- Sorting is achieved by droplet formation.

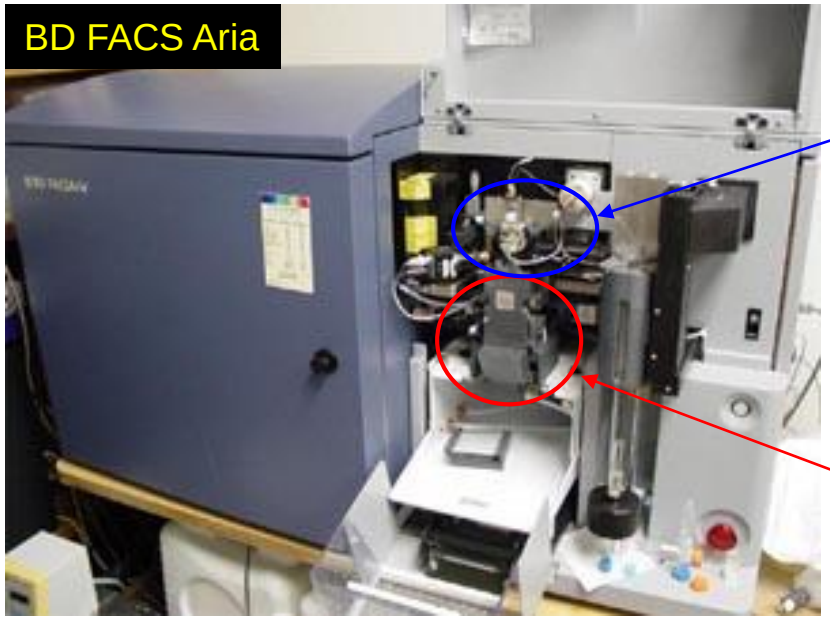
- The basic components of any sorter are:
  1. A droplet generator
  2. A droplet charging and deflecting system
  3. A collection component
  4. The electronic circuitry for coordinating the timing and generation of droplet-charging pulses

