Critical Role of Flow Cytometry in Stem Cell Transplantation and Cellular Therapy Laboratories

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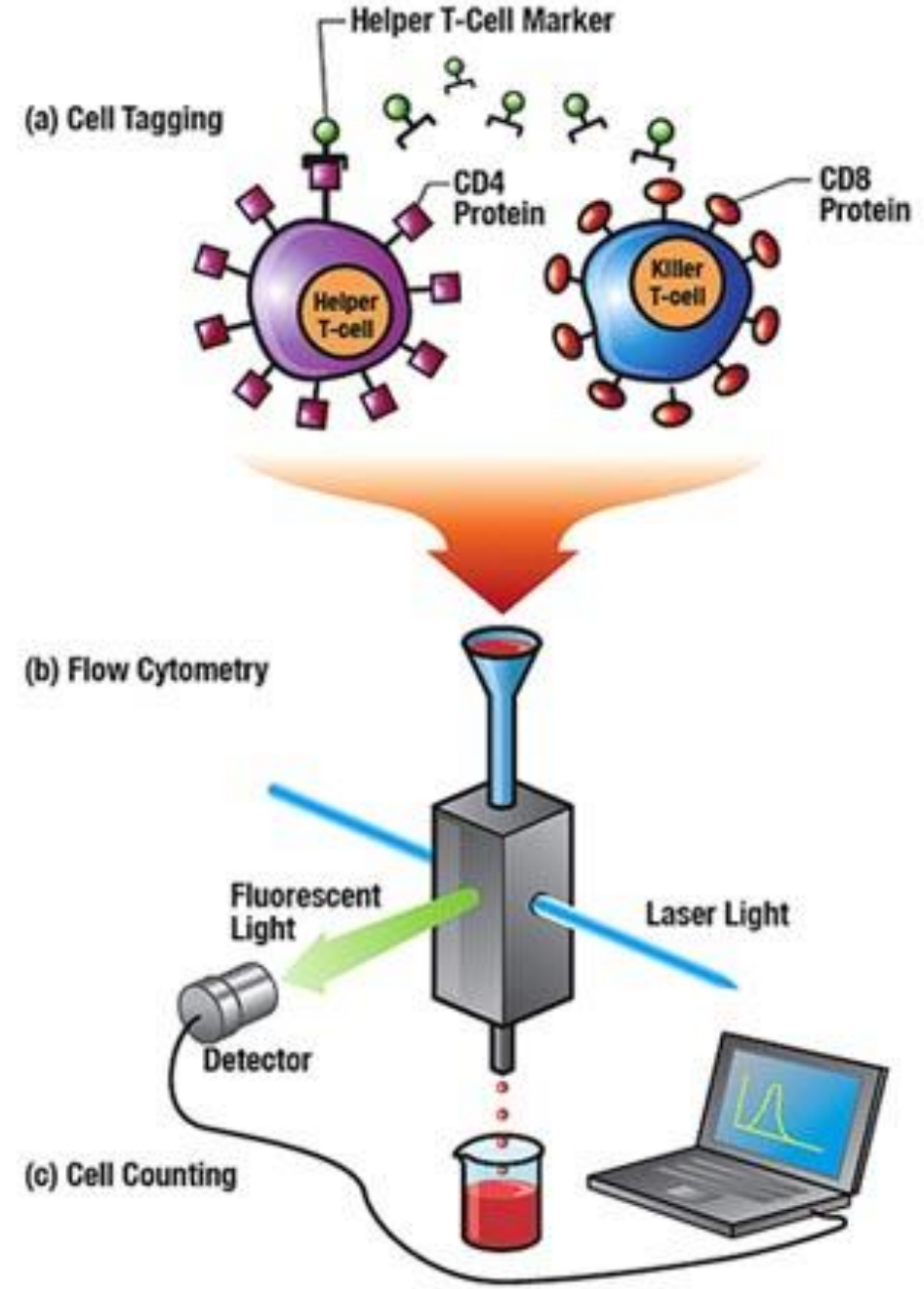
Retired January 2019

Applications in Flow Cytometry

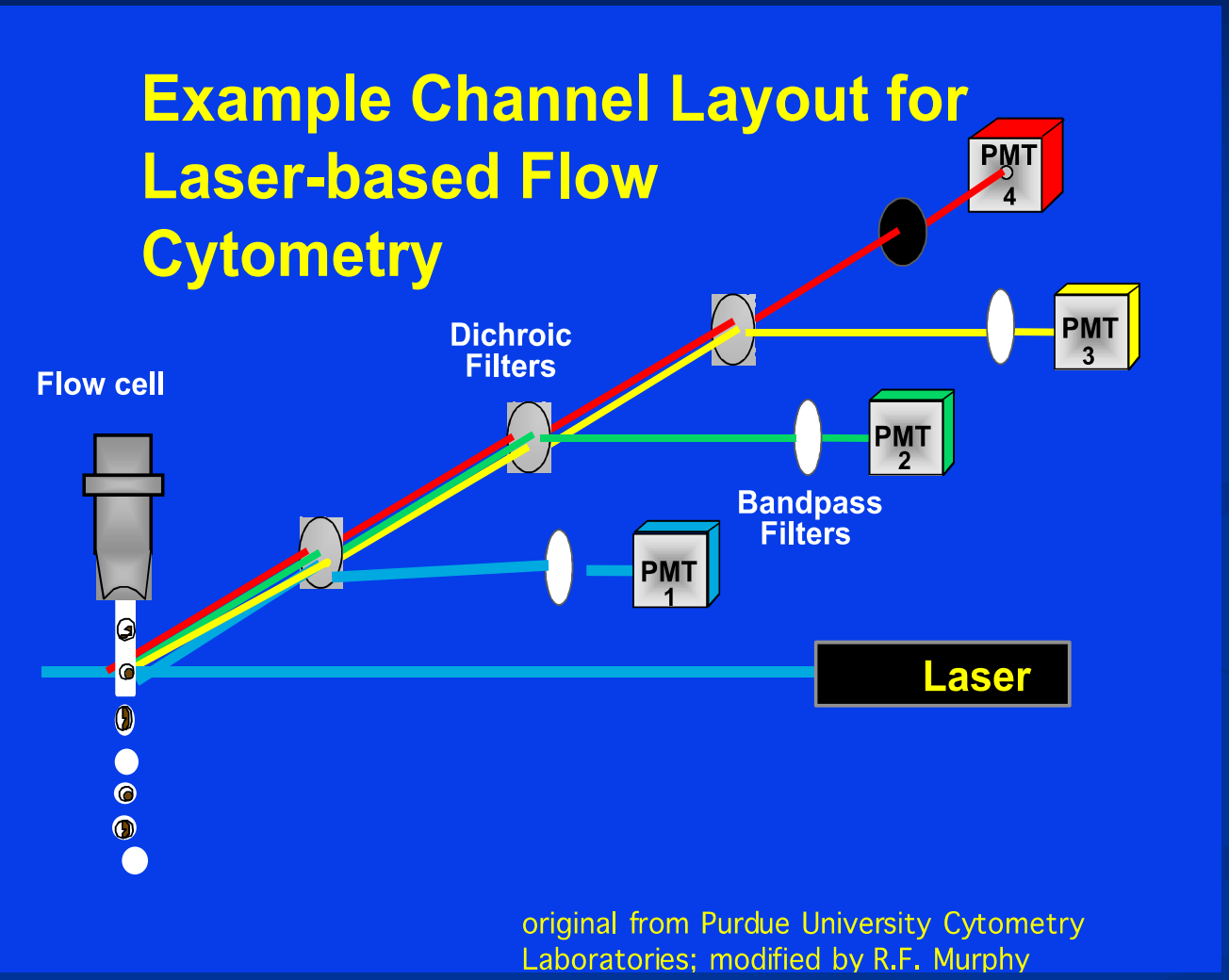
Virtual Talk: 28 March 2022



Basics of a Flow Cytometer:

An automated Fluorescence Microscope

FLOW CYTOMETRY - 101

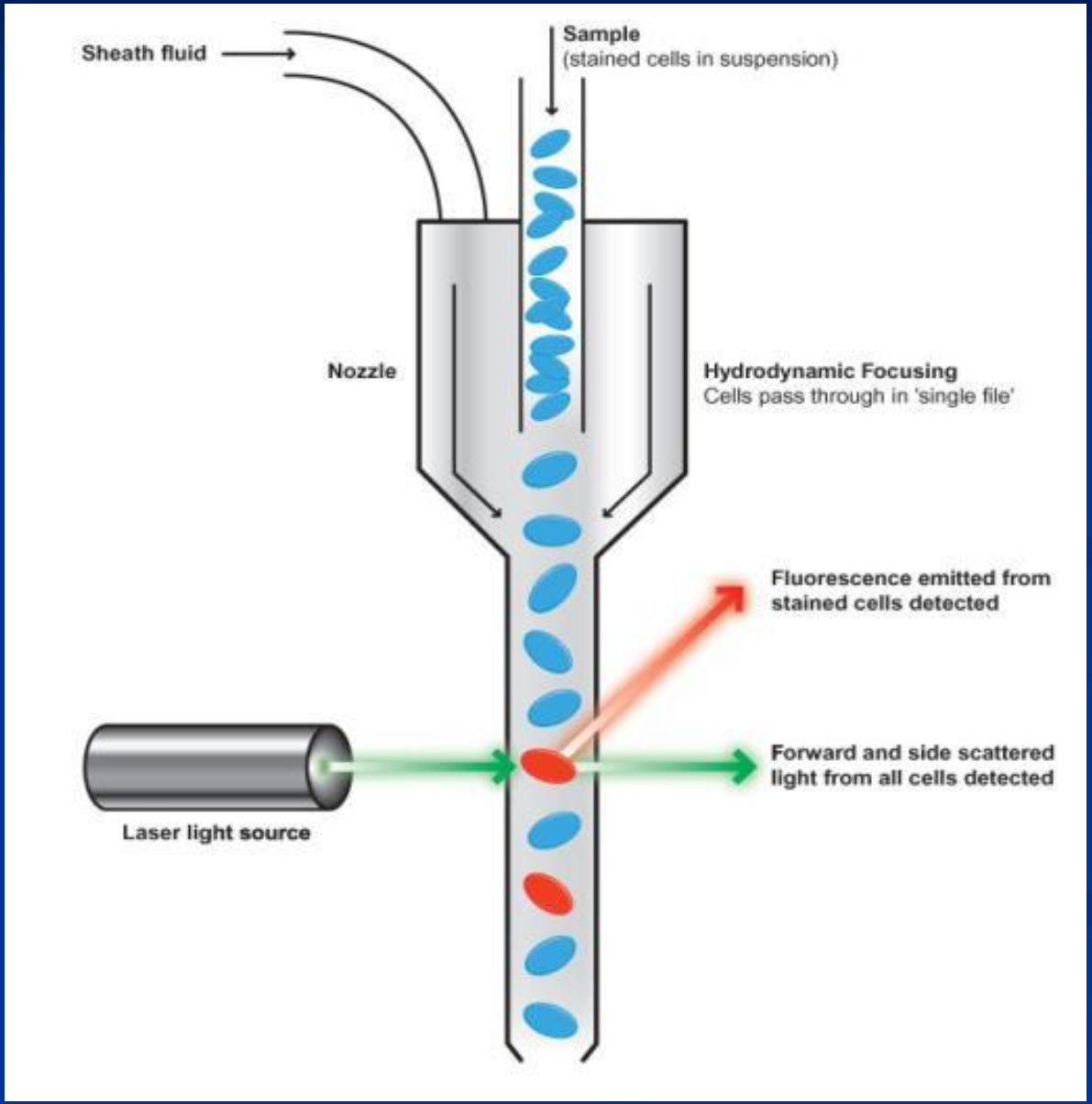


FLOW CYTOMETRY:

The measurement of cells in a flow system that has been designed to deliver particles (cells) in single file past a point of measurement

A **Flow Cytometer** consists of a light source (laser), a lens system to focus the light, a flow cell, optical components to direct light to the detectors, electronic components to convert the light signals and a computer to analyse the data.

FLOW Cytometry - 101:
The Flow Cell and Hydrodynamic Focusing

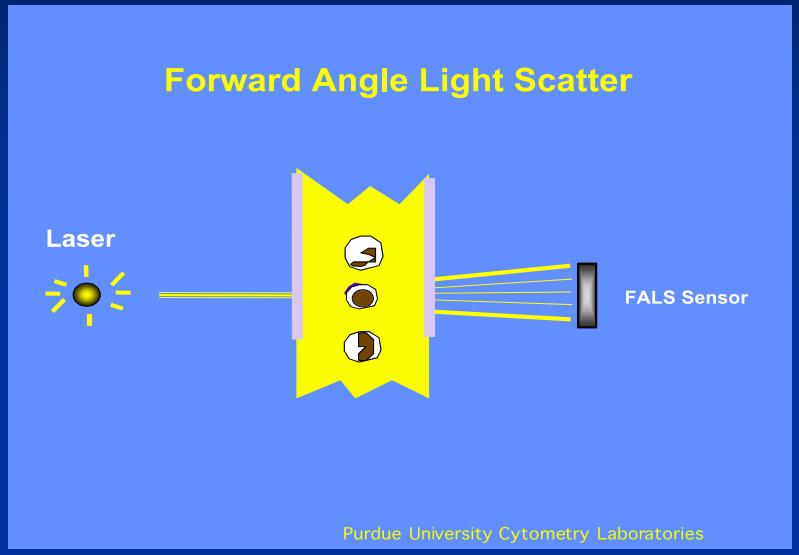


Cytometer measures
A. light scatter

and

B. fluorescence emissions

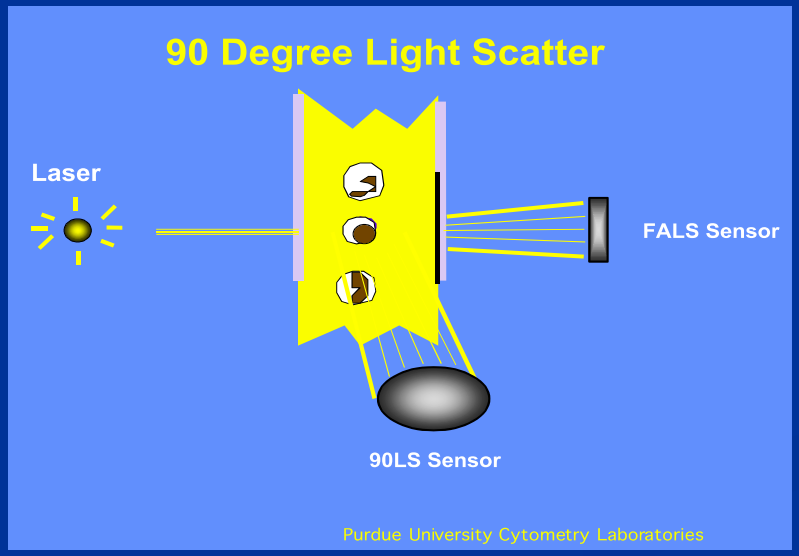
What is Light Scatter?



