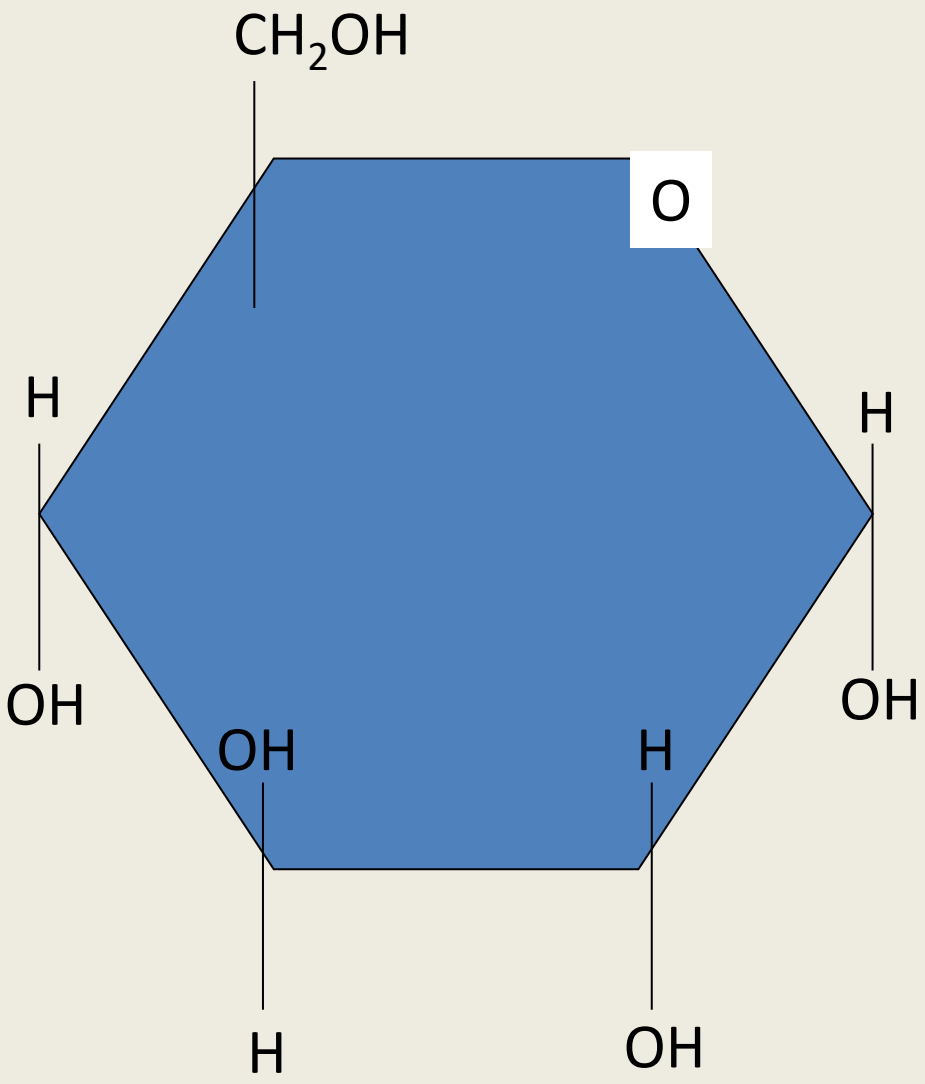
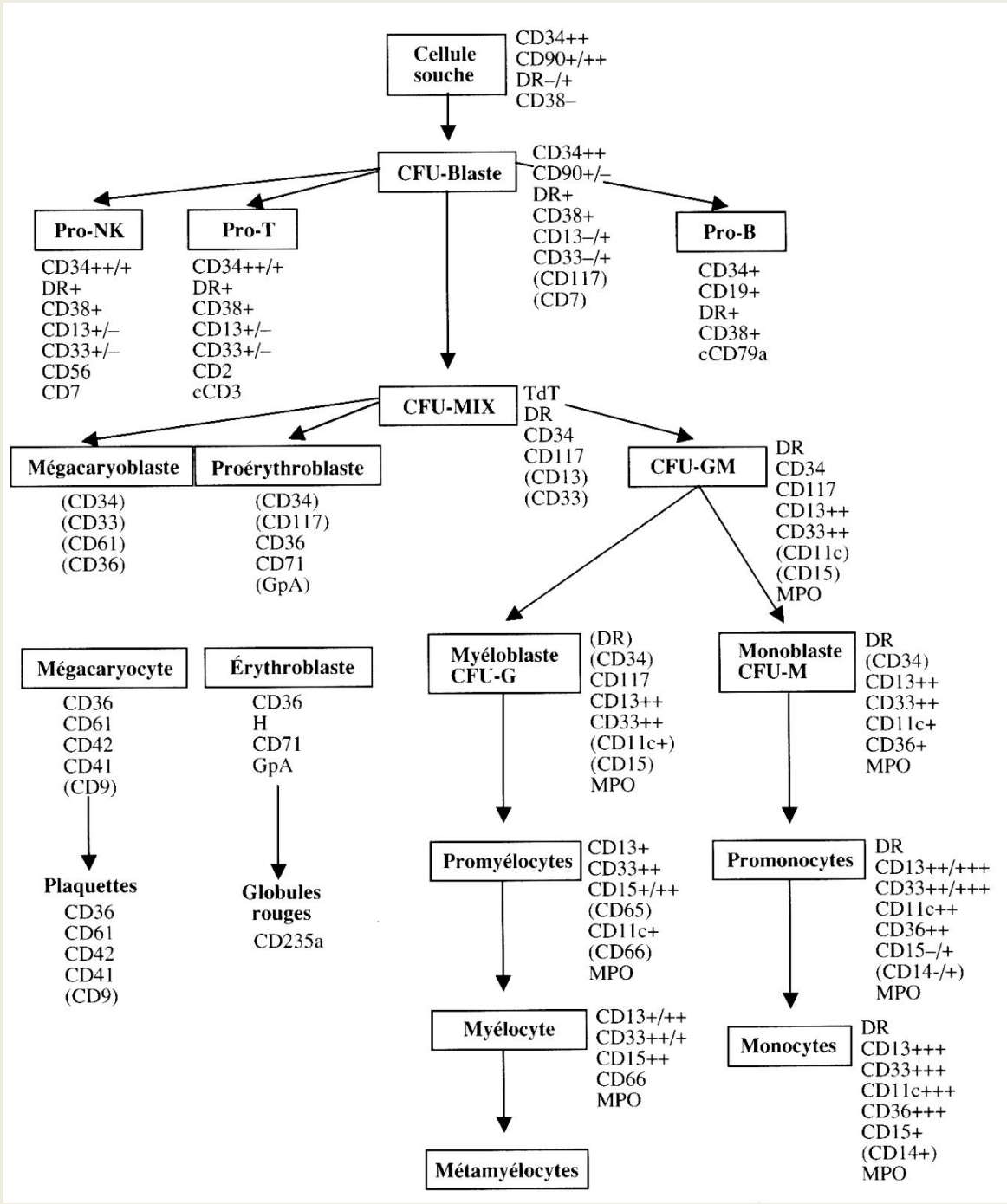
Method validation in flow cytometry Discussion of guidelines