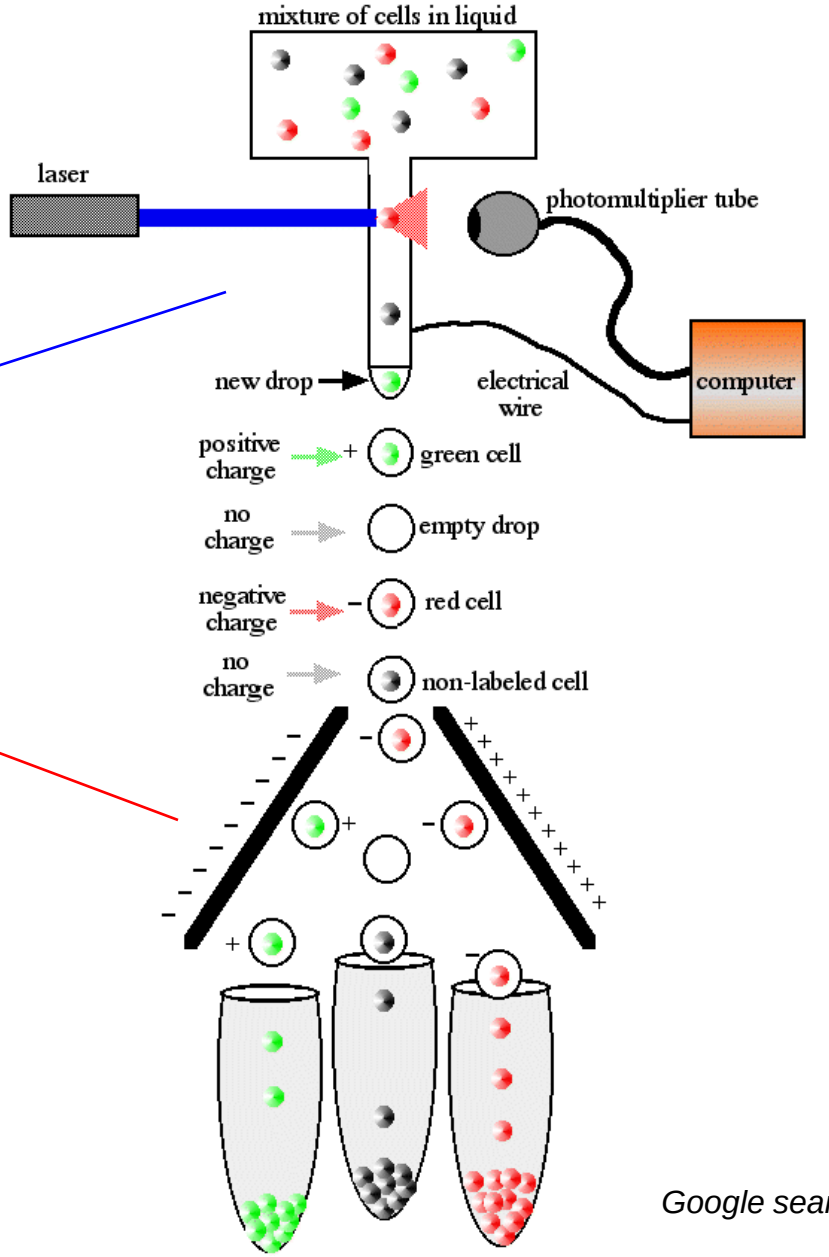
Cell Sorting



- The flow chamber is attached to a piezoelectric crystal, which vibrates at a certain frequency so that when the fluid carrying the cells passes through the nozzle, forming a jet in air with a velocity of 15 m/s, the vibration causes the jet to break up in precisely uniform droplets, approximately 30,000 to 40,000/s.
- Each droplet, when separated from the jet, can be charged and deflected by a steady electric field and is collected in a receptacle.
- Almost every cell is isolated in a separate droplet.
- When the cell is analyzed a sorting decision is made, and until the proper electrical charge pulse is applied to the droplet containing the cell, there is a transit time determined by several factors, such as flow velocity, droplet separation, and the cell preparation. If two cells cannot be separated the sorting is aborted.