As the cells move through the flow cell past the laser, cells cause the laser light to be “scattered”

Light scattered at 0º angle is called Forward Scatter (FSC / FALS).

FSC is related to the size of the cells

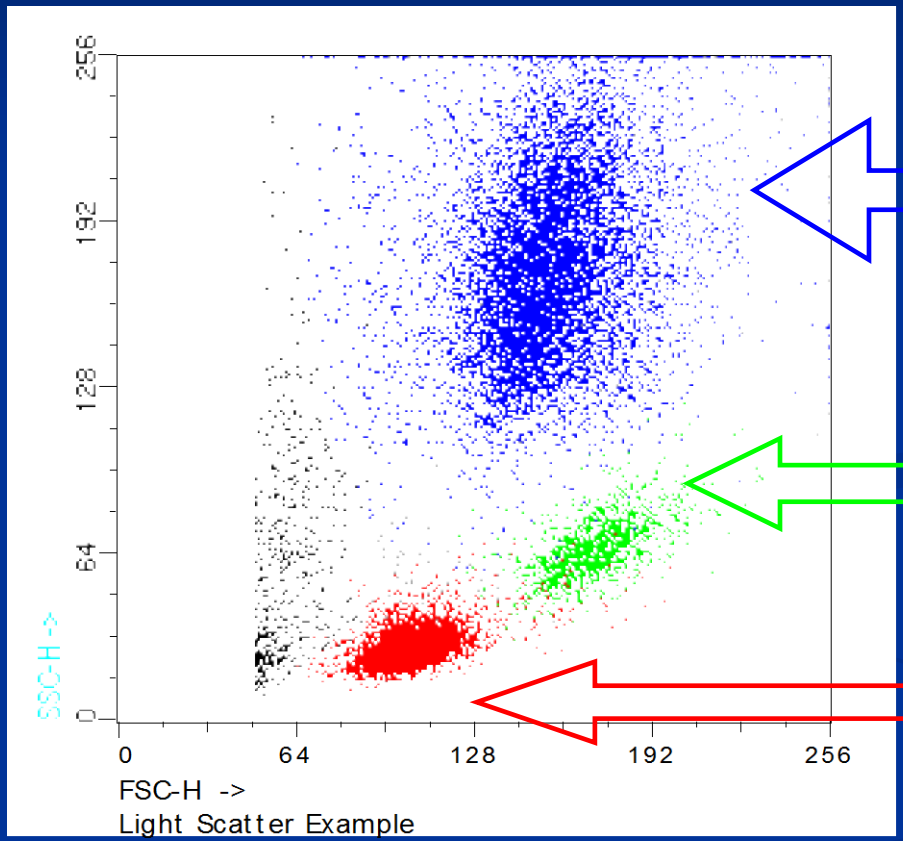


Light Scattered at 90º angle is called Side Scatter (SSC / RALS).

SSC is related to the complexity of the cells i.e. cytoplasmic granularity, vacuoles, indented/lobulated nucleus

Light scatter plot

Increase in Complexity



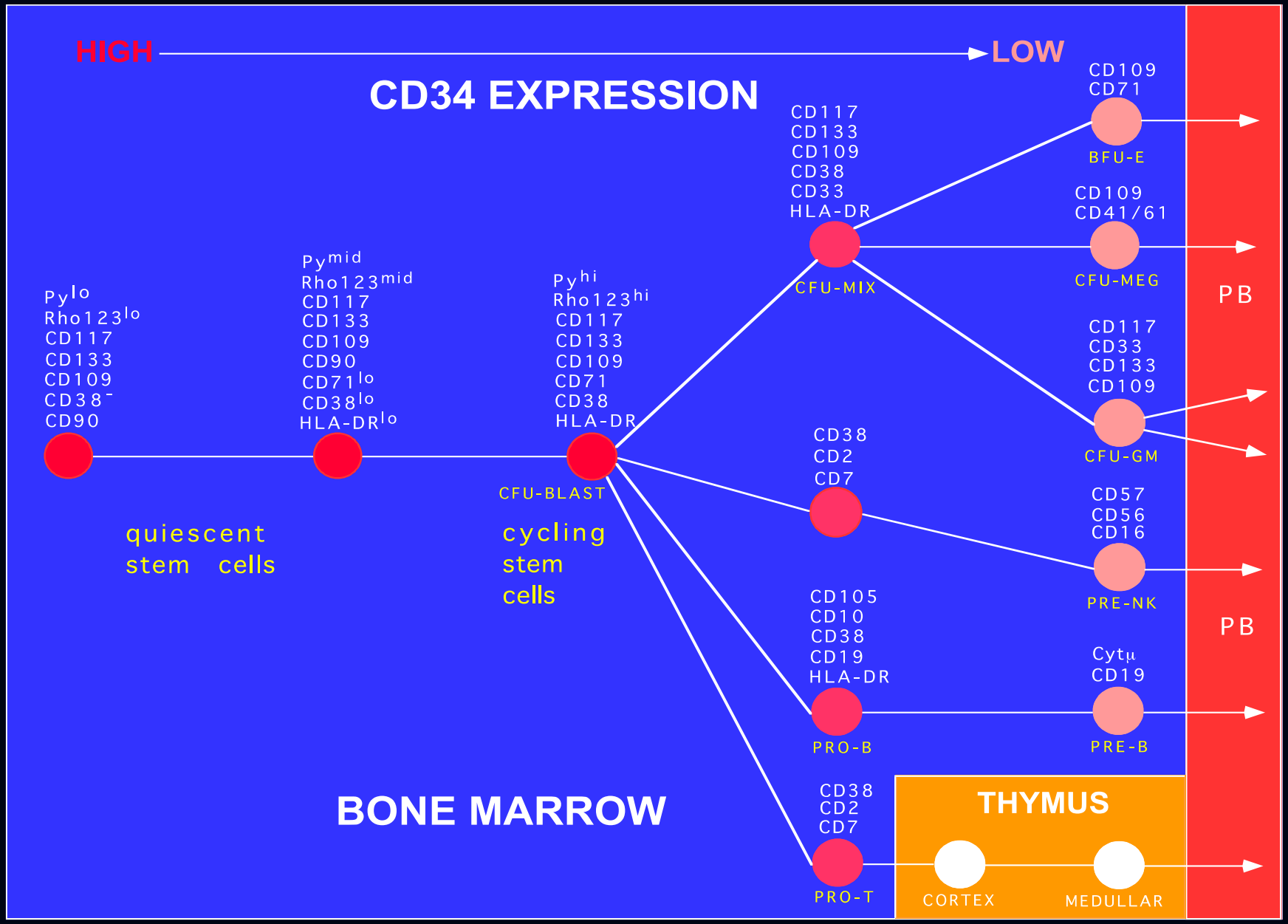
Granulocytes
+++ size
+++ complexity

Monocytes
++ size
++ complexity

Lymphocytes
+ size
+ complexity

Increase in ‘Size’

The role of CD34+ cells in Hematopoietic Stem Cell Transplantation



Sources of Hematopoietic Stem Cells for Bone Marrow Transplantation

BONE MARROW

Thomas et al, 1957

PERIPHERAL BLOOD (PB)

McCreadie et al, 1971, Korbling et al, 1980

CHEMOTHERAPY-MOBILIZED PB

Juttner et al, 1985, Reiffers et al, Korbling et al, Kessinger et al, 1986

CYTOKINE MOBILIZED PB

Siena et al, 1989, Chao et al, 1993

CORD BLOOD

Christenson et al, 1987, Gluckman et al, Broxmeyer et al, 1989

Counting CD34+ cells provides critical information to the Transplant Physician

Number of CD34+ cells in peripheral blood after mobilization with cytokines and/or chemotherapy predicts 'yield' of CD34+ cells in apheresis product

AND:

Number of CD34+ cells collected predicts time to engraftment after autologous or allogeneic HSC transplantation

BUT:

The use of mobilized peripheral blood for HSCT initially evolved without a consensus means to assess the engraftment potential of the HSC product

New Assay Design:
Gather Scientific Knowledge

What Flow Cytometric methods are available?

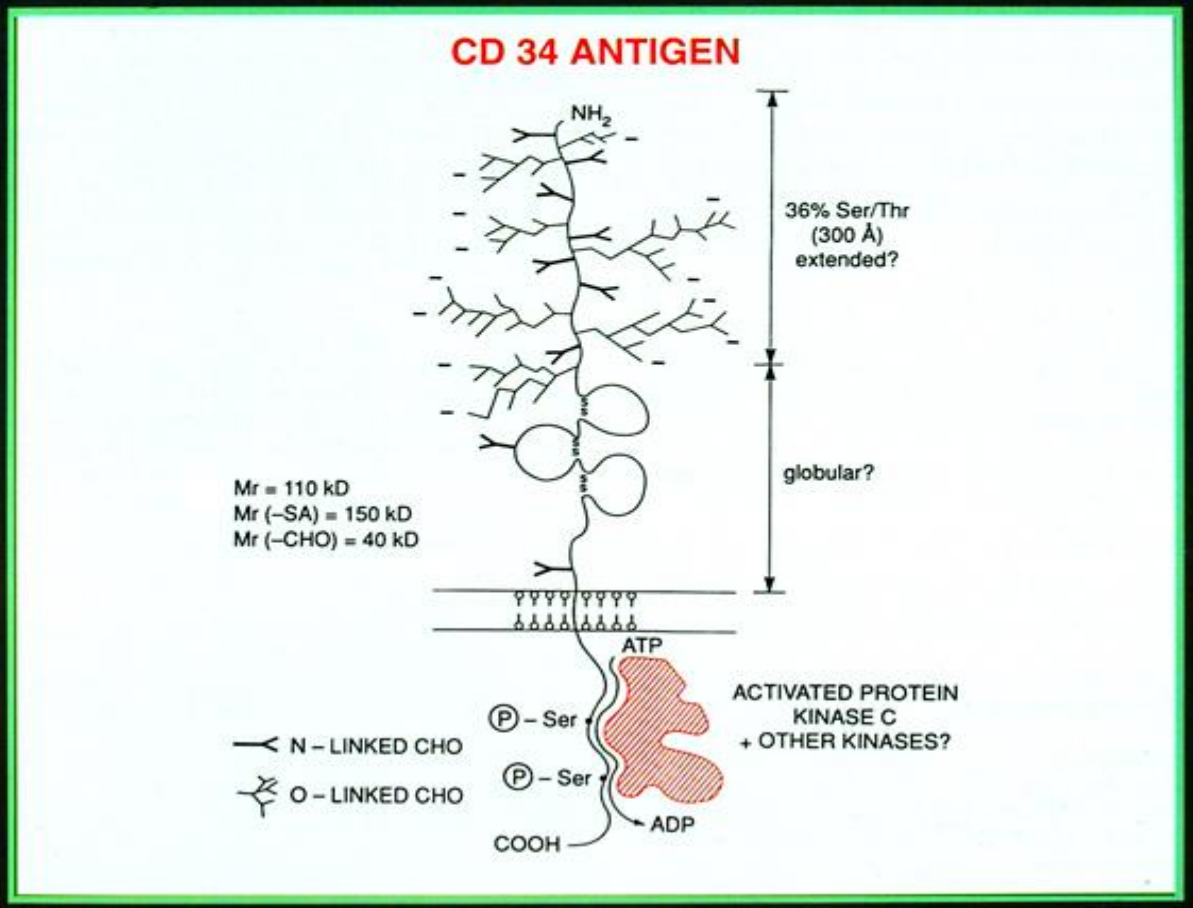
What is the science behind them?

What is the basis of antibody and/or antibody-conjugate selection?

What are the requirements of the assay?

- Simple methodology
- Suitable for all sources of HSCs (BM, mPB, CB etc)
- Suitable for all Flow Cytometers with 4 or more PMTs
- Rapid
- Accurate at level of clinical decision-making (10 cells/μL)

The Journal of
BIOLOGICAL REGULATORS
& Homeostatic Agents
JBRHA



Volume 15 - Number 1 January - March 2001

CD34 is a highly O-glycosylated cell-surface molecule

Lanza F, Healy L, Sutherland DR. Structural and functional features of the CD34 antigen: An update. J Biol Regulators and Homeostatic Agents 15: 1-13, 2001.

CD34 Antibodies: Epitope Considerations

Not all CD34 monoclonal antibodies detect all Glycoforms of CD34 Antigen!!

CD34 Epitopes:

CLASS I (MY10, B1.3C5, 12.8, ICH3)
- neuraminidase and O-sialo-glycoprotease sensitive

CLASS II (QBEnd10, 9C5, 11.A.10)
- O-sialo-glycoprotease sensitive

CLASS III (TUK3, 8G12, 581)
- Insensitive to both enzymes

Sutherland DR et al. Differential sensitivity of CD34 epitopes to cleavage by *Pasteurella haemolytica* glycoprotease: implications for purification of CD34-positive progenitor cells. Exp Hematol 20: 590-599, 1992.
Greaves MF, et al and Sutherland DR.
Report on the CD34 cluster workshop. In: Leukocyte Typing V; Proceedings of the Vth HLDA Workshop (Schlossman S., et al eds.) Oxford University Press, Oxford pp 840-846, 1995.

CD34 Antibodies: Conjugate Considerations

- Class I antibodies fail to detect all glycoforms of CD34
- Class I antibodies conjugated with negatively-charged fluorochromes e.g. FITC lose binding efficiency

- Class II antibodies detect all glycoforms of CD34
- Class II antibodies conjugated with negatively-charged fluorochromes e.g. FITC lose binding efficiency

- Class III antibodies detect all glycoforms of CD34
- Class III antibodies still fully functional regardless of conjugated form

Sutherland DR, Anderson L, Keeney M, Nayar R, Chin-Yee I. The ISHAGE Guidelines for CD34+ Cell Determination by Flow Cytometry. J Hematother 3:213-226, 1996.

Lanza F, Healy L, Sutherland DR. Structural and functional features of the CD34 antigen: An update. J Biol Regulators and Homeostatic Agents 15: 1-13, 2001.