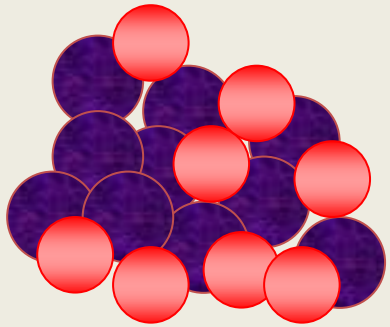
A. Gothot

Liège University Hospital

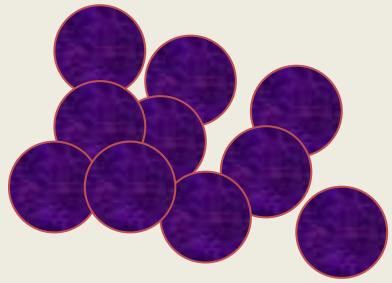

CH2OH
O
H
H
OH
OH
OH
H
H
OH


Cellule souche
CD34++
CD90+/++
DR–/+
CD38–
CFU-Blaste
CD34++
CD90+/–
DR+
CD38+
CD13–/+
CD33–/+
(CD117)
(CD7)
Pro-NK
CD34++/+
DR+
CD38+
CD13+/–
CD33+/–
CD56
CD7
Pro-T
CD34++/+
DR+
CD38+
CD13+/–
CD33+/–
CD2
cCD3
Pro-B
CD34+
CD19+
DR+
CD38+
cCD79a
CFU-MIX
TdT
DR
CD34
CD117
(CD13)
(CD33)
Mégacaryoblaste
(CD34)
(CD33)
(CD61)
(CD36)
Proérythroblaste
(CD34)
(CD117)
CD36
CD71
(GpA)
CFU-GM
DR
CD34
CD117
CD13++
CD33++
(CD11c)
(CD15)
MPO
Mégacaryocyte
CD36
CD61
CD42
CD41
(CD9)
Plaquettes
CD36
CD61
CD42
CD41
(CD9)
Érythroblaste
CD36
H
CD71
GpA
Globules rouges
CD235a
Myéloblaste CFU-G
(DR)
(CD34)
CD117
CD13++
CD33++
(CD11c+)
(CD15)
MPO
Monoblaste CFU-M
DR
(CD34)
CD13++
CD33++
CD11c+
CD36+
MPO
Promyélocytes
CD13+
CD33++
CD15+/++
(CD65)
CD11c+
(CD66)
MPO
Promonocytes
DR
CD13++/+++
CD33++/+++
CD11c++
CD36++
CD15–/+
(CD14-/+)
MPO
Myélocyte
CD13+/++
CD33++/+
CD15++
CD66
MPO
Monocytes
DR
CD13+++
CD33+++
CD11c+++
CD36+++
CD15+
(CD14+)
MPO
Métamyélocytes