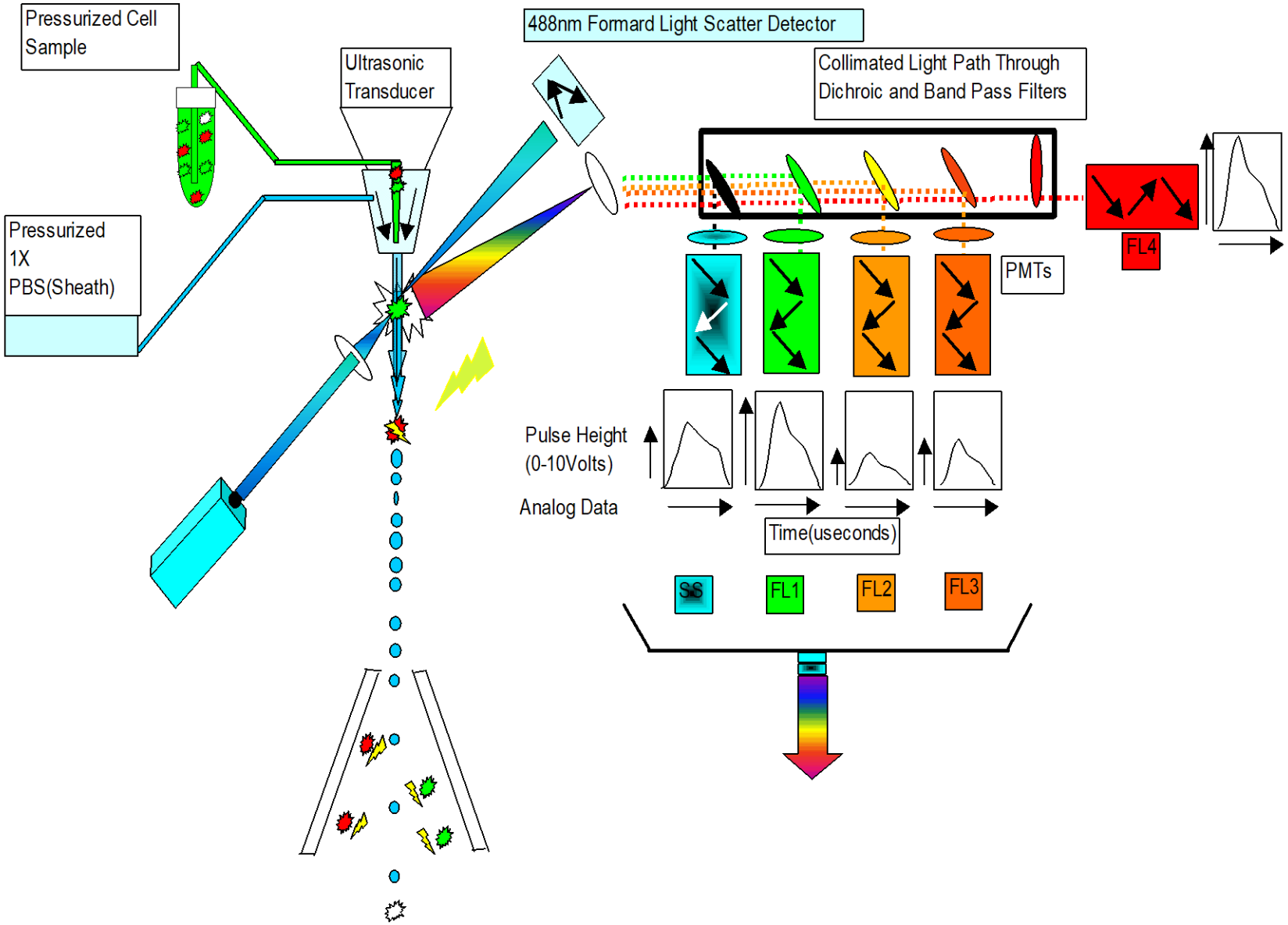
FACS sorting





Google search

Flow Cytometry and sorting



Ab-coated Magnetic Beads

Positive selection

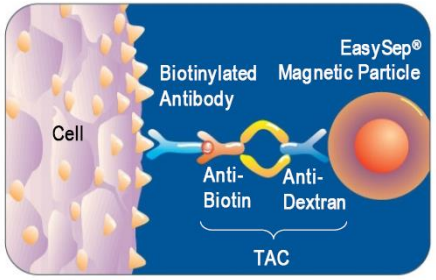
excellent purity (rare cell enrichment) and recovery

negative selection

removal of unwanted cells

if no specific Ab is available for target cells

if binding of the Abs to the target cells is not desired (activation, suppression)



TAC; bispecific tetrameric Ab complex

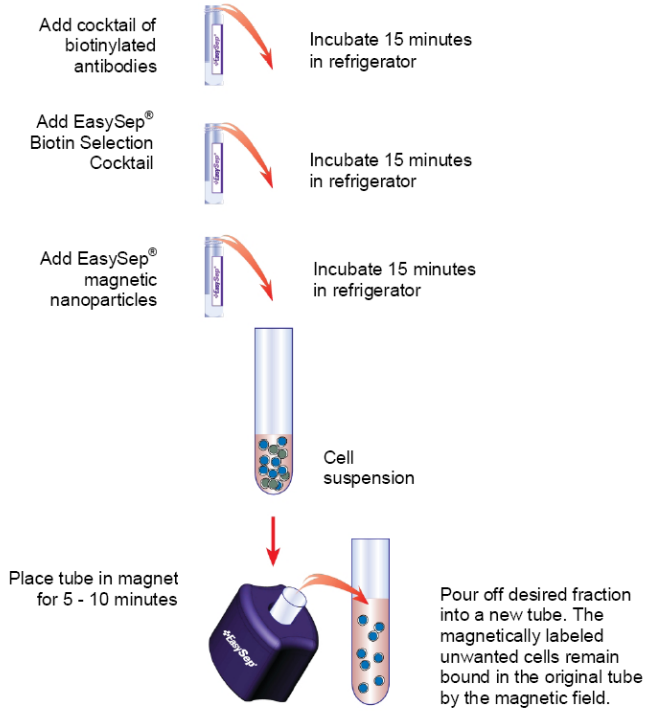
Commercial available sources for magnetic beads