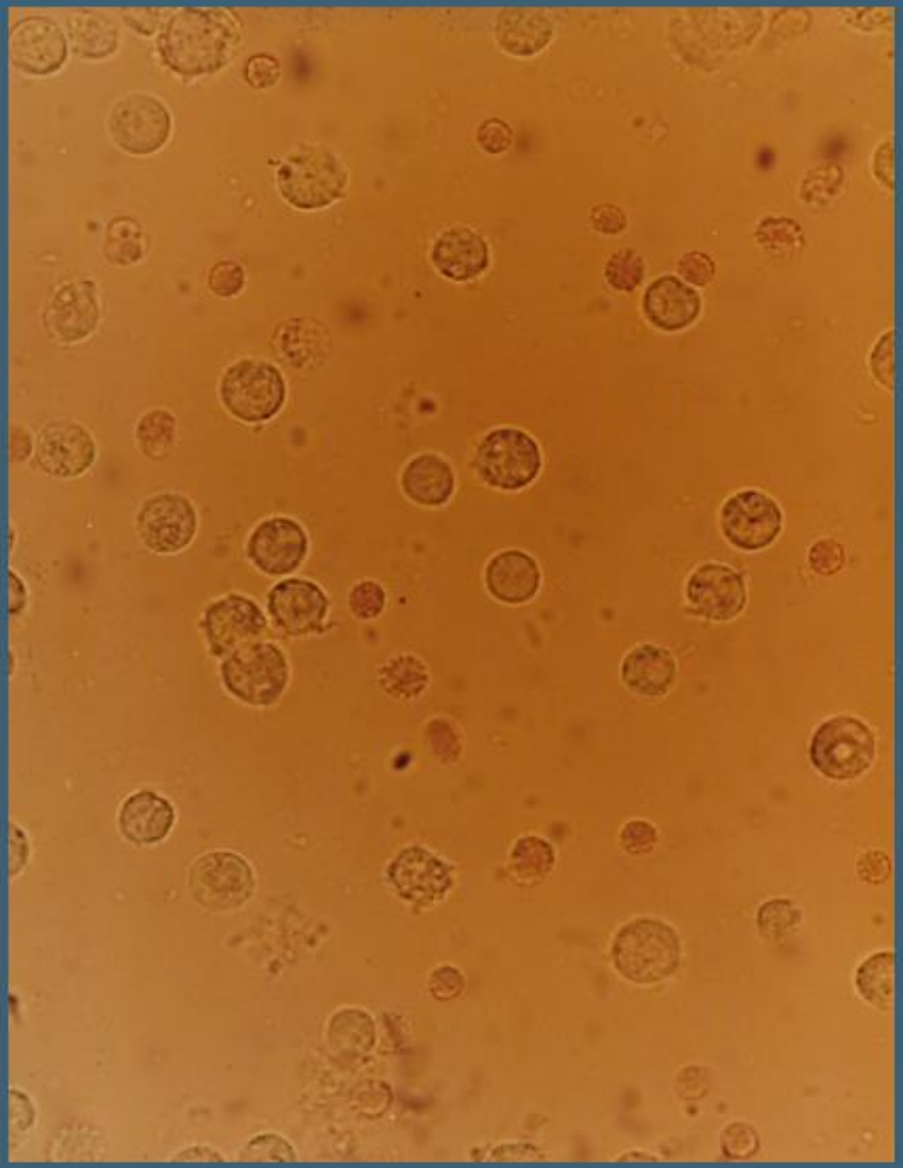
Use of CD45 allows a more reliable denominator to calculate '%CD34+'

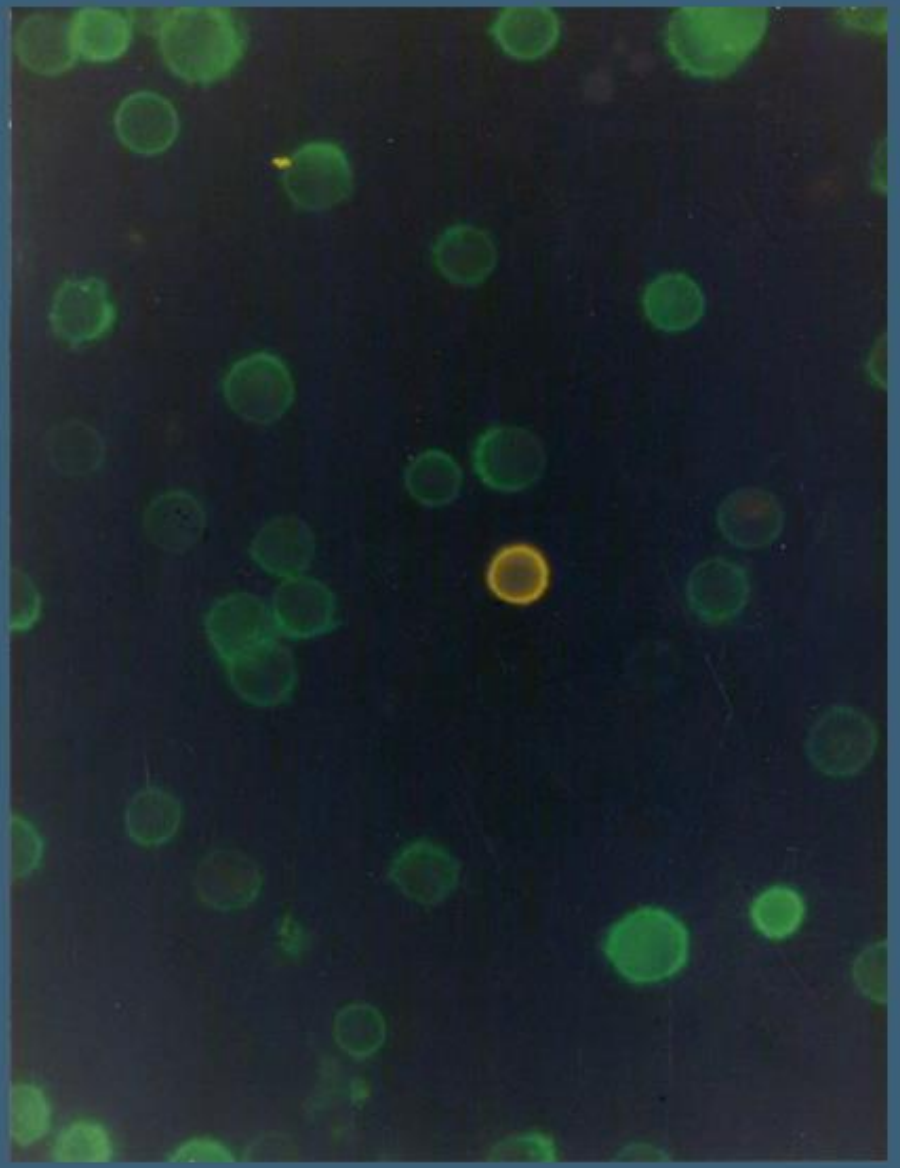
CD45 Antibodies: Clone and Conjugate Considerations

- Need to use a 'Pan-CD45' clone;
  NOT CD45RO, NOT CD45RA, NOT CD45RB
- At least 1 Pan-CD45 clone, IOL1-B (Beckman Coulter) detects a sialic acid-dependent epitope
- So we need to use a Pan-CD45 epitope that also detects ALL glycoforms of CD45

Sutherland DR, Anderson L, Keeney M, Nayar R, Chin-Yee I. The ISHAGE Guidelines for CD34+ Cell Determination by Flow Cytometry. J Hematother 3:213-226, 1996.

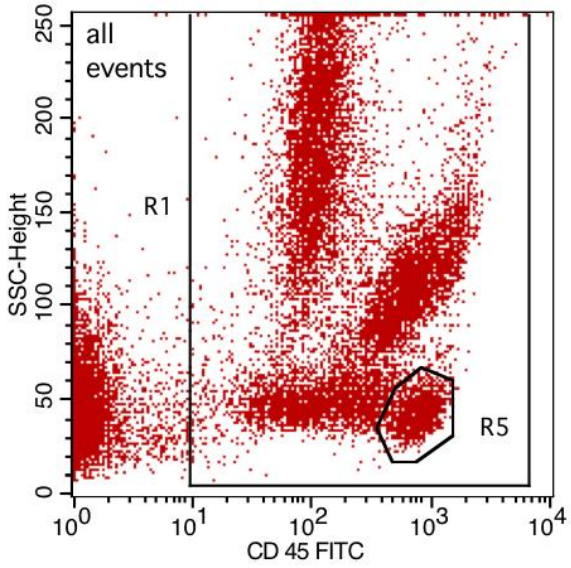
Lanza F, Healy L, Sutherland DR. Structural and functional features of the CD34 antigen: An update. J Biol Regulators and Homeostatic Agents 15: 1-13, 2001.

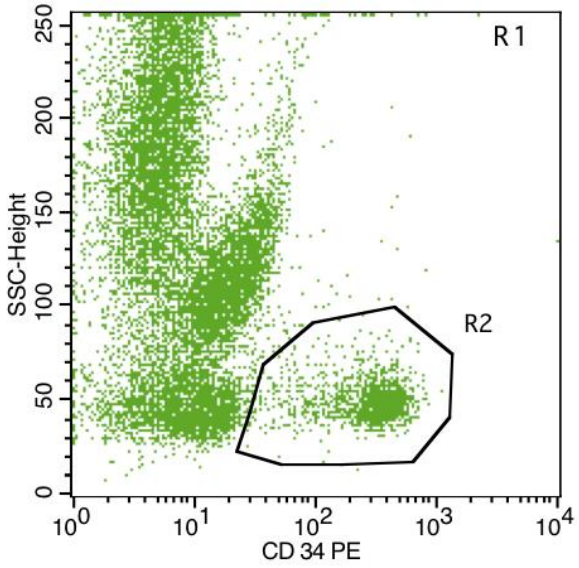




‘pan’ CD45FITC + Class III CD34PE to detect CD34+ cells by microscopy

PBSC:CD34/CD45 & Boolean gating 1993





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Total Events: 30000

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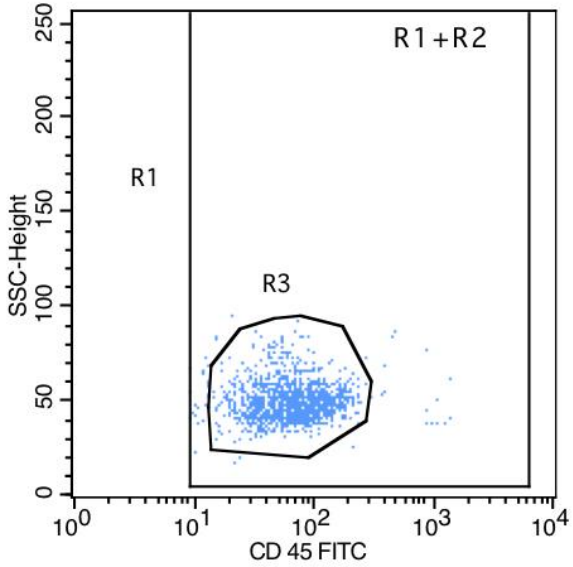
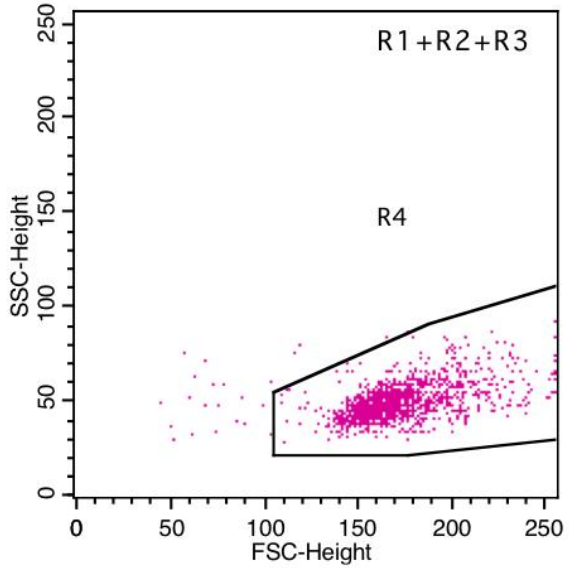
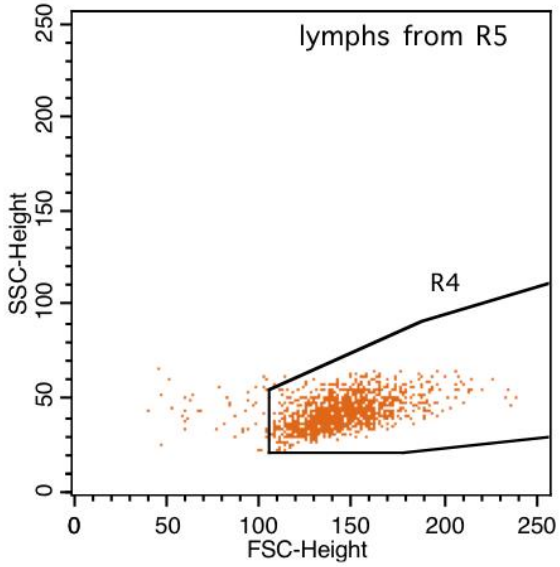
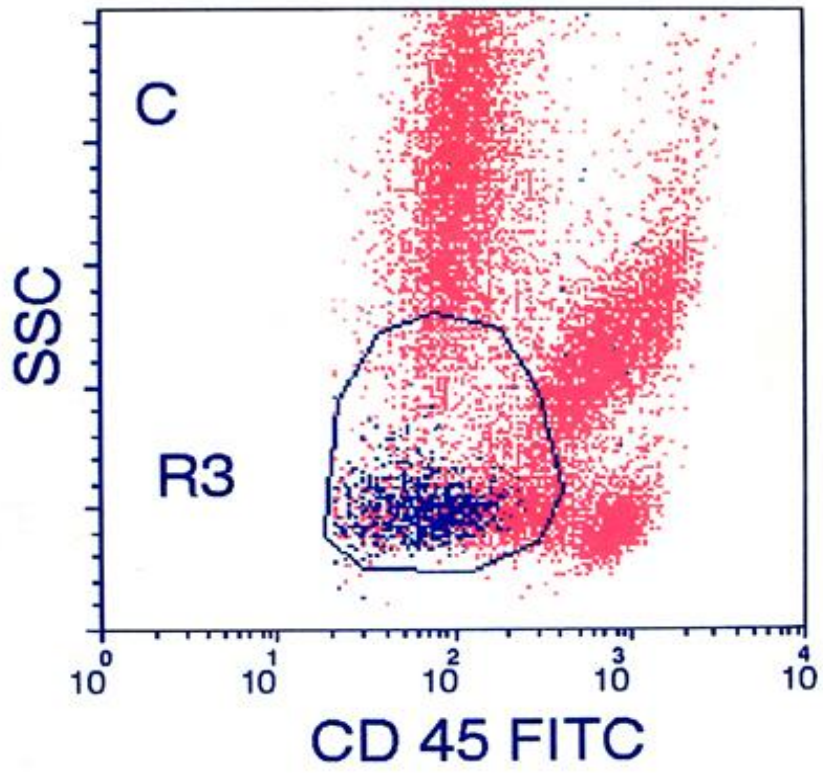
G3	1549	10.04	5.16
G4	1527	9.89	5.09







Experimental Hematology
Volume 22
Number 10
September 1994
Official Publication of the International Society for Experimental Hematology
Peter J. Quesenberry, Editor



CD45 vs. side-scatter analysis

Sensitive detection and enumeration of CD34+ cells in peripheral and cord blood by flow cytometry.

Sutherland DR, Keating A, Nayar R, Anania S, and Stewart AK. Experimental Hematology 22:1003-1010, 1994.

A LIFE-CHANGING EVENT!!

JOURNAL OF HEMATOTHERAPY 5:213–226 (1996)
Mary Ann Liebert, Inc.