Blood
Marrow
Lymph node
CSF

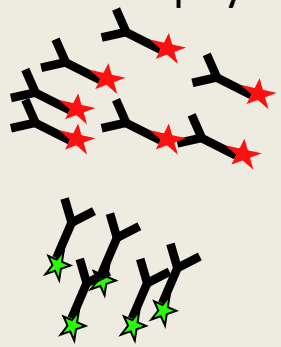


Dissociation
Red cell lysis



Incubation with fluorescence-tagged antibodies

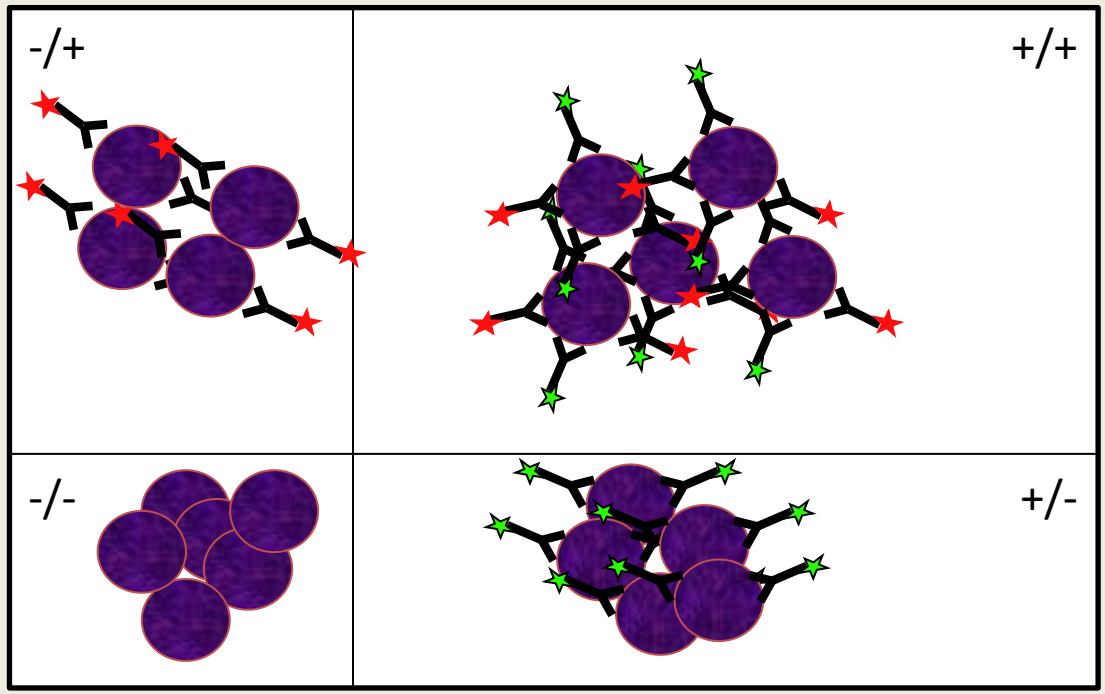
phycoerythrin



fluorescein

cytometer

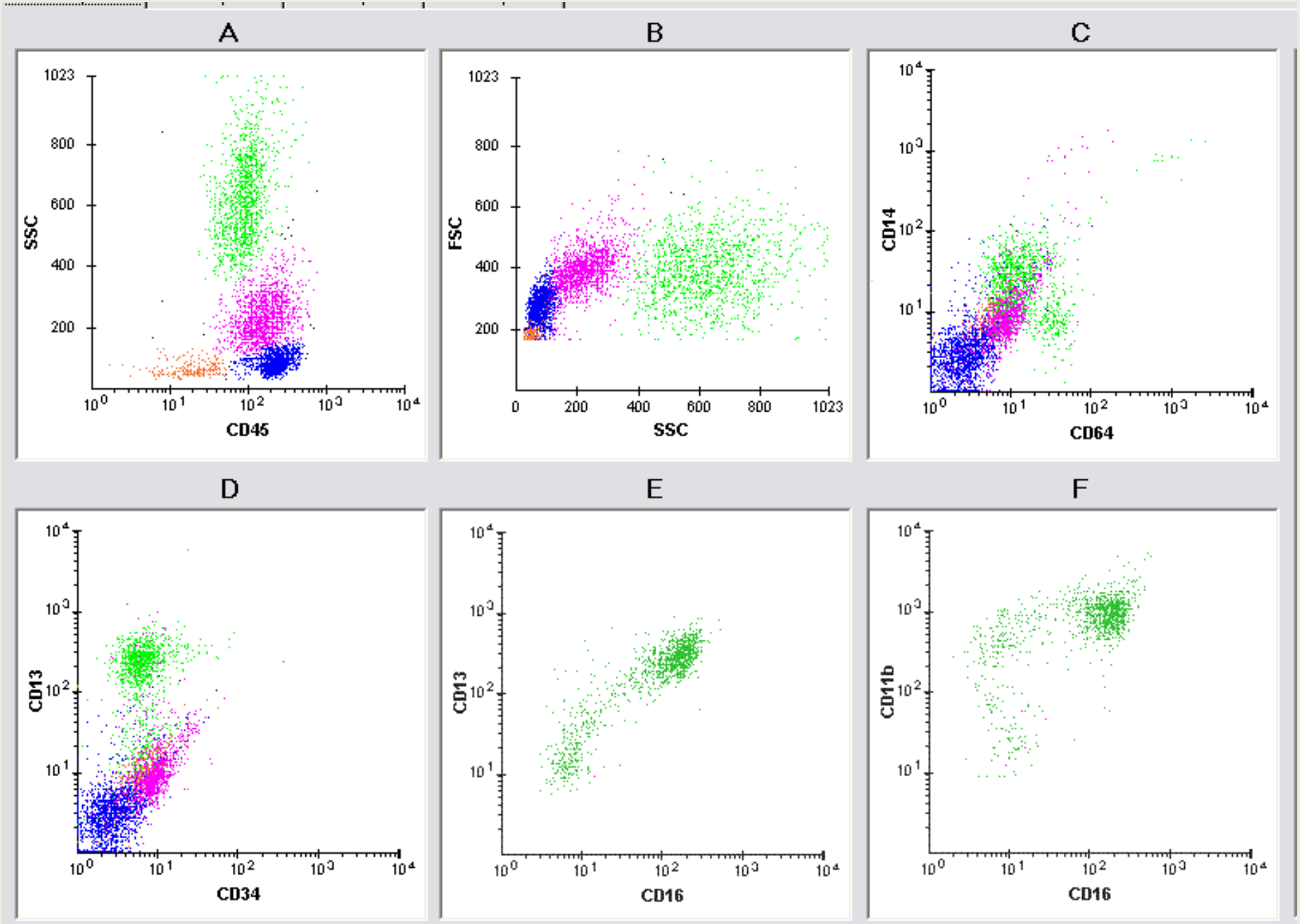
Dot plot



Fluorochrome	Abbreviation	Excitation max (nm)	Emisson max (nm)
Cascade blue		380, 401	419
Cascade yellow		399	549
Pacific blue		410	455
Alexa 488*		495	519
Fluorescein isothiocyanate*	FITC	494	519
Phycoerythrin*	PE	496, 546	578
Texas red*	ECD	595	615
PE-cyanine 5*	PC5/PE-Cy5	496, 546	667
PE-cyanine 5.5*	PC5.5/PE-Cy5.5	495, 564	696
PE-cyanine 7*	PC7/PE-Cy7	495, 564	767
Peridinin-chlorophyll*	PerCP	482	678
PerCP-cyanine 5.5	PerCP-Cy5.5	482	678
Allophycocyanin*	APC	650	660
APC-cyanine 7	APC-Cy7	650	785

What is your favourite colour?

In clinical flow cytometry (2024): standard = 8 to 12 colour combinations


A
SSC
CD45
B
FSC
SSC
C
CD14
CD64
D
CD13
CD34
E
CD13
CD16
F
CD11b
CD16

Cytometry Part B (Clinical Cytometry) 84B:135–142 (2013)

Original Article

Accreditation of Flow Cytometry in Europe

Ulrich Sack,[1*] David Barnett,[2] Gulderen Yanikkaya Demirel,[3] Chantal Fossat,[4] Stephan Fricke,[5] Nikolitsa Kafassi,[6] Thomas Nebe,[7] Katherina Psarra,[8] Jörg Steinmann,[9] and Claude Lambert[10]

What should be done to support accreditation in flow cytometry?

- Recommendation for daily cytometer operation
- Offering comparative calibration procedures between laboratories
- Advice on how to validate cytometric protocols
- Recommendation of harmonised validated and well documented antibody panels with known reference values and clear indications
- Organization of interlaboratory comparisons of best practices