Stemcell technologies, Miltnyi Biotec (MACS),

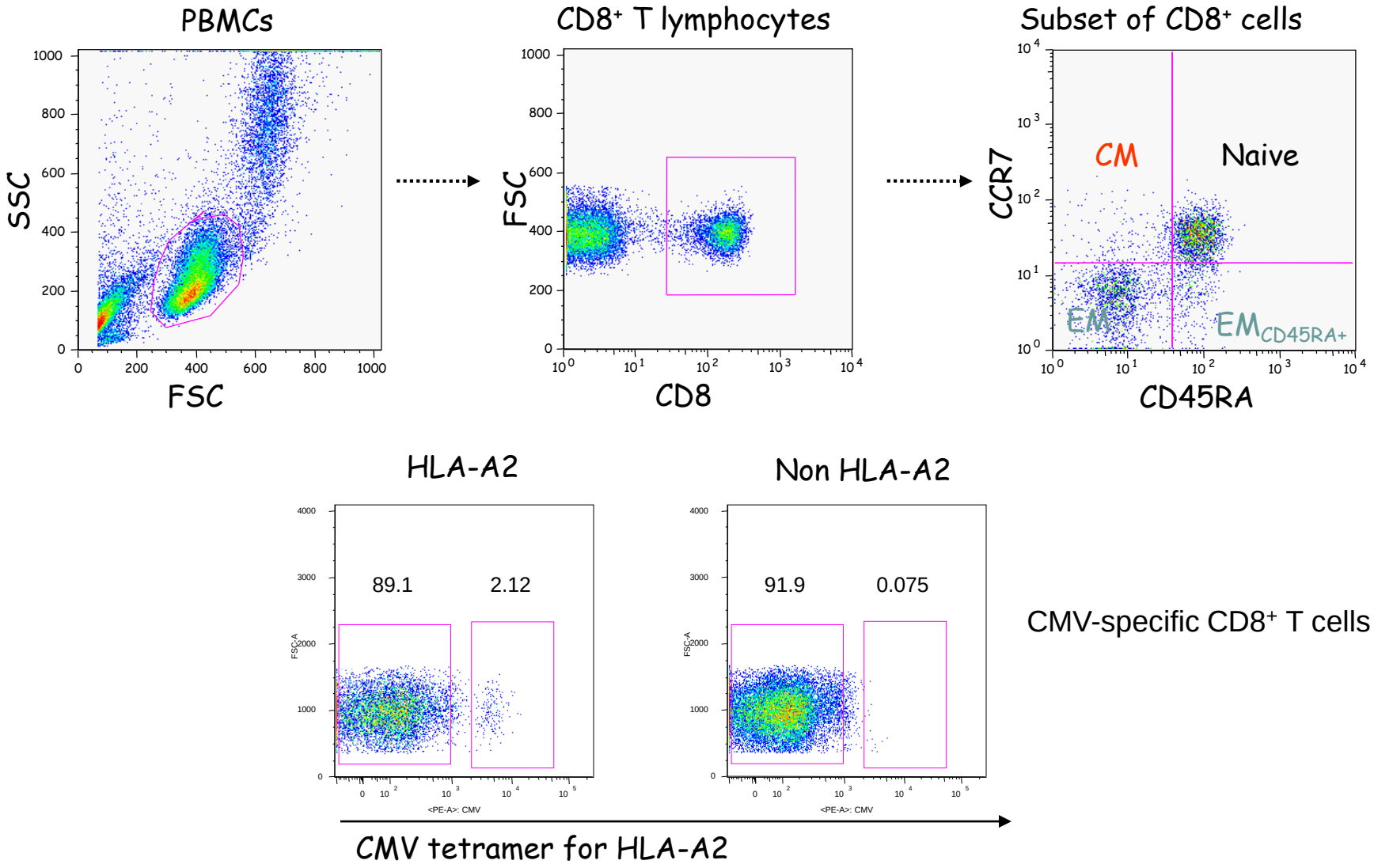
Dynal, Proimmune *etc*

Note for positive selection;

1. MACS magnetic beads are biodegradable and typically disappear after a few days in culture.
2. Because EasySep magnetic particles (~150 nm) are tiny, they do not interfere with downstream application.
3. In case of Dynal superparamagnetic beads (2.8 um), there is a step for separating magnetic beads.