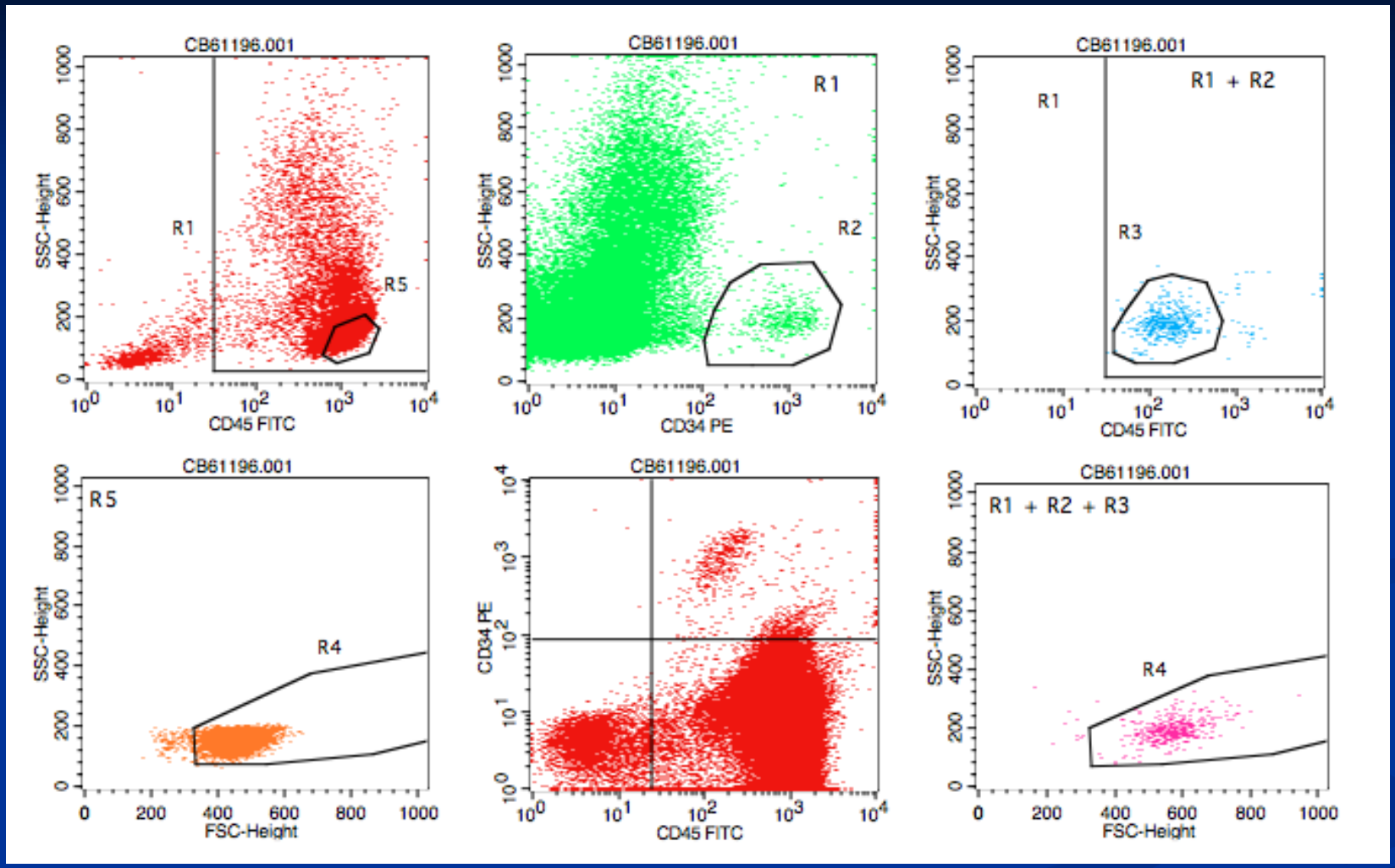
The ISHAGE Guidelines for CD34+ Cell Determination by Flow Cytometry

D. ROBERT SUTHERLAND,[1] LORI ANDERSON,[2] MICHAEL KEENEY,[2] RAKASH NAYAR,[1] and IAN CHIN-YEE[2]

ABSTRACT

The increased use of Peripheral Blood Stem Cells (PBSC) to reconstitute hematopoiesis in autotransplant and, more recently, allotransplant settings has not been associated with a consensus means to quality control the PBSC product. Since the small population of cells that bear the CD34 antigen are thought to be responsible for multilineage engraftment, graft assessment by flow cytometric quantitation of CD34+ cells should provide a rapid, reliable, and reproducible assay. Unfortunately, although a number of flow cytometric assays for CD34 enumeration have been described, the lack of a standardized method has led to the generation of widely divergent data. Furthermore, none of these assays has been validated as to interlaboratory reproducibility and suitability for widespread clinical application. In early 1995, the International Society of Hematotherapy and Graft Engineering (ISHAGE) established a Stem Cell Enumeration Committee, the mandate of which was to validate a simple, rapid, and sensitive flow cytometric method to quantitate CD34+ cells in peripheral blood and apheresis products. We also sought to establish its utility on a variety of flow cytometers in clinical laboratories and its reproducibility between transplant centers. Here, we describe the four-parameter flow methodology adopted by ISHAGE for validation in a multicenter study in North America.

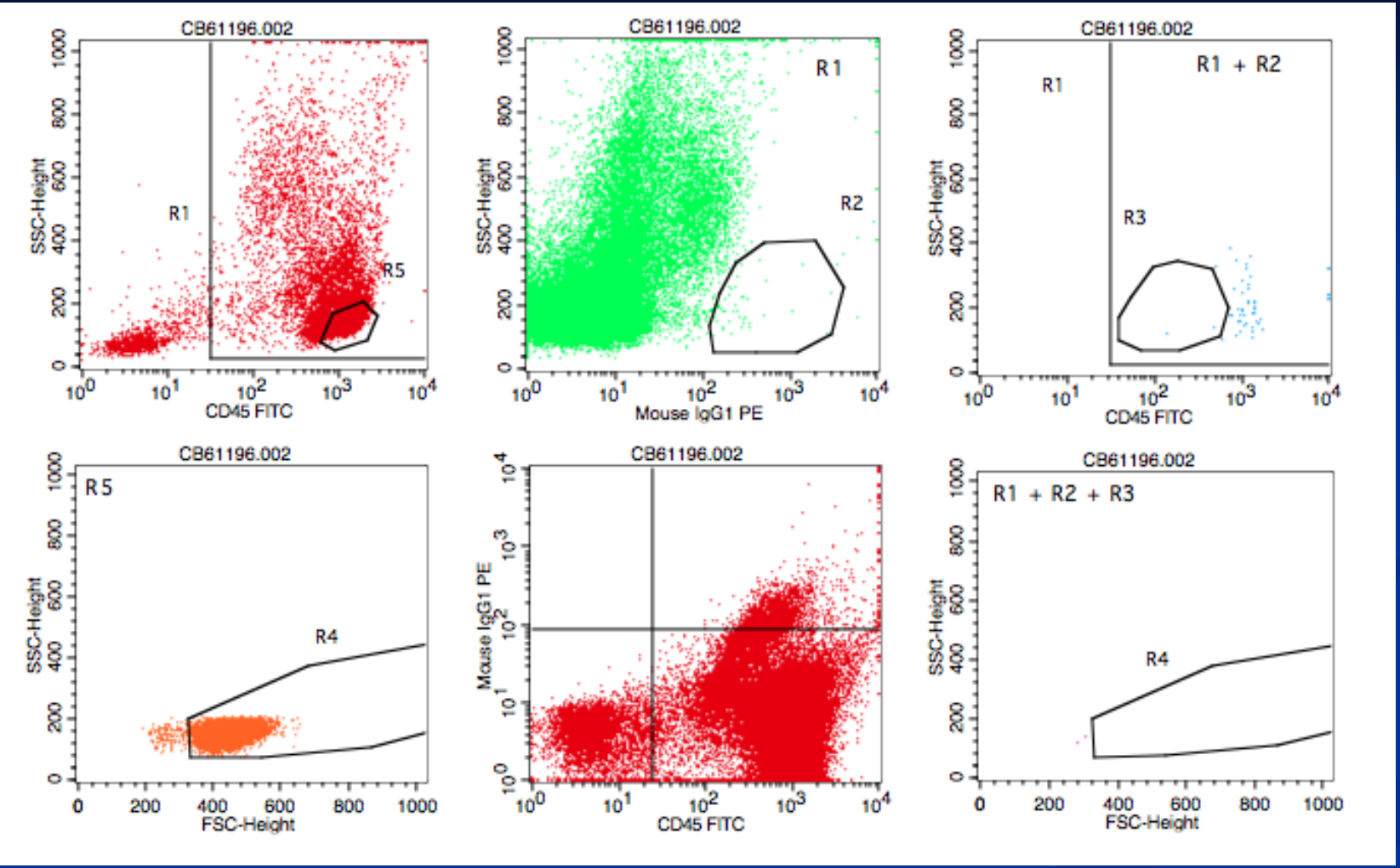
ISHAGE Guidelines 1996: Dual Platform



Fresh Cord Blood sample CD45/CD34

Sutherland DR, Anderson L, Keeney M, Nayar R, Chin-Yee I.
The ISHAGE Guidelines For CD34+ Cell Determination By Flow Cytometry.
J. Hematotherapy 3:213-226, 1996.

ISHAGE Guidelines 1996: Dual Platform



CD45FITC/IgG1PE: Isotype controls Useless!

Sutherland DR, Anderson L, Keeney M, Nayar R, Chin-Yee I.
The ISHAGE Guidelines For CD34+ Cell Determination By Flow Cytometry.
J. Hematotherapy 3:213-226, 1996.

Dual Platform (DP) to Single Platform (SP) and other Refinements (1998)

Eliminated redundant isotype control

Added a fluorescent counting beads to make the method single platform

Added a viability dye (7-AAD)

Single platform absolute counting of viable CD34+ cells in 45 minutes

Cytometry (Communications in Clinical Cytometry) 34:61–70 (1998)

Original Articles

Single Platform Flow Cytometric Absolute CD34+ Cell Counts Based on the ISHAGE Guidelines

Michael Keeney,[1]* Ian Chin-Yee,[1] Karin Weir,[1] Jan Popma,[1] Rakash Nayar,[2] and D. Robert Sutherland[2]

[1]The London Health Sciences Centre, London, Ontario, Canada

[2]Oncology Research, The Toronto Hospital, Ontario, Canada

In concert with the International Society of Hematotherapy and Graft Engineering (ISHAGE), we previously described a set of guidelines for detection of CD34+ cells based on a four-parameter flow cytometry method (CD45 FITC/CD34 PE staining, side and forward angle light scatter). With this procedure, an absolute CD34+ count is generated by incorporating the leukocyte count from an automated hematology analyser (two-platform method). In the present study, we modified the basic ISHAGE method with the addition of a known number of Flow-Count™ fluorospheres. To reduce errors inherent to sample washing/centrifugation, we implemented ammonium chloride lyse, no-wash no-fix sample processing. These modifications convert the basic protocol into a single-platform method to determine the absolute CD34 count directly from a flow cytometer and form the basis of the Stem-Kit from Coulter/Immunotech. A total of 72 samples of peripheral blood, apheresis packs, and cord blood were analysed and compared using the ISHAGE protocol with or without the addition of fluorescent microspheres. Comparison of methods showed a high correlation coefficient ($r = 0.99$), with no statistically significant difference or bias between methods ($P > 0.05$). Linearity of the absolute counting method generated an R^2 value of 1.00 over the range of 0–250/μl. Precision of the absolute counting method measured at three concentrations of CD34+-stabilised KG1a cells (Stem-Trol, COULTER®) generated a coefficient of variation (C.V.) ranging from 4% to 9.9%. In a further modification of the single-platform method, the viability dye 7-amino actinomycin D was included and demonstrated that both viable and nonviable CD34+ cells could be identified and quantitated. Together, these modifications combine the accuracy and sensitivity of the original ISHAGE method with the ability to produce an absolute count of viable CD34+ cells. It is the accurate determination of this value that is most clinically relevant in the transplant setting. These modifications may improve the interlaboratory reproducibility of CD34 determinations due to the reduction in sample handling and calculation of results. Cytometry (Comm. Clin. Cytometry) 34:61–70, 1998.

Single Platform (SP) ISHAGE Protocol

Any clinical cytometer with 4 or more PMTs

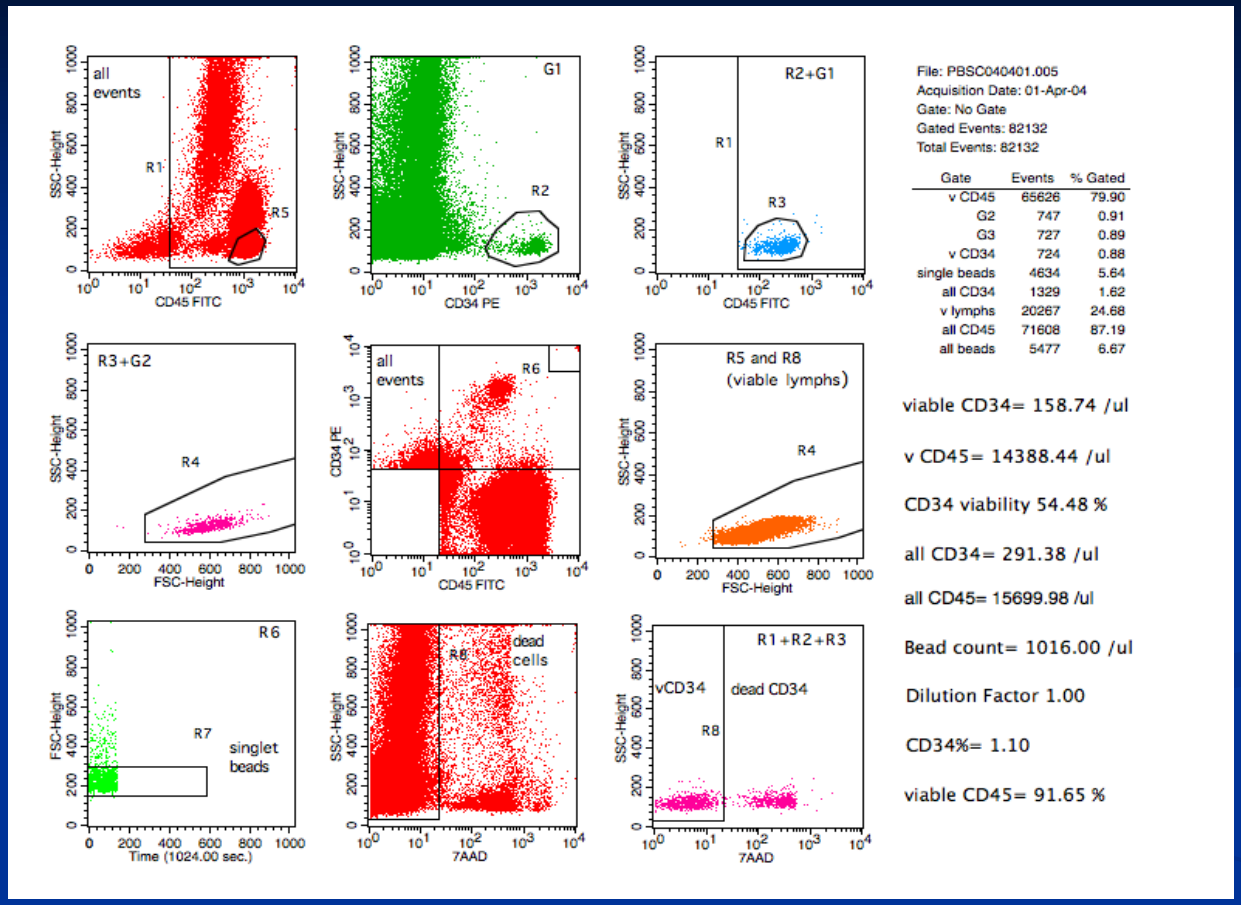
- Pan-CD45 FITC (detects all isoforms and glycoforms)
- Pan-CD34 PE (class III, detects all glycoforms)
- Viability dye (7-AAD, - include for all samples)
- Pipettable Fluorospheres (Stem-Kit™ [Beckman], CD34 Count Kit [DAKO]) or Trucount™ tubes [BD]
- Reverse-pipetting of sample (and beads) mandatory
- Sequential boolean gating strategy