- Providing an open access library of ‘‘reference cases’’ and variants (images and FCS files)

Cytometry Part B (Clinical Cytometry) 72B:S3 (2007)

Perspective

2006 Bethesda International Consensus Conference on Flow Cytometric Immunophenotyping of Hematolymphoid Neoplasia

Maryalice Stetler-Stevenson,[1*] Bruce Davis,[2] Brent Wood,[3] and Raul Braylan[4]

Leukemia (2012) **26**, 1908–1975

www.nature.com/leu

Open

ORIGINAL ARTICLE

EuroFlow antibody panels for standardized *n*-dimensional flow cytometric immunophenotyping of normal, reactive and malignant leukocytes

JJM van Dongen[1], L Lhermitte[2], S Böttcher[3], J Almeida[4], VHJ van der Velden[1], J Flores-Montero[4], A Rawstron[5], V Asnafi[2], Q Lécrevisse[4], P Lucio[6], E Mejstrikova[7], T Szczepański[8], T Kalina[7], R de Tute[5], M Brüggemann[3], L Sedek[8], M Cullen[5], AW Langerak[1], A Mendonça[6], E Macintyre[2], M Martin-Ayuso[9], O Hrusak[7], MB Vidriales[10] and A Orfao[4] on behalf of the EuroFlow Consortium (EU-FP6, LSHB-CT-2006-018708)

Cytometry Part B (Clinical Cytometry) 84B:315–323 (2013)

Validation of Cell-based Fluorescence Assays: Practice Guidelines from the ICSH and ICCS – Part V – Assay Performance Criteria

Brent Wood,[1] Dragan Jevremovic,[2] Marie C. Béné,[3] Ming Yan,[4] Patrick Jacobs,[5] Virginia Litwin[6*]; on behalf of ICSH/ICCS Working Group[1]

bjh guideline

Guidelines on the use of multicolour flow cytometry in the diagnosis of haematological neoplasms

Ulrika Johansson,[1] David Bloxham,[2] Stephen Couzens,[3] Jennifer Jesson,[4] Ricardo Morilla,[5] Wendy Erber,[6] Marion Macey[7] and British Committee for Standards in Haematology