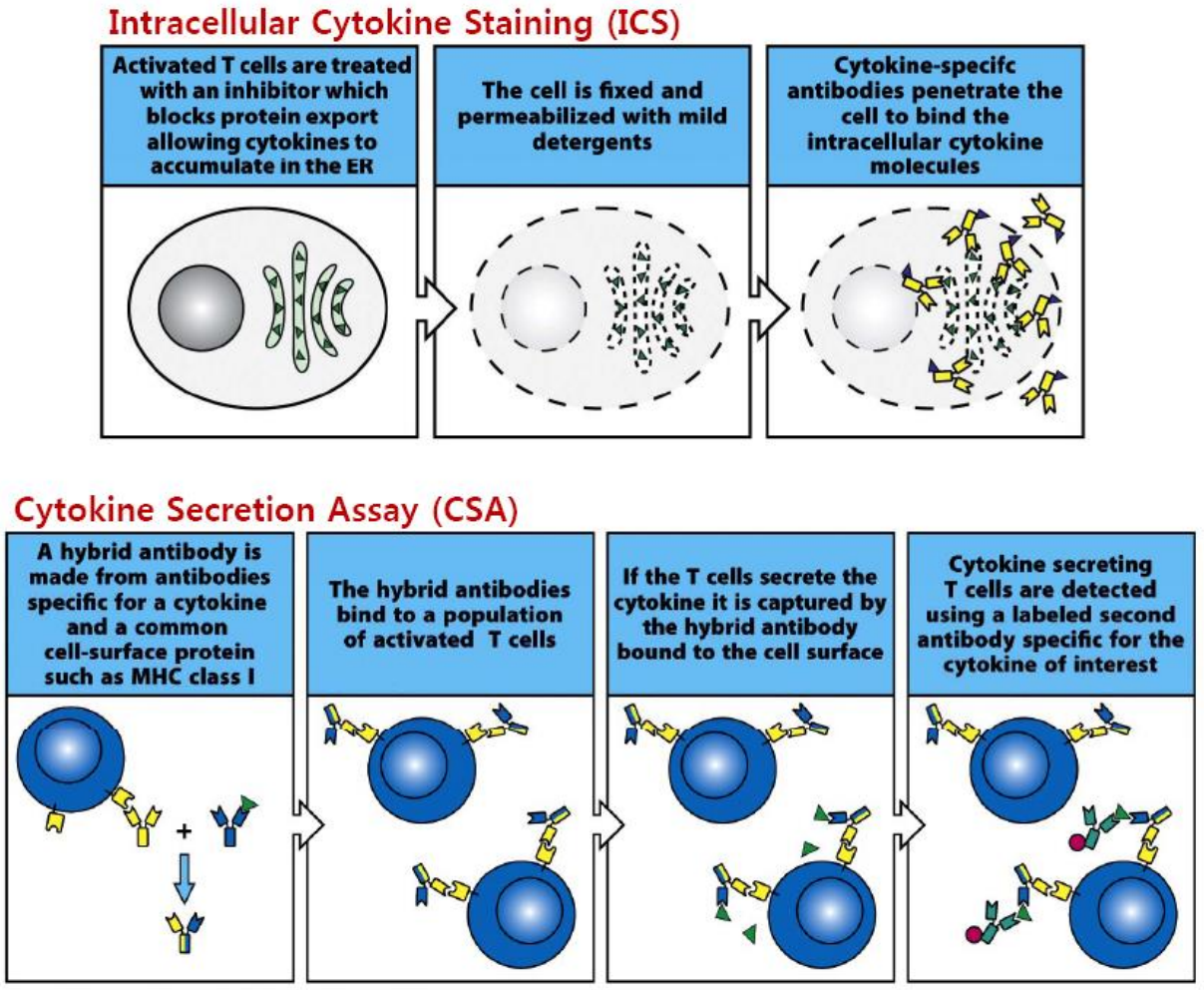


1. Surface phenotype, Ag-specific T cells



CMV-specific CD8⁺ T cells

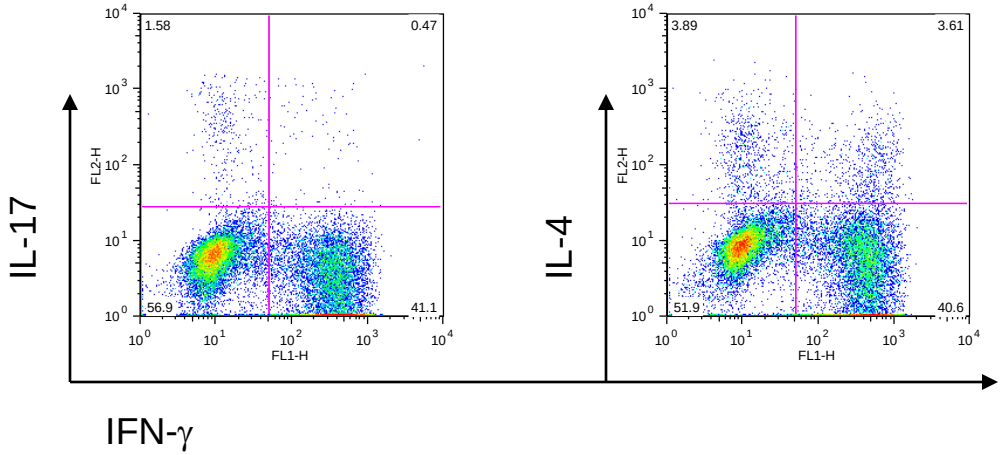
2. Cytokine productions



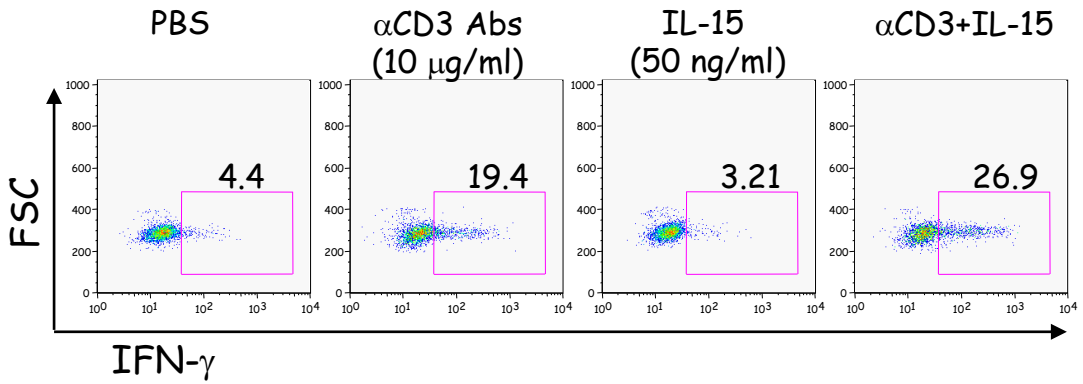
Fixative; PFA
Perm; Sapoinin, PEG (BD Perm II solution for human)