    - to identify 'true' $CD34^{+}$ cells:

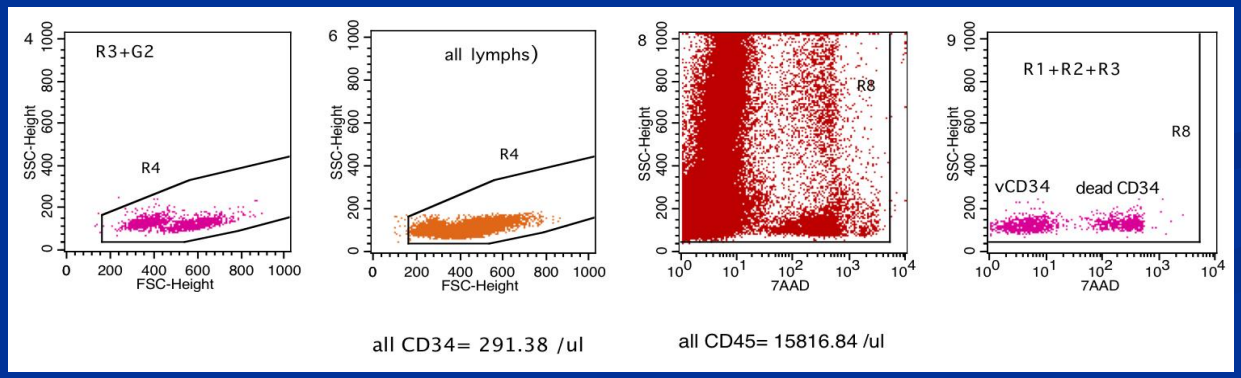
        - $CD34^{+}$, $CD45^{dim}$, $SSC^{low/int}$, $FSC^{low/int}$

Keeney M, Chin-Yee I, Weir K, Popma J, Nayar R, Sutherland DR.
Single platform flow cytometric absolute CD34+ cell counts based on the ISHAGE Guidelines.
Cytometry 34: 61-67, 1998

Beckman Stem-Kit on BD FACSCalibur

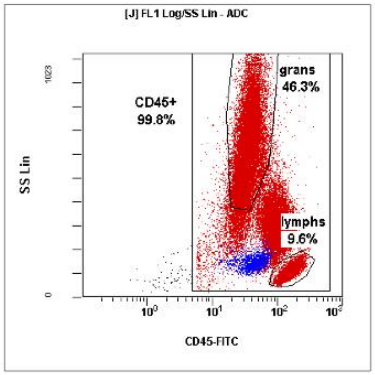


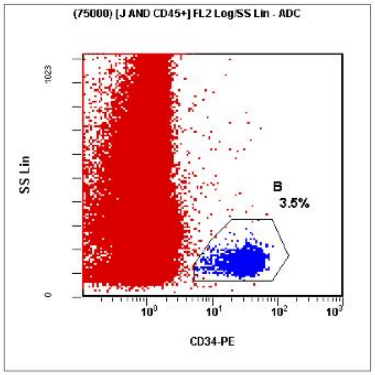
Gate	Events	% Gated
v CD45	65626	79.90
G2	747	0.91
G3	727	0.89
v CD34	724	0.88
single beads	4634	5.64
all CD34	1329	1.62
v lymphs	20267	24.68
all CD45	71608	87.19
all beads	5477	6.67

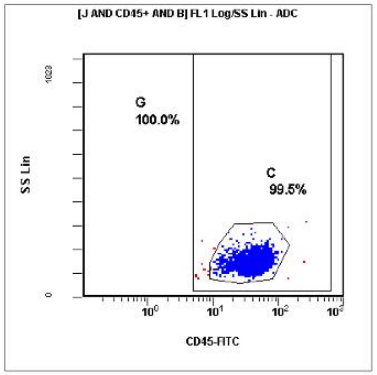


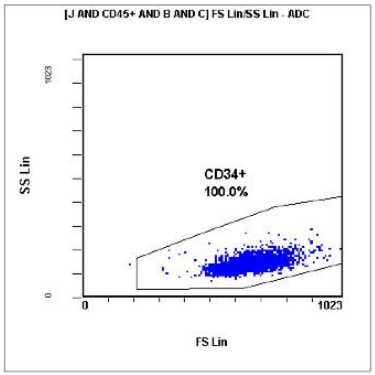
Importance of viability assessment
Viable cells only (7-AAD-negative)

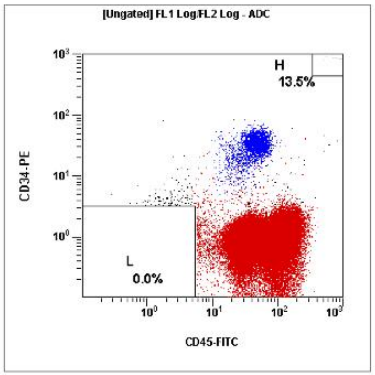
All cells: (live plus dead)

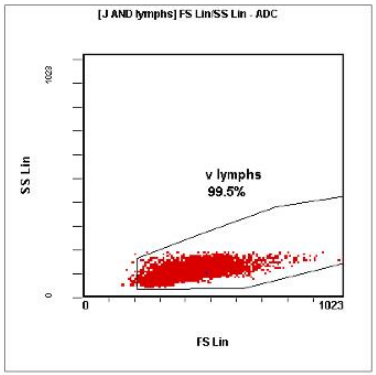


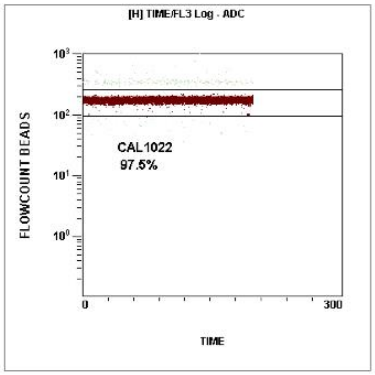


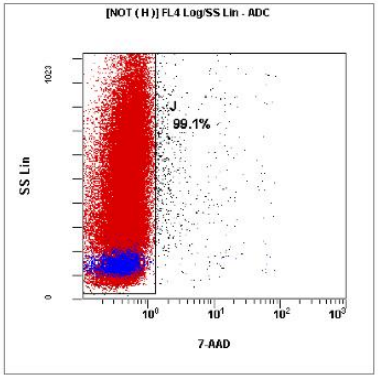


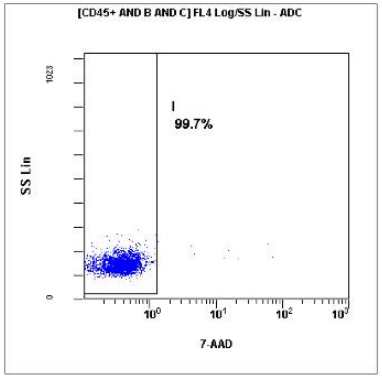












[Ungated] Legend

Color	Name	% Gated	% Total	Number	Cells/µL
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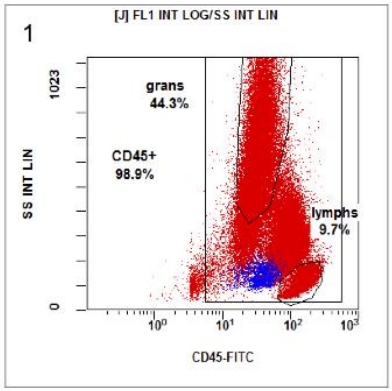
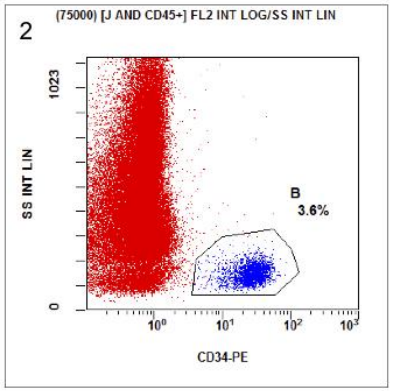
	ALL CD34+ (C)	3.01	3.01	2662	233.81
	v CD45+	85.53	85.53	75564	6636.85
0x0100001A	ALL CD45+	86.49	86.49	76416	6711.68
	CAL	13.17	13.17	11636	1022.00

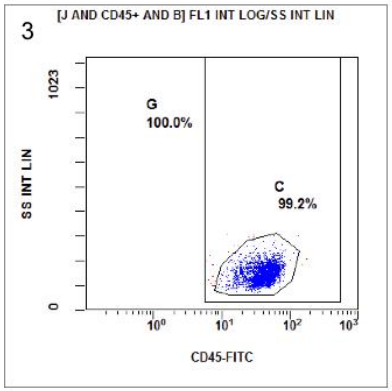
Assay Validation

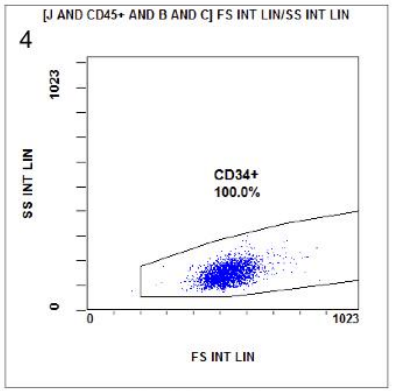
Old Cytometer Beckman FC500

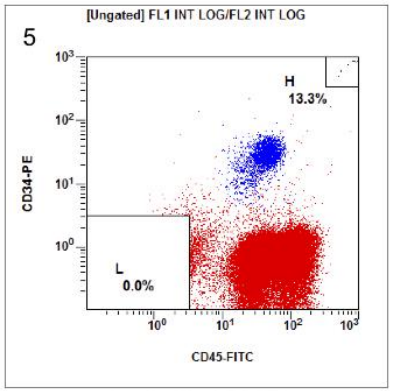
Stem-Kit™
ISHAGE manual protocol
Beckman Coulter FC500™

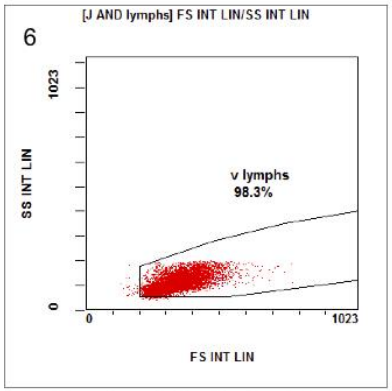


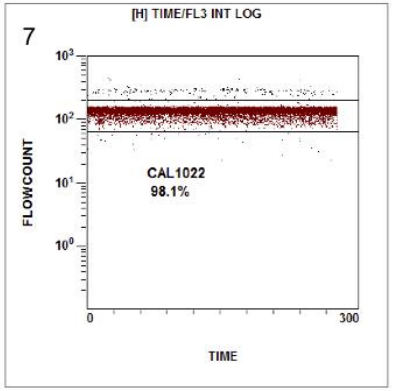


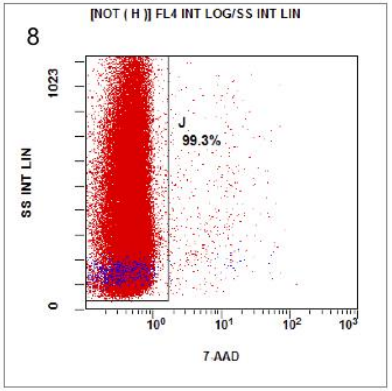


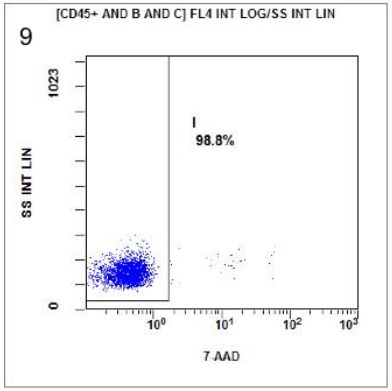












[Ungated] Legend

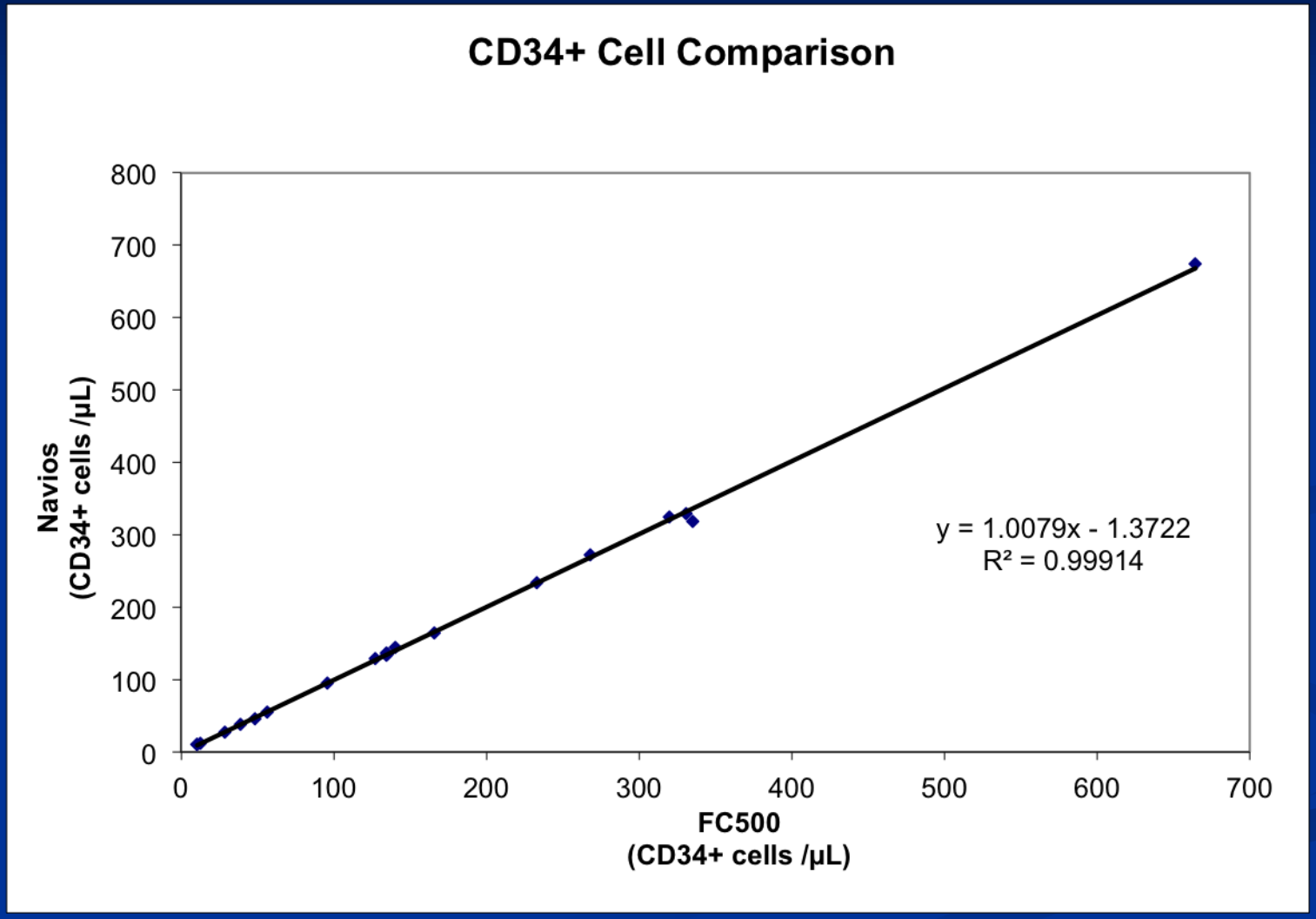
Color	Name	% Gated	% Total	Number	Cells/µL
	v CD34+	3.00	3.00	2644	234.32
	ALL CD34+	3.04	3.04	2676	237.16
	v CD45+	85.15	85.15	75011	6647.70
	ALL CD45+	86.65	86.65	76336	6765.12
	CAL	13.09	13.09	11532	1022.00