Guidelines of international workshops
Main references

- US-Canadian consensus 1997 → Bethesda conference 2006 (Cytometry B, 72B:S3, 2007)
  - Medical indications
  - **Training/education**
  - **Report format**
  - Optimal reagents
- EuroFlow consortium 2012 (Leukemia, vol 26, 2012)
  - **Instrument standardization**
  - **Antibody panels**
- ICCS/ICSH 2012 (Cytometry B, 84B, 2013)
  - Preanalytical issues
  - Analytical issues (instrument setting, compensation, controls)
  - Postanalytical issues (reports, education, QC)
  - **Assay performance criteria** (quantitative assays) – part V
- BCSH 2014 (BJH, 165:455, 2014)
  - **Process validation** for diagnosis of hematological neoplasms
  - Concise, practical and graded recommandations on all aspects of qualitative FC

Cytometry Part B (Clinical Cytometry) 84B:315–323 (2013)

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I. Performance assessment of (quasi)quantitative assays ICSH guidelines – part V

Lymphocyte subsets

Immune deficiencies

CD34+ enumeration

Paroxysmal Nocturnal Hemoglobinuria (PNH)

Measurable Residual Disease (MRD)

Accuracy

- Agreement between the average of a series of test results and an accepted reference value
- Alternatives to reference materials: EQA programs or externalized IQC
  - Z-score and/or mean bias of 10 samples from an EQA program
  - Requirements:
    - 90% agreement, i.e., 9/10 samples within z-scores <2.5 (UK NEQAS)
    - Maximal bias (Ricos, www.westgard.com) for cells counts (hemogram):

		typical value (% leucocytes)
Neutrophils:	9.25%	65%
Lymphocytes:	9.19%	30%
Monocytes:	13.2%	10%
Eosinophils:	19.8%	4%
Basophils:	15.4%	1%

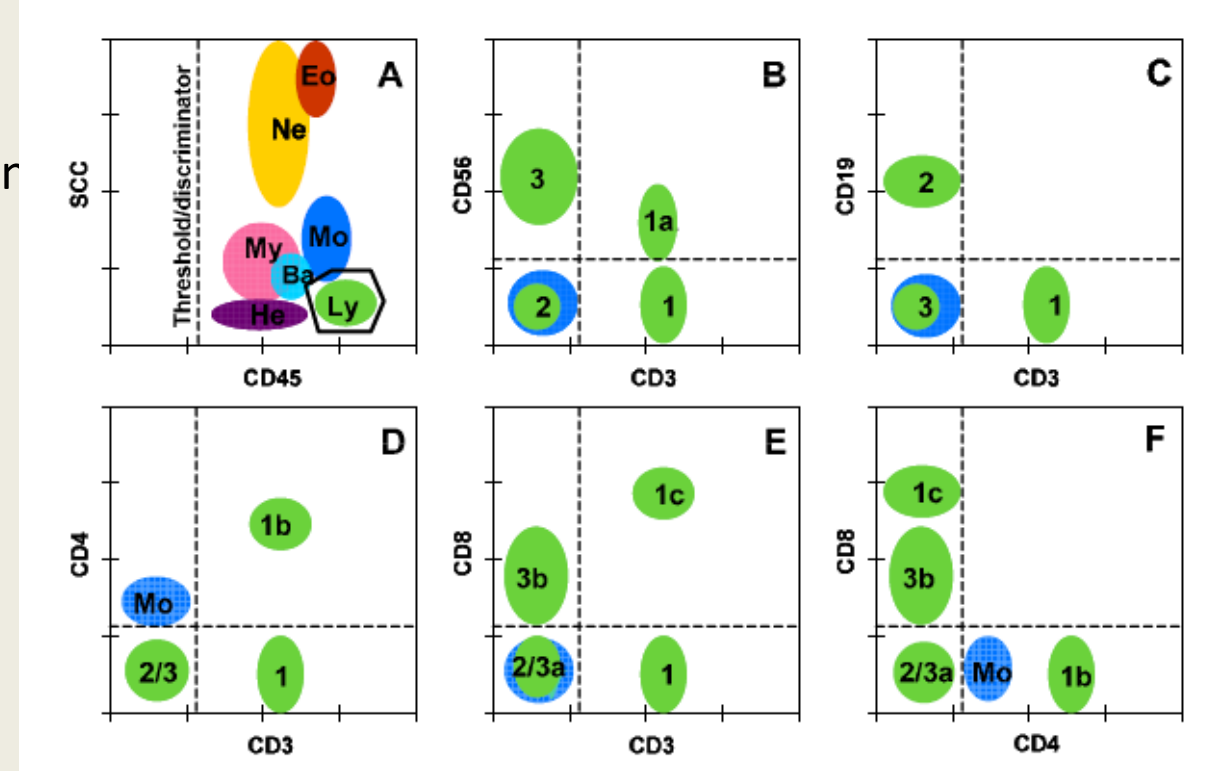
Bias

Cell Population	Your Results (cells/µL)	Robust Mean (cells/µL)	
CD34 Absolute Values	12.60	13.20	4.5%

Cell Population	Your Results (%)	Median Result (%)	Lower Quartile (%)	Upper Quartile (%)	
Red Blood Cells PNH Clone	1.31	1.26	1.10	1.59	4.0%
Monocytes PNH Clone	2.00	2.19	0.62	3.27	8.7%
Granulocytes PNH Clone	3.23	3.13	2.74	3.50	3.2%