Adapted from KAIST (Shin, EC)

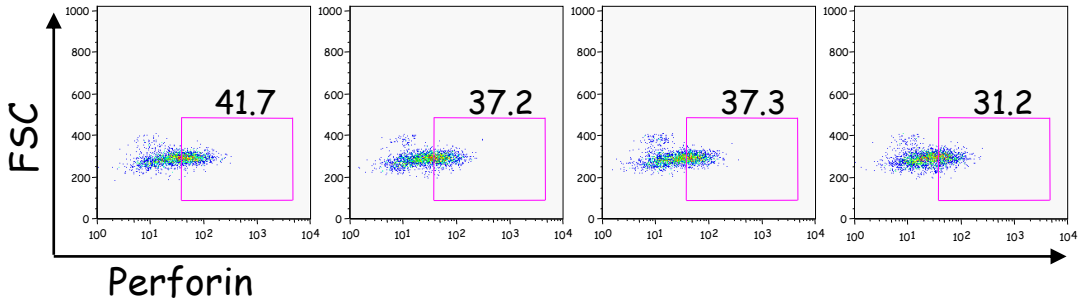
Representative cytokine staining



Sort CD4⁺ cells from PBMC
Stimulate cells with PMA/ION
in the presence of GolgiStop®
Fix and Perm with BD buffer
Stain cells with Abs against IFN-γ, IL-17 and IL-4



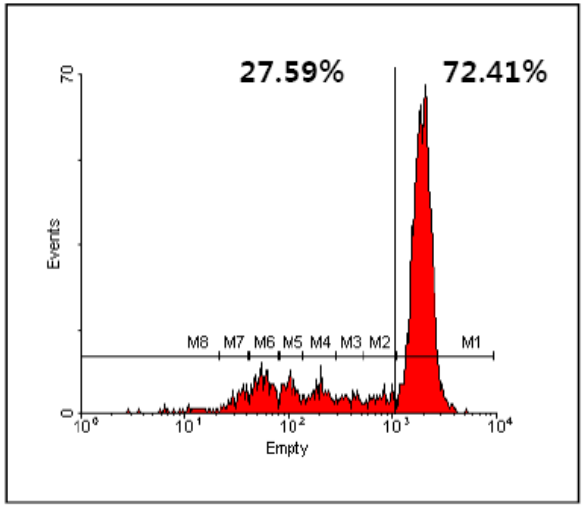
+ αCD28 (2 μg/ml)
+ αCD49d (2 μg/ml)



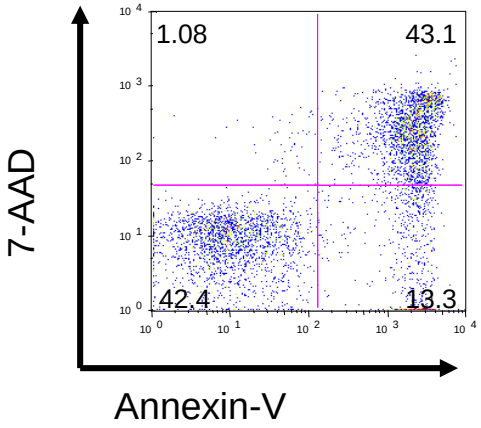
Stain cells with Abs for surface Ags
Stimulate cells with indicated cytokine and/or Abs
in the presence of Golgiplug®
Fix and Perm with BD buffer
Stain cells with Abs against IFN-γ and perforin