Assay Validation

New Cytometer Beckman Navios

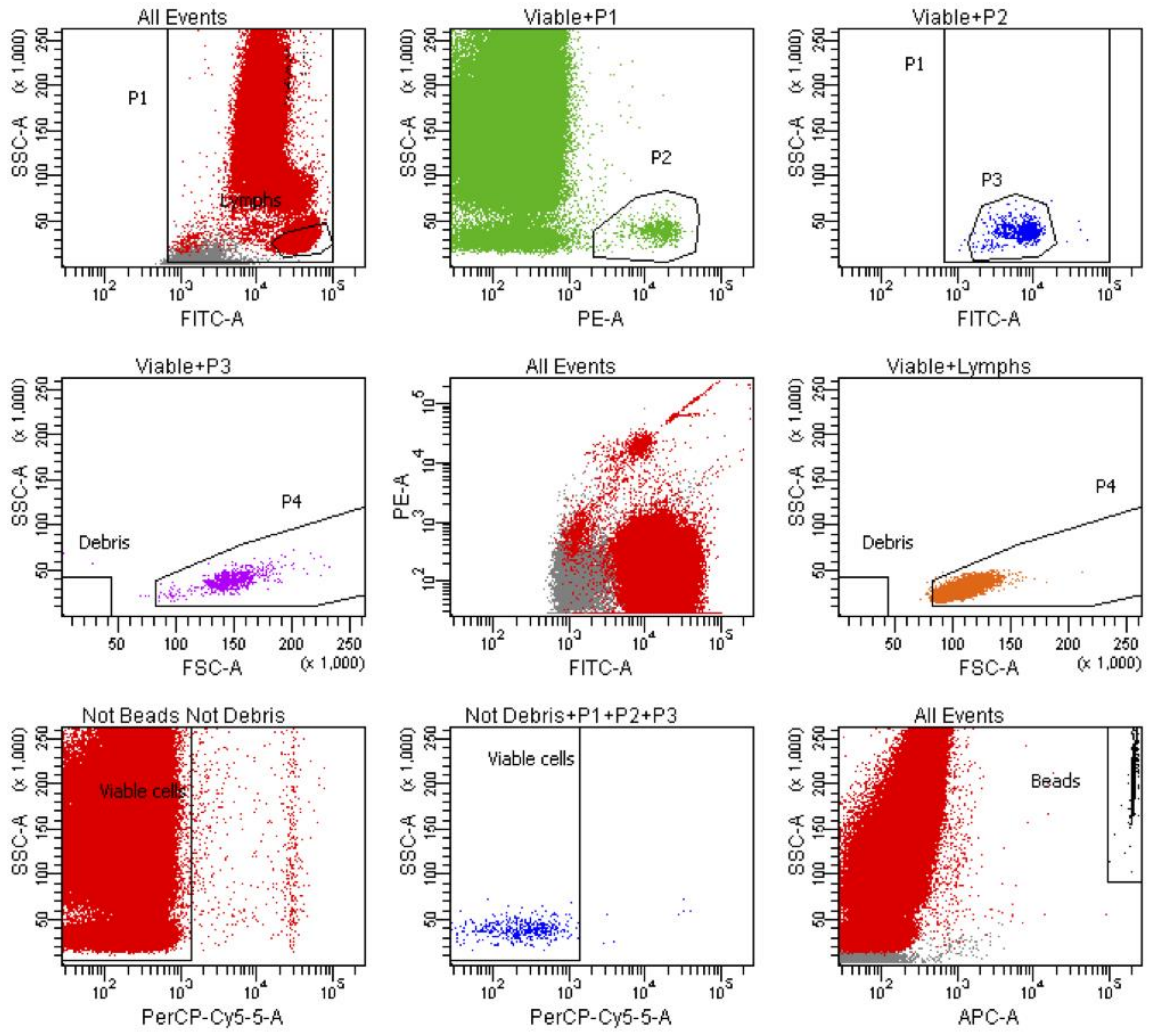
Stem-Kit™
ISHAGE manual protocol
Beckman Coulter Navios™

Assay Validation
FC500 versus Navios



20 fresh PB or PBSC samples acquired on both instruments

TruCount-ISHAGE (SCE-Kit™) on BD Canto II



Specimen Name: 96 hours
Tube Name: GC05-1-96
Record Date: Apr 19, 2013 12:06:02 PM

Tube: GC05-1-96

Population	#Events	%Total
All Events	295,771	100.0
Debris	18,769	6.3
Not Debris	277,002	93.7
Beads	3,724	1.3
Not Beads	273,278	92.4
P1	273,262	92.4
P2	766	0.3
P3	749	0.3
P4	738	0.2
Lymphs	30,826	10.4
Viable cells	272,518	92.1
Viable Lymphs	30,811	10.4
Viable P1	272,515	92.1
Viable P2	750	0.3
Viable P3	742	0.3
Viable P4	737	0.2

Viable CD34 = 98.84/ul
Viable CD45 = 36548.76/ul
Total CD34 = 100.45/ul
Total CD45 = 36648.95/ul
CD34 Viability = 98.39%
CD45 Viability = 99.72%

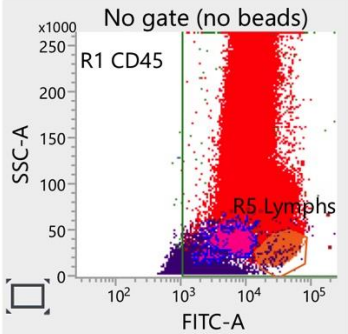
Calculations
Viable CD34 cells/ul = ([Viable P4] x Bead Count x DF) / (beads x SV)
Viable CD45 cells/ul = ([Viable P1] x Bead Count x DF) / (beads x SV)
Total CD34 cells/ul = ([P3] x Bead Count x DF) / (beads x SV)
Total CD45 cells/ul = ([P1] x Bead Count x DF) / (beads x SV)
CD34 Viability = [Viable P4] / [P3] x 100
CD45 Viability = [Viable P1] / [P1] x 100

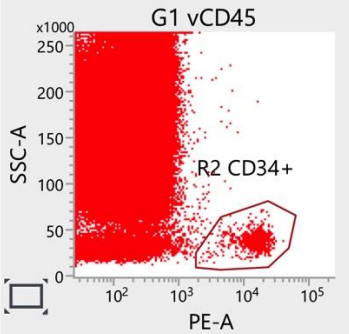
Bead Count: 49945
Sample Volume (SV): 100
Dilution Factor (DF): 1
Bead Lot#

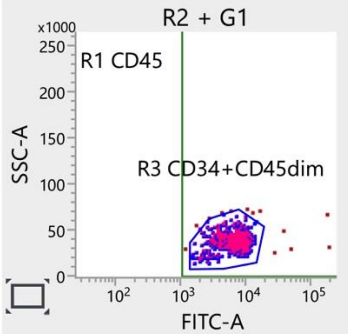
BD FACSuite template for ISHAGE (RUO)

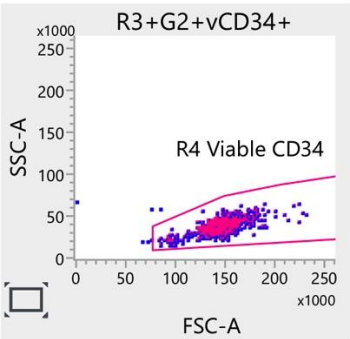
Trucount-based, Single Platform, Semi-automated Analysis

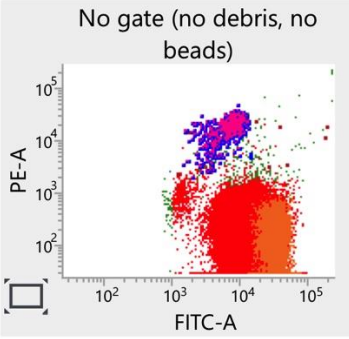
For new BD Lyric and Canto*

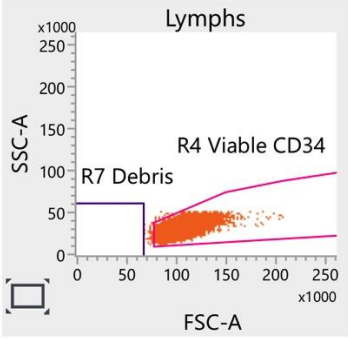


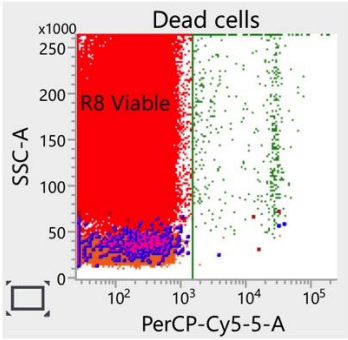


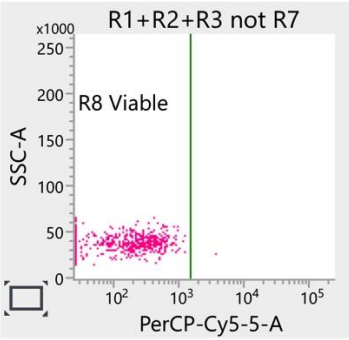


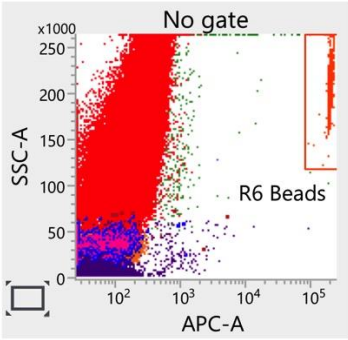












Bead Count = 49,500.0

Bead Lot# = 12,344.0

Dilution factor = 1

Sample volume = 100 ul

RESULTS

tCD34: Total CD34 = 98.0 /ul

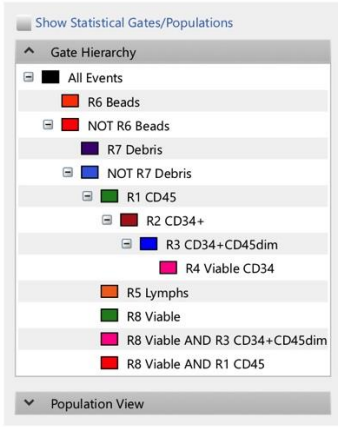
%vCD34: CD34 Viability = 100 %

vCD34: Viable CD34 = 97.6 /ul

tCD45: Total CD45 = 36,245.9 /ul

%vCD45: CD45 Viability = 99.8 %

vCD45: Viable CD45 = 36,164.1 /ul



Research use only.
not intended for diagnostic use

Statistics

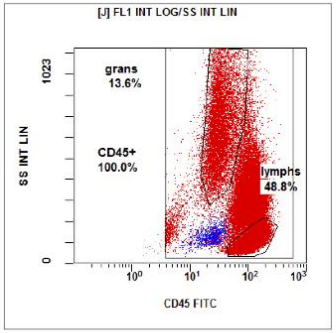
Name	Events	% Total	% Parent	% Grandparent
GC05-1-96:All Events	295,771	100.00	***	***
GC05-1-96:R7 Debris	19,459	6.58	6.66	6.58
GC05-1-96:R6 Beads	3,722	1.26	1.26	***
GC05-1-96:R1 CD45	272,540	92.15	99.98	93.32
GC05-1-96:R2 CD34+	750	0.25	0.28	0.28
GC05-1-96:R3 CD34+CD45dim	737	0.25	98.27	0.27
GC05-1-96:R4 Viable CD34	731	0.25	99.19	97.47
GC05-1-96:R5 Lymphs	31,495	10.65	11.55	10.78
GC05-1-96:R8 Viable	271,974	91.95	99.77	93.13
GC05-1-96:R8 Viable AND R3 CD34+CD45dim	734	0.25	0.27	0.25
GC05-1-96:R8 Viable AND R1 CD45	271,925	91.94	99.76	93.11

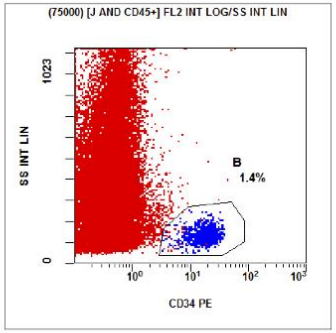
Courtesy Dr. Ahmad Al-Attar

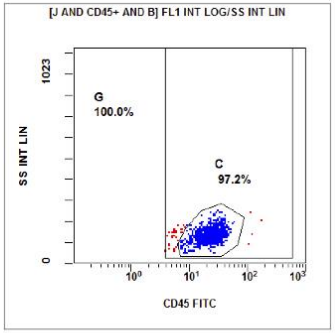
ISHAGE Single Platform Protocol for Allograft Assessment

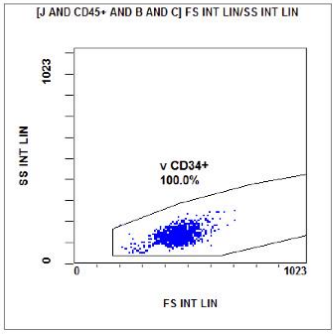
Beckman Coulter
FC500/Navios

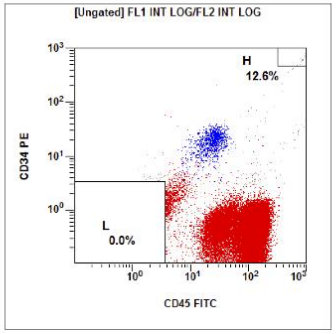
CD45-FITC
CD34-PE
7-AAD
CD3-PECy7
Flowcount beads