Specificity

- Antibody specificity
  - Leucocyte differentiation antigen workshop: www.hcdm.org
- Gating strategies:
  - Cell subset of interest included
  - Exclusion of other subsets
  - Exclusion of doublets

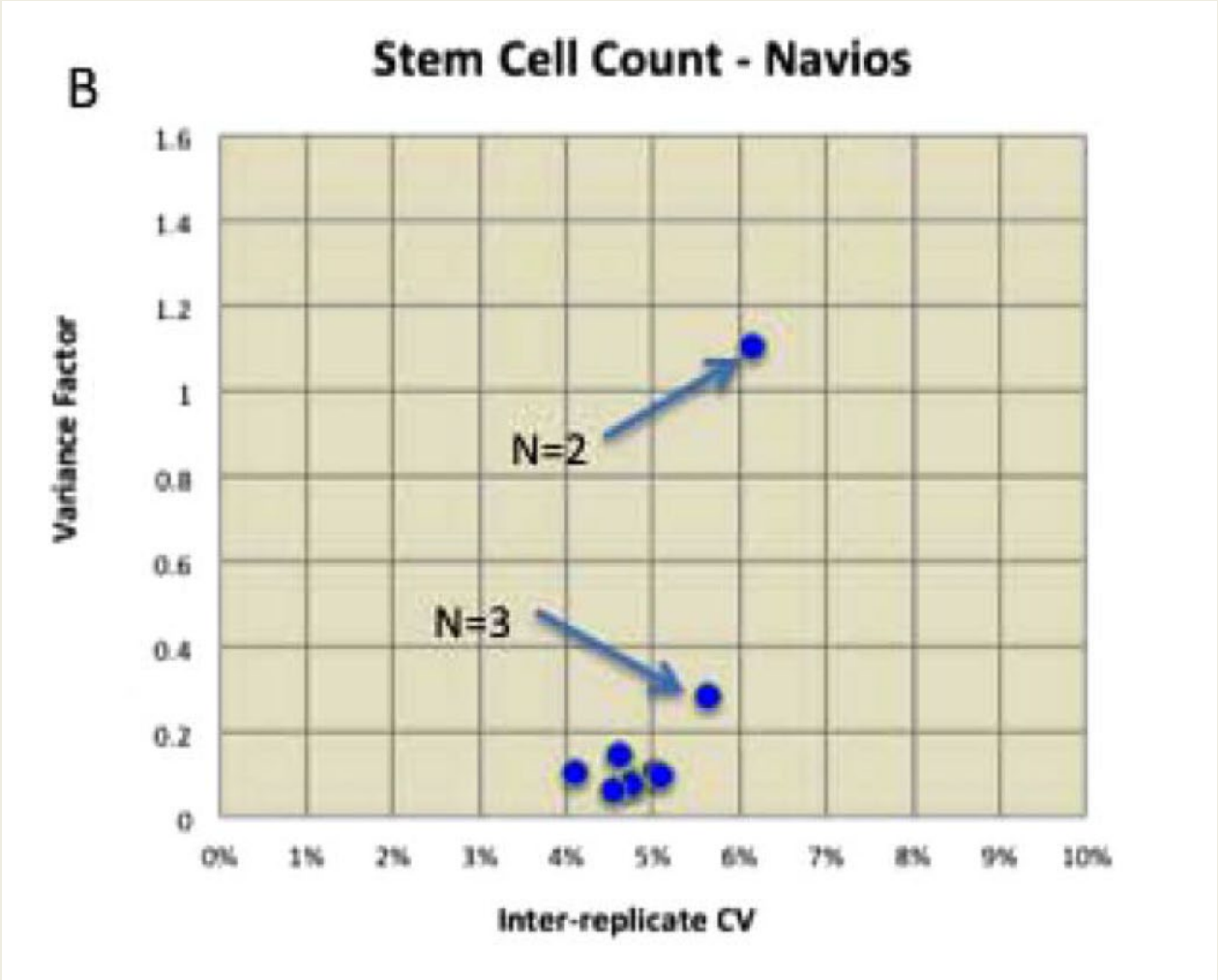


Analytical sensitivity

- Limit of blank (LOB)
  - Highest apparent signal measure with a negative sample
  - $Mean_{blank} + 1{,}645\ SD_{blank}$
  - 95% of negative samples < LOB
- Limit of detection (LOD)
  - $LOB + 1{,}645\ SD_{low\ positive}$
  - 95% of low positive samples > LOD

Experimental plan for LOB/LOD

- Blank samples:
  - FMO: may understimate background (background generated by the test reagent not measured)
  - Isotypic control: must match exactly the test reagent
  - Internal cell controls: ex CD3 expression of B lymphocytes
  - Confirm that 95% of results < LOB
- Low positive:
  - Typically clinically relevant low positive: ex 0,01 % PNH cells or 10 CD34+ cells/µl
  - Confirm that 95% of results > LOD


B
Stem Cell Count - Navios
Variance Factor
1.6
1.4
1.2
1
0.8
0.6
0.4
0.2
0
N=2
N=3
0%
1%
2%
3%
4%
5%
6%
7%
8%
9%
10%
Inter-replicate CV