3. Cell proliferation, Cell cycle, Apoptosis

CFSE; cell proliferation



Apoptosis



Cell Cycle

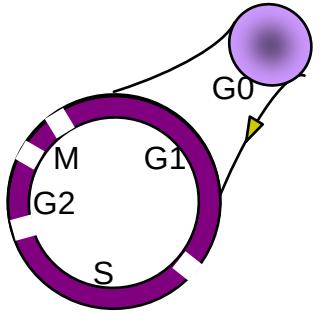
Go : 2n
(Gap0) resting state

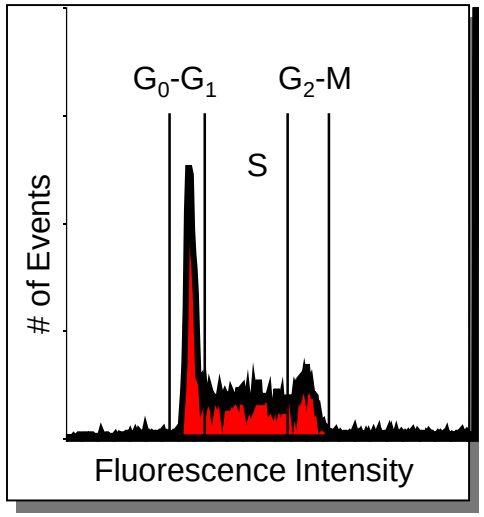
G1 : 2n
(Gap1) RNA & protein synthesis to prepare for S phase

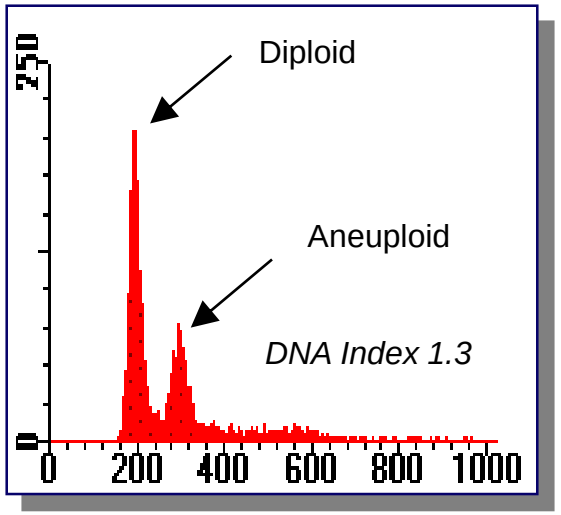
S : 2n~4n
(Synthesis) DNA Synthesis

G2 : 4n
(Gap2) RNA & protein synthesis before cell division

M : 4n
(Mitosis) preparation for daughter cell production







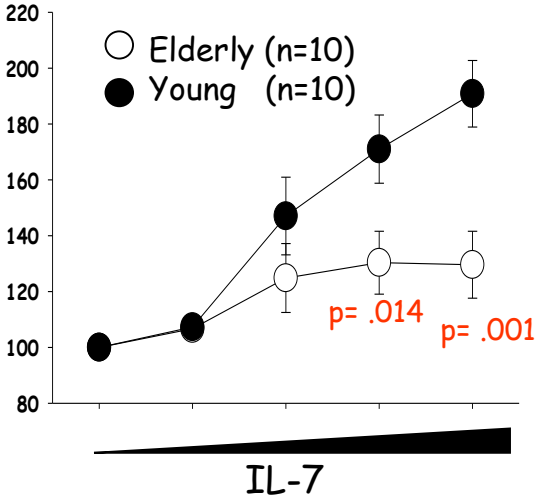
Adapted from BD biosciences

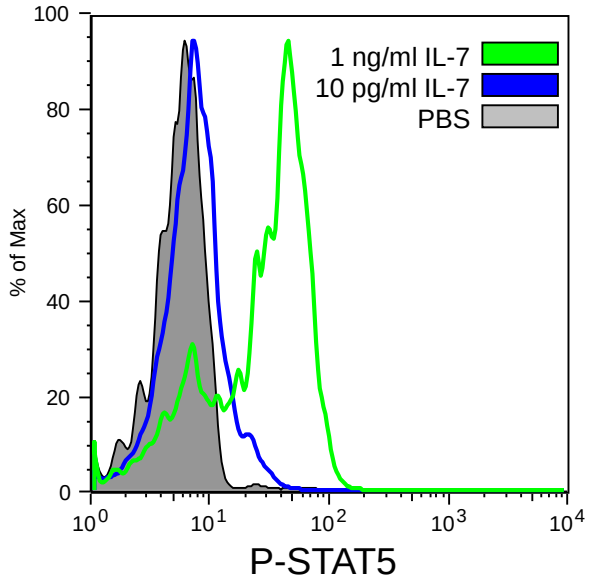
4. Intracellular protein

Phospho protein;

p-STAT1, p-STAT5
KINASES (p38 MAPK,P44/42 MAPK, JNK/SAP).
Members of cell survival pathways (AKT/PKB)
T cell activation pathway (TYK2)
p-ERK