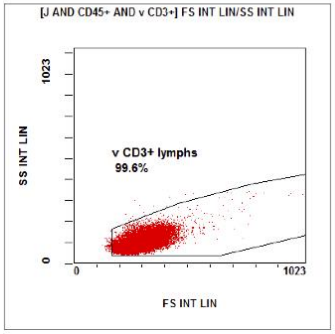


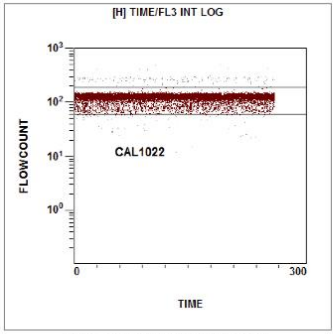


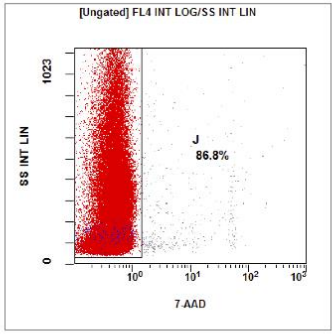


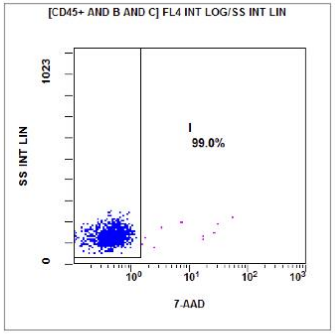


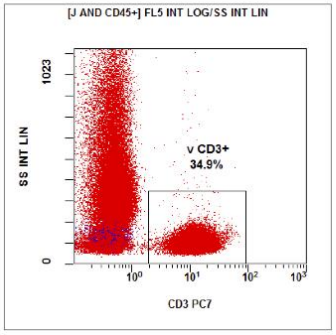








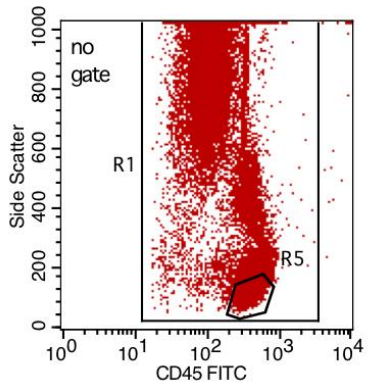


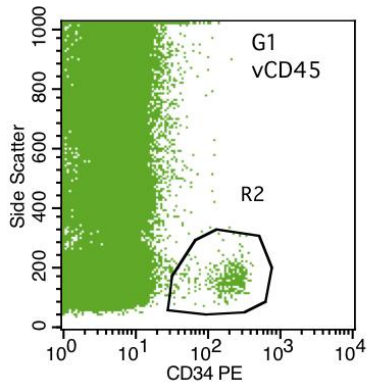


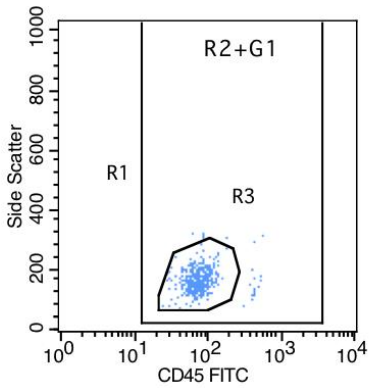
[Ungated] Legend

Color	Name	% Gated	% Total	Number	Cells/μL
	v CD34+	1.18	1.18	1022	97.50
	ALL CD34+	1.19	1.19	1032	98.45
	v CD45+	86.74	86.74	75166	7170.69
	ALL CD45+	87.36	87.36	75707	7222.31
	v CD3+ LYMPHS	30.25	30.25	26214	2500.77
	CAL	12.36	12.36	10713	1022.00

SP ISHAGE Protocol for Allograft Assessment

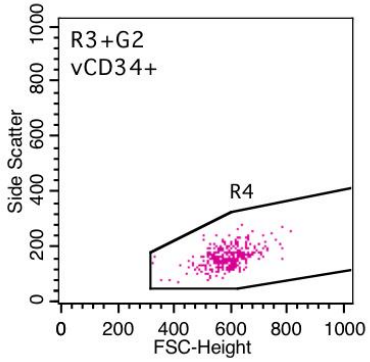


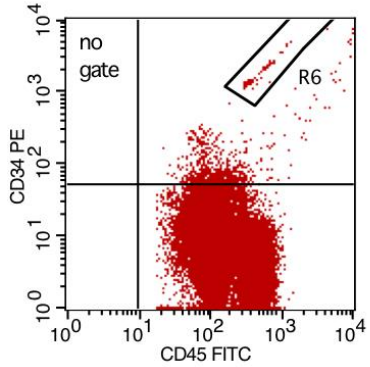


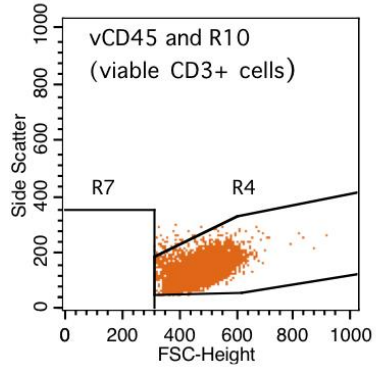


File: CB CD3APC truco49800.fcs
Acquisition Date: 20-Dec-13
Gate: No Gate
Gated Events: 131730
Total Events: 131730

Gate	Events	% Gated
v CD45	105902	80.39
G2	383	0.29
G3	362	0.27
v CD34	362	0.27
Beads	4654	3.53
all CD34	362	0.27
v T cells	26608	20.20
all CD45	125557	95.31
Debris	1476	1.12
Alt beads	4659	3.54







viable CD34= 38.74 /ul

all CD34= 38.74 /ul

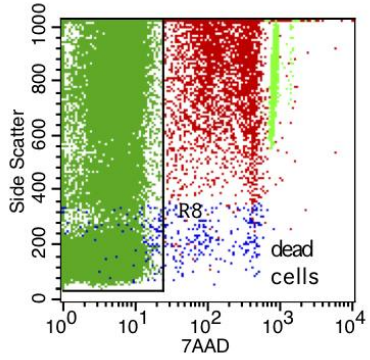
CD34 viability= 100.00 %

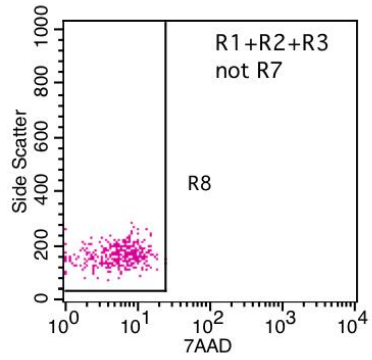
viable CD3 cells= 2847.18 /ul

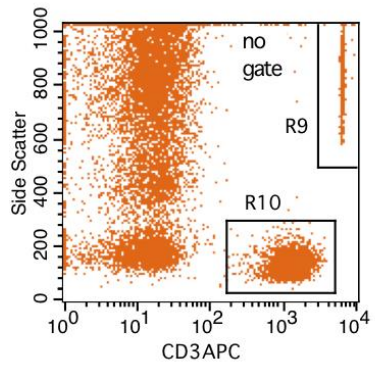
viable CD45= 11332.01 /ul

all CD45= 13435.19 /uL

% Viable CD45= 84.35







bead count = 49800.00

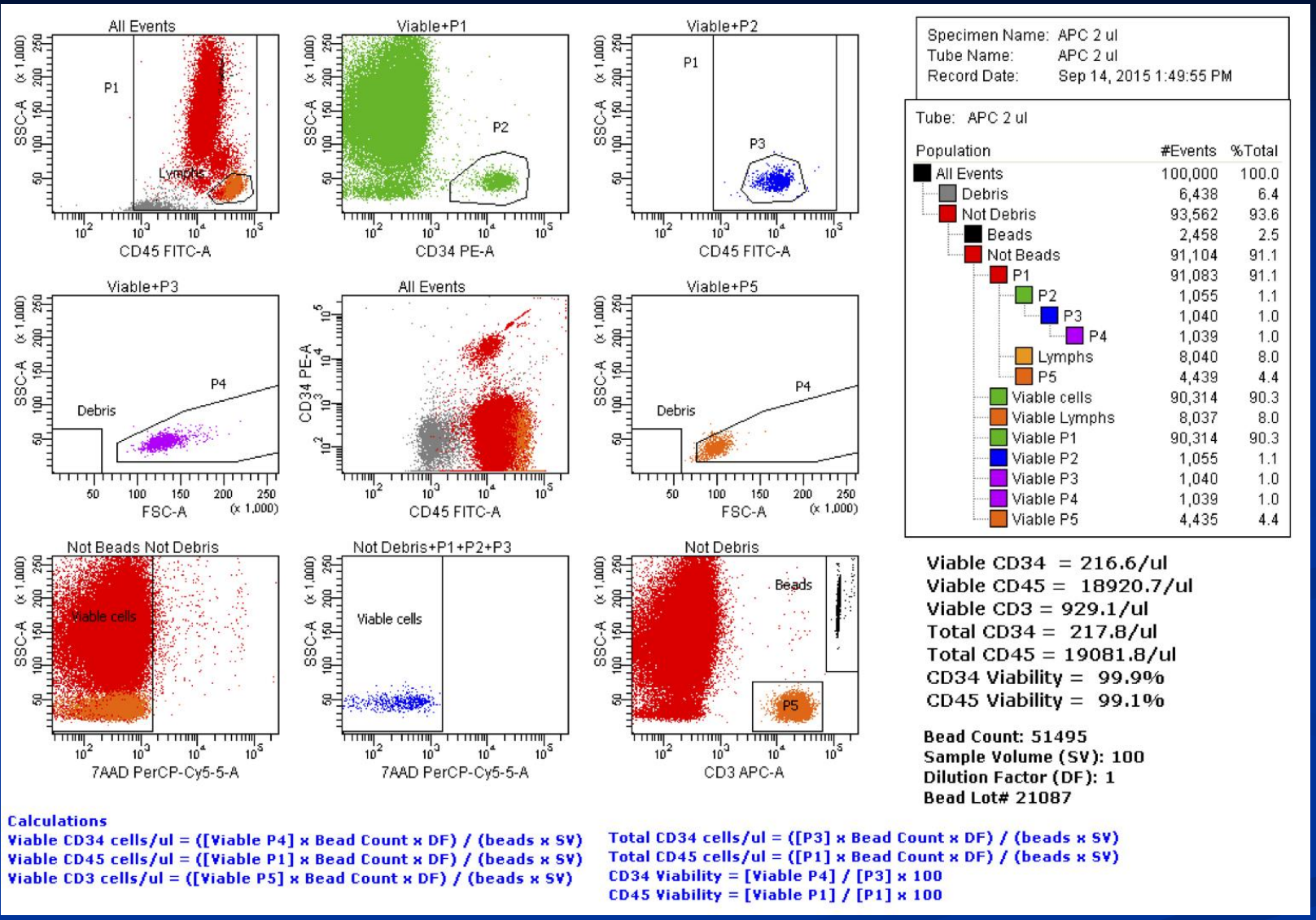
dilution factor 1.00

Sample volume 100.00 ul

BD
Calibur

CD45-FITC
CD34-PE
7-AAD
CD3-APC
Trucount

SP ISHAGE Protocol for Allograft Assessment



Specimen Name: APC 2 ul
Tube Name: APC 2 ul
Record Date: Sep 14, 2015 1:49:55 PM

Tube: APC 2 ul

Population	#Events	%Total
All Events	100,000	100.0
Debris	6,438	6.4
Not Debris	93,562	93.6
Beads	2,458	2.5
Not Beads	91,104	91.1
P1	91,083	91.1
P2	1,055	1.1
P3	1,040	1.0
P4	1,039	1.0
Lymphs	8,040	8.0
P5	4,439	4.4
Viable cells	90,314	90.3
Viable Lymphs	8,037	8.0
Viable P1	90,314	90.3
Viable P2	1,055	1.1
Viable P3	1,040	1.0
Viable P4	1,039	1.0
Viable P5	4,435	4.4

Viable CD34 = 216.6/ul
Viable CD45 = 18920.7/ul
Viable CD3 = 929.1/ul
Total CD34 = 217.8/ul
Total CD45 = 19081.8/ul
CD34 Viability = 99.9%
CD45 Viability = 99.1%

Bead Count: 51495
Sample Volume (SV): 100
Dilution Factor (DF): 1
Bead Lot# 21087

Calculations

Viable CD34 cells/ul = ([Viable P4] x Bead Count x DF) / (beads x SV)
Viable CD45 cells/ul = ([Viable P1] x Bead Count x DF) / (beads x SV)
Viable CD3 cells/ul = ([Viable P5] x Bead Count x DF) / (beads x SV)

Total CD34 cells/ul = ([P3] x Bead Count x DF) / (beads x SV)
Total CD45 cells/ul = ([P1] x Bead Count x DF) / (beads x SV)
CD34 Viability = [Viable P4] / [P3] x 100
CD45 Viability = [Viable P1] / [P1] x 100

BD
Canto II

CD45-FITC
CD34-PE
7-AAD
CD3-APC
Trucount

SP ISHAGE Protocol for Allograft Assessment

Addition of CD3 conjugate to Single Platform ISHAGE protocol allows:

1. Simultaneous Enumeration of absolute viable CD34+ and viable CD3+ cell numbers
2. High-sensitivity detection and enumeration of contaminating CD3+ cells in CD34-selected samples for non-matched transplants
3. Accurate enumeration of viable CD3+ cells for Donor Lymphocyte Infusions can be performed using this same protocol
4. Can be used on any cytometer with 4 or more PMTs

CD34+ CELL ENUMERATION: FAQ

Michael Keeney and D. Robert Sutherland

http://www.cytometry.org/public/index.php

TABLE OF CONTENTS

CD34+ CELL ENUMERATION: FAQ
http://www.cytometry.org/public/index.php

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SP ISHAGE WITH VIABILITY ASSESSMENT

Non-fresh samples MUST be analyzed only with Single Platform ISHAGE

- shipped, CD34-selected, purged or manipulated

Post-thawed samples MUST be analyzed only with SP ISHAGE because the '%CD34' value in DP ISHAGE increases due to loss of most granulocytes post thaw

If pre-freeze WBC count is used with post thaw '%CD34+' in DP ISHAGE, more than 100% recovery of CD34+ cells is almost guaranteed!

- how this happens is still a mystery to some!!

Hematology analyzers inaccurate on post-thawed samples !!

ENUMERATING VIABLE CD34+ CELLS IN POST-THAWED SAMPLES

How are samples processed/frozen?- many differences

How are samples thawed?