Experimental plan for LOB/LOD

Negative sample and low positive sample	Replicates (i.e., 5 ≠ staining procedures)	# list mode files	Day
1	5	5 LMD/replicate	1
2	5	5 LMD/replicate	1
3	5	5 LMD/replicate	2
4	5	5 LMD/replicate	2
5	5	5 LMD/replicate	3

5 negative samples, 5 replicate stainings, 5 acquisitions = 125 results
5 low positive samples, 5 replicate stainings, 5 acquisitions = 125 results

« Functional » sensitivity

- Lower limit of quantification (LLOQ)
  - ≥ LOD
  - Lowest level of measurand that can be reliably detected and whose total error (TE = bias + 2 SD) meets a desired specification
- Bias unknown →TE = 2 x %CV
- Assay 5 replicates of a low positive sample
  - CV < 10% or < 20% (if frequency ~ 1:1000 or less)

Imprecision

Intra-assay imprecision

- 5 samples spanning the clinical range
- Calculate mean, SD, %CV
- Target imprecision?
  - CV < 10% or < 20% (if frequency ~ 1:1000 or less)
- How many replicates?
  - **Not more than 4 for most assays**

Cytometry Part B (Clinical Cytometry) 84B:329–337 (2013)

Original Article

Determination of Optimal Replicate Number for Validation of Imprecision Using Fluorescence Cell-Based Assays: Proposed Practical Method

Bruce H. Davis,[1*] Christine E. McLaren,[2] Anthony J. Carcio,[1] Linda Wong,[1] Benjamin D. Hedley,[3] Mike Keeney,[3] Adam Curtis,[1] and Naomi B. Culp[1]

[1]Trillium Diagnostics, LLC, Bangor, Maine 04401

[2]Department of Epidemiology, University of California Irvine, Irvine, California 92697-7550

[3]London Laboratory Services Group, London Health Sciences Centre, London, Ontario N6A 5W9, Canada

Our findings indicate that for the four assays selected for analysis, replicates of less than five would be sufficient to achieve detection of imprecision.

Imprecision

Inter-assay imprecision

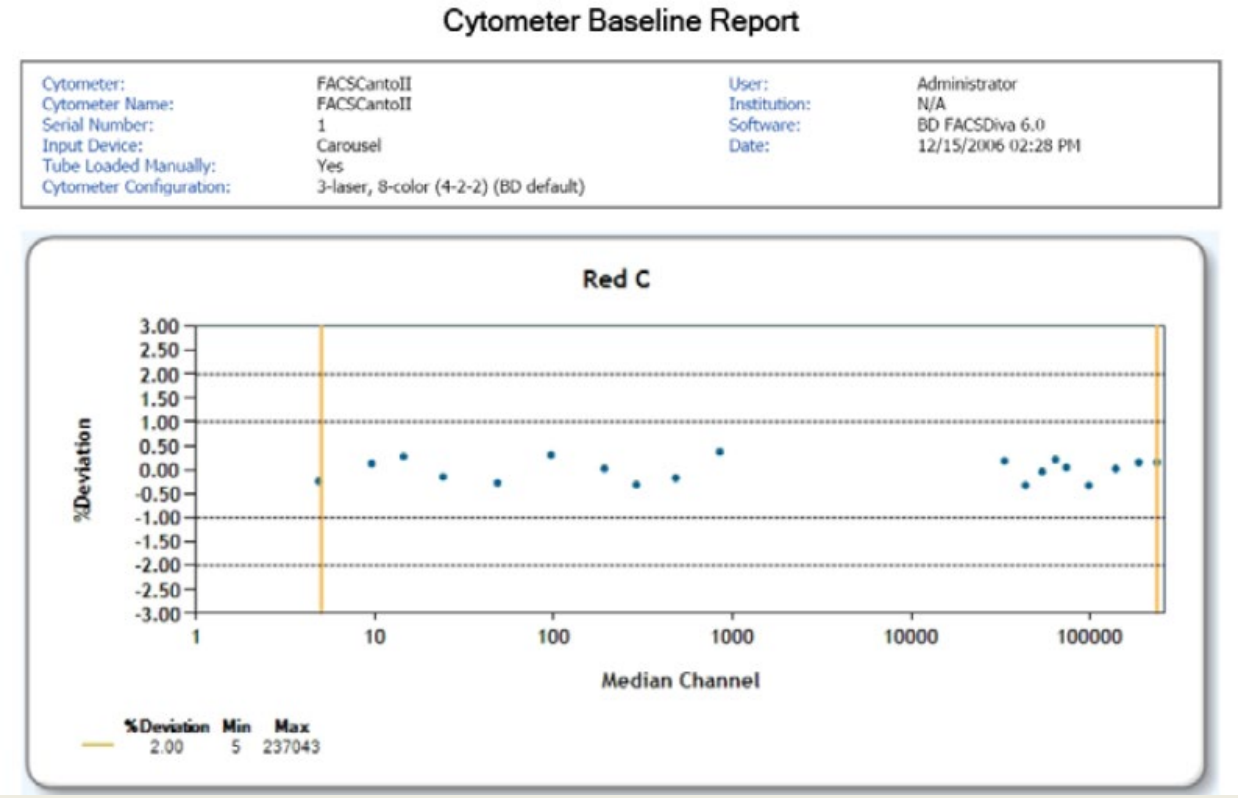
- 2 levels of stabilized control samples assayed in triplicates over 3 days
- If unavailable, multiple runs on the same day with intercalating shutdowns/restart/instrument monitoring procedures
- Calculate mean, SD and %CV