Granzyme, Perforin

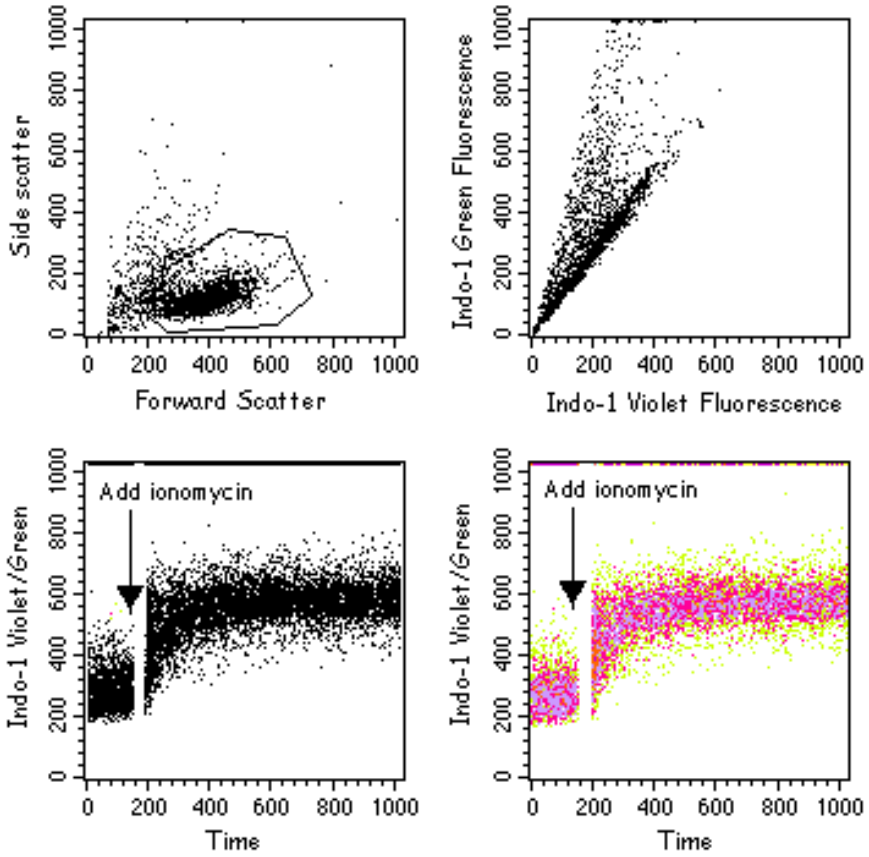


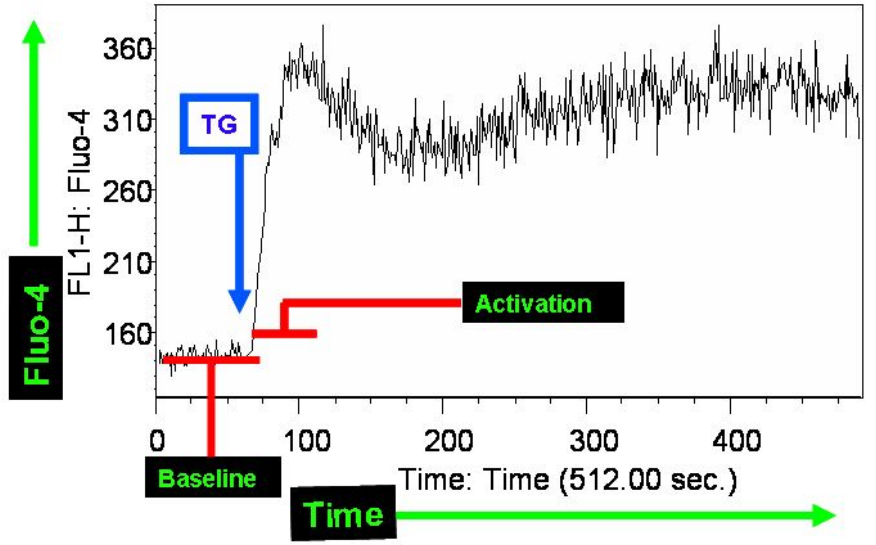


Stain cells with Abs for surface Ags
Stimulate cells in the presence
or absence of IL-7
Fix with 2% formalin
Permeabilize with 90% methanol
Stain cells with Abs for p-STAT5

Fixative; PFA
Perm; Methanol

5. Intracellular Calcium





Legend. Jurkat T-cells were loaded with 1 μM Fluo-4 for 45 min at 37°C and adjusted to 1 x 10^6/ml in calcium free PBS. After a 30 second baseline was collected, thapsigargin (Tg) (5 μg/ml) an endoplasmic reticulum (ER) ATPase inhibitor was added. The subsequent release of internal stores of calcium from the ER into the cytoplasm was detected by Fluo-4 (activation phase) before moving to miotchondria.

UV (em 390_violet & 500_green)
Indo-1

488 (blue laser)
Fluo-4

Google search