If samples diluted post thaw,

- Which diluent and how much, and at what temperature?
- How is it done; dump dilution or drop-wise and how slowly?

Is centrifugation/washing employed after dilution?

How is staining performed;

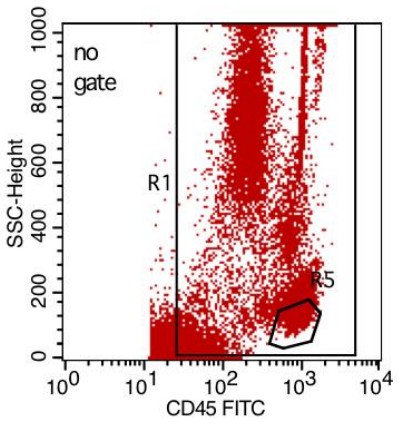
- In the cold or room temperature
- How long?

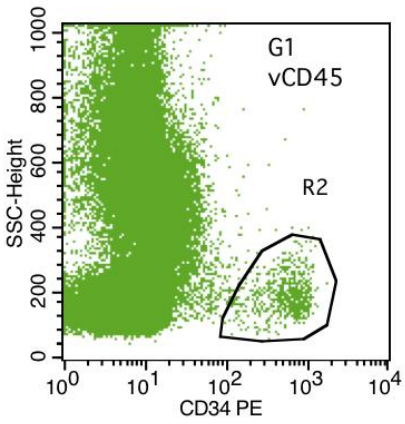
After staining, is lysing agent used?

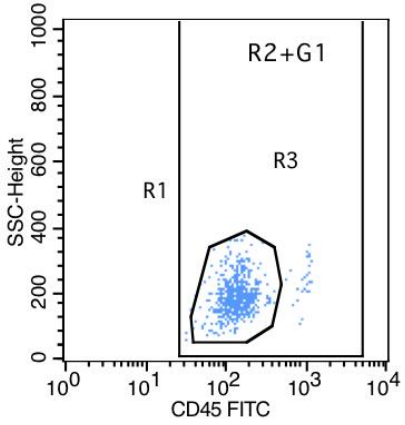
- Lysing agents increase prep time by 10 minutes at room temp during which cell death and apoptosis are increased

Despite Kit manufacturers' recommendations, lysing agents ARE NOT recommended by authors of ISHAGE protocols!!

POST-THAWED CORD BLOOD; NH_4Cl LYSE

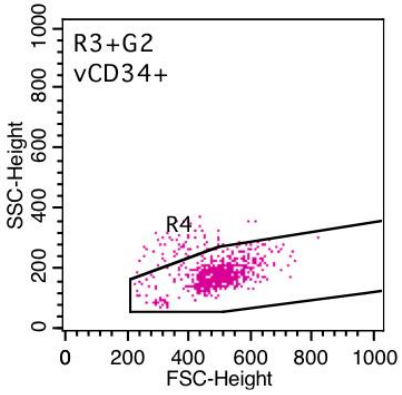


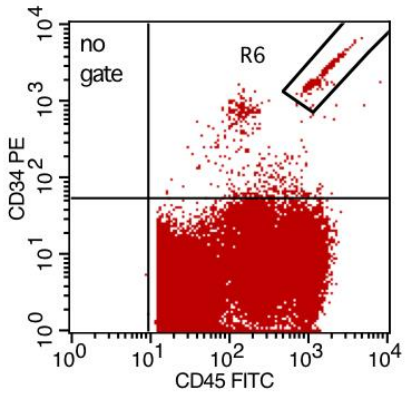


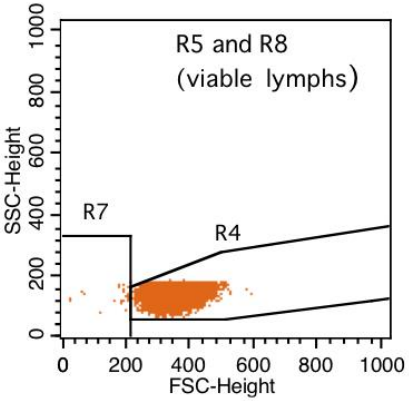


File: 031006.003
Sample ID: PW-A lyse
Acquisition Date: 10-Mar-06
Gate: No Gate
Gated Events: 280825
Total Events: 280825

Gate	Events	% Gated
v CD45	61100	21.76
G2	665	0.24
G3	636	0.23
v CD34	573	0.20
Beads	16868	6.01
all CD34	662	0.24
lymphs	19296	6.87
all CD45	99663	35.49
Debris	164087	58.43
R9 beads	29	0.01







viable CD34= 33.83 /ul

all CD34= 39.09 /ul

CD34 viability= 86.56 %

viable CD45= 3607.75 /ul

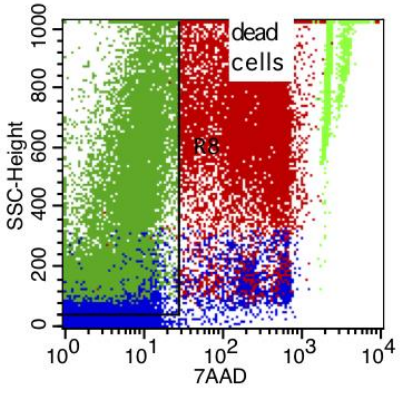
all CD45= 5884.77 /uL

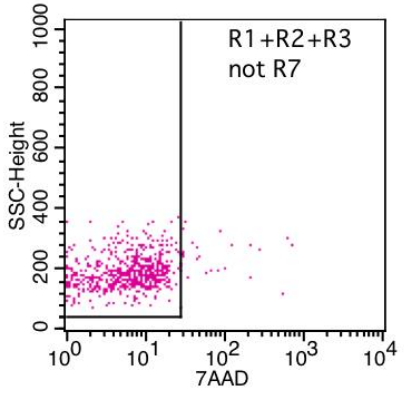
% Viable CD45= 61.31

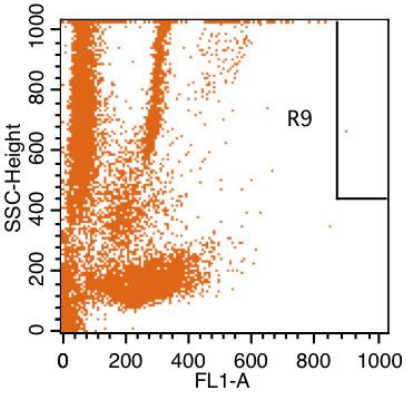
bead count = 49800.00

dilution factor 1.00

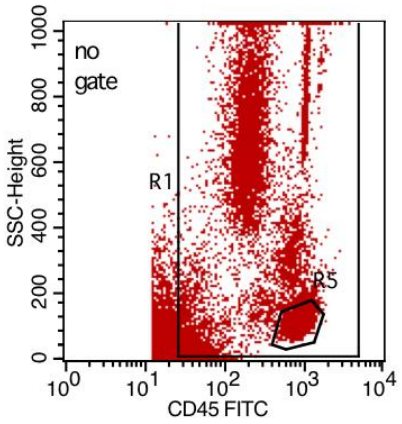
Sample volume 50.00 ul

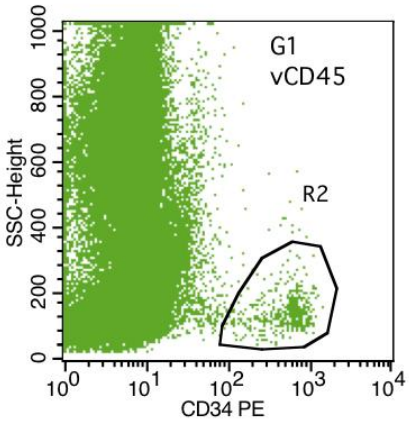


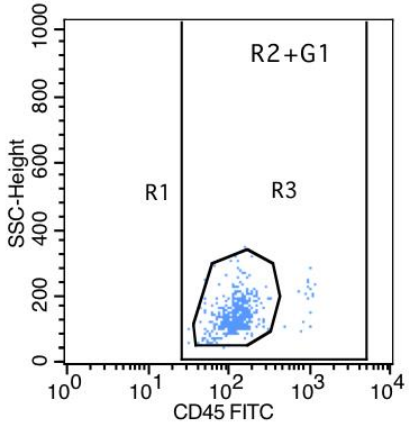




POST-THAWED CORD BLOOD; NO LYSE

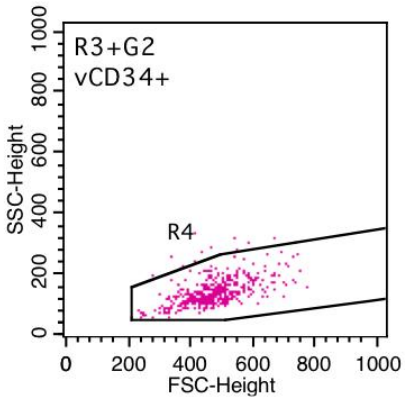


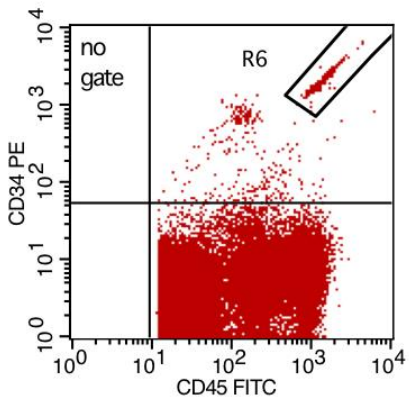


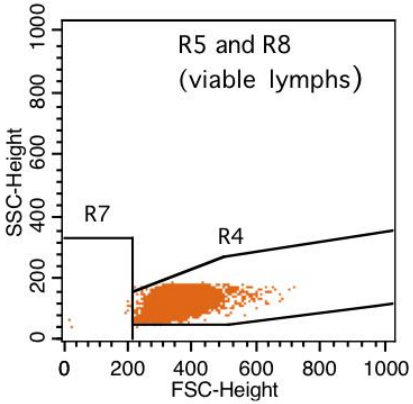


File: 031006.004
Sample ID: PW-A without lyse pbs/ bs
Acquisition Date: 10-Mar-06
Gate: No Gate
Gated Events: 189427
Total Events: 189427

Gate	Events	% Gated
v CD45	65093	34.36
G2	475	0.25
G3	451	0.24
v CD34	440	0.23
Beads	10795	5.70
all CD34	469	0.25
lymphs	17547	9.26
all CD45	78115	41.24
Debris	92965	49.08
R9 beads	28	0.01







viable CD34= 40.60 /ul

all CD34= 43.27 /ul

CD34 viability= 93.82 %

viable CD45= 6005.80 /ul

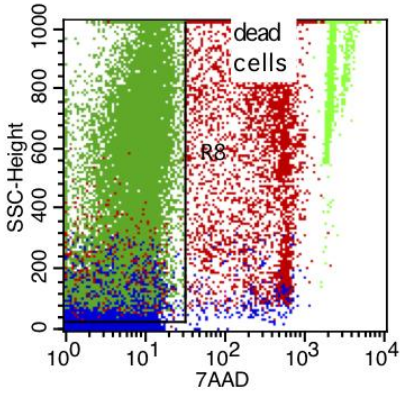
all CD45= 7207.28 /uL

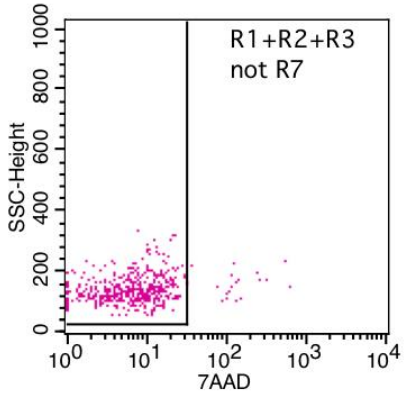
% Viable CD45= 83.33

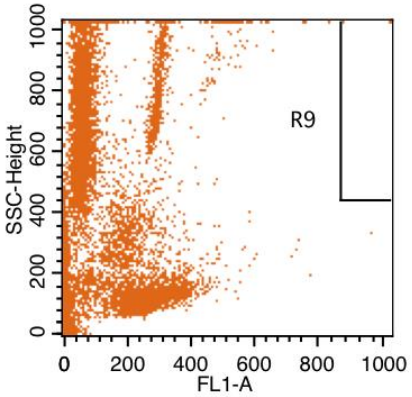
bead count = 49800.00

dilution factor 1.00

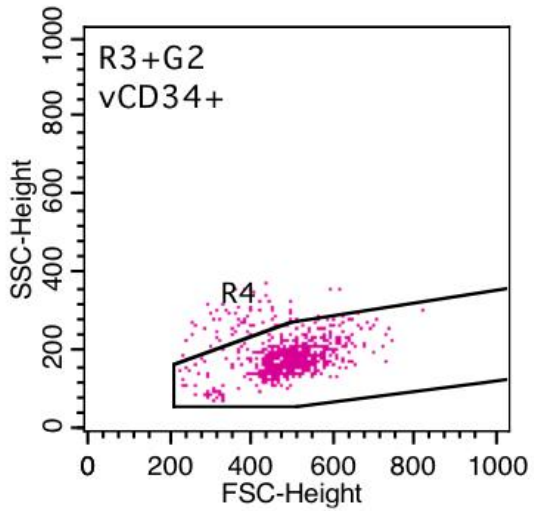
Sample volume 50.00 ul







NH_4CL-LYSE
10 min @ RT



viable CD34= 33.83 /ul

all CD34= 39.09 /ul

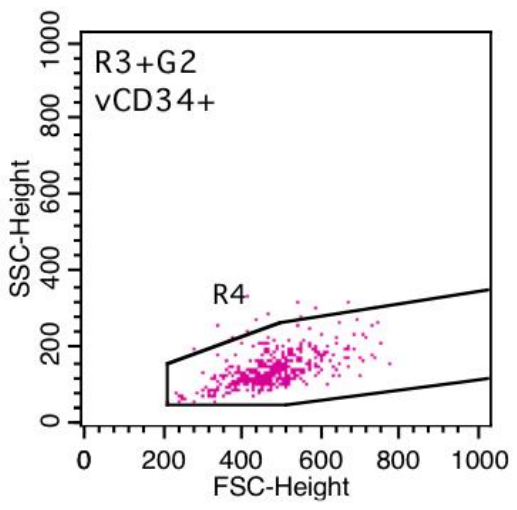
CD34 viability= 86.56 %

viable CD45= 3607.75 /ul

all CD45= 5884.77 /uL

% Viable CD45= 61.31

No LYSE
Acquired ASAP



viable CD34= 40.60 /ul

all CD34= 43.27 /ul

CD34 viability= 93.82 %

viable CD45= 6005.80 /ul

all CD45= 7207.28 /uL

% Viable CD45= 83.33

Fig 56-6 from Sutherland DR, Keeney M. Enumeration of CD34+ cells by Flow Cytometry. In: AABB Cell Therapy Technical Manual, Bethesda MD. 2nd Edition. Chapter 56; pp 558-569, 2016.

Acknowledgments

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Ahmad Al-Attar