Imprecision (intra- and inter-assay)

- **~ frequency of target population**
- **Number of events collected**
  - **In MRD: 4-5 million cells**

Instrument linearity

Instrument linearity is assessed by the manufacturer's procedure (range for each channel): check that bright positives are within the linearity range



Analyte linearity

- Lymphs subsets, MRD, CD34+ cells
  - Serial dilutions of a stained sample in unstained cells (-CD19)
  - Deviation from expected result < 10% or <20% for rare events

			B cells	% lymphs		
1	0,7	1,2	2,7	6,7	9,2	13,5
2	0,7	1,3	2,7	6,7	9,3	13,7
3	0,8	1,2	2,3	6,6	9,1	13,5
4	0,6	1,2	2,6	6,6	9,1	13,1
obtained mean	0,700	1,225	2,575	6,650	9,175	13,450
reference mean						13,45
dilution factor	0,05	0,10	0,20	0,50	0,70	1,00
expected value	0,673	1,345	2,690	6,725	9,415	
difference (abs)	-0,028	0,120	0,115	0,075	0,240	
difference (%)	-3,93	9,80	4,47	1,13	2,62	
evaluation limits (abs)	0,07	0,13	0,27	0,67	0,94	
evaluation limits	10%	10%	10%	10%	10%	
corr coeff	0,999835					

Carry-over

- Carry-over is instrument-dependent (not sample or assay-dependent)
  - Flow cytometer
  - Lyse-wash assistant
  - Sample prep assistant

!! Each instrument should be tested separately

- 3 replicates of CLL sample (h1-h2-h3) + 3 replicates of unstained sample (l1-l2-l3): record % CD19+CD5+ cells
- % carry-over: (l1-l3)/(h3-l3)
- Acceptable carry-over?
  - Should be undetectable, if not contact technical service
  - < LOD

Unprocessed specimen stability

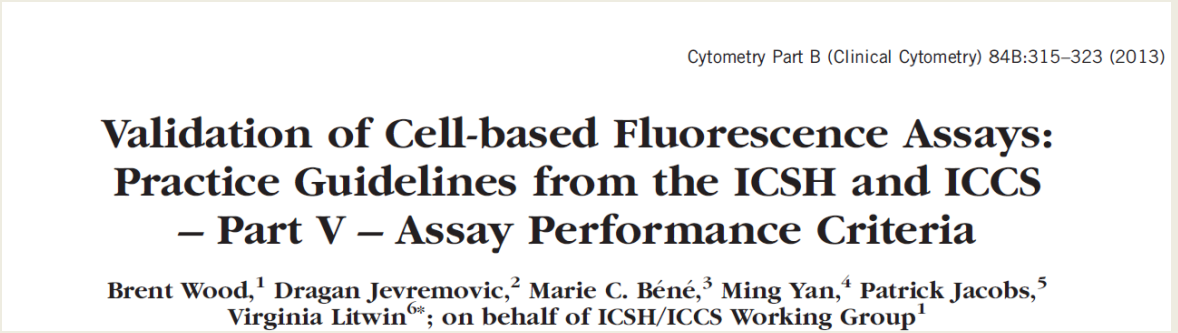
- Assay 5 healthy and diseased samples within 2h of collection and at various time points
- Storage conditions as for regular samples (RT)
- Stability must be evaluated for ≠ anticoagulants
- Record measurand and viability
- Performance criteria
  - < 20% change from baseline

Processed specimen stability

- How soon processed samples should be evaluated?
- Record measurand within 1h staining and at various time points
- Storage conditions same as for regular samples (4°c, in the dark)
- Performance criteria same as for unprocessed

Reagent stability

- Use reagents within manufacturer’s specifications
- If expired, equivalent performance must be documented
- Cocktails must be tested for stability under storage conditions and shelf-life as in routine
  - Compare cocktail results with single colour stainings
  - Use cocktail at various time points
- Specification criteria: < 20% deviation (2 interbatch CV)
  - 10% is the acceptable interbatch CV for reagent manufacturers



Cytometry Part B (Clinical Cytometry) 84B:315–323 (2013)

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Brent Wood,[1] Dragan Jevremovic,[2] Marie C. Béné,[3] Ming Yan,[4] Patrick Jacobs,[5] Virginia Litwin[6*]; on behalf of ICSH/ICCS Working Group[1]

II. Performance assessment of qualitative assays

Leukemia/lymphoma phenotyping

Accuracy

- Not relevant – absence of standards
- Comparison of flow cytometric assays to expected results with regard to clinical and other laboratory findings
  - 20 normal/20 abnormal samples per antibody combination

Analytical sensitivity (LOD/LOB)

- Distinction of an abnormal population from normal cells; recognition of abnormal level of antigen expression

BUT:

- Great variability in immunophenotype-related diseases
- Composition of normal subsets (internal controls) is variable

$\rightarrow$ assay sensitivity is likely to vary on a per sample basis

$\rightarrow$ <u>impossible to define general recommandations</u>

Clinical specificity and sentivity

- Specificity = TN/(TN+FP)
- Sensitivity = TP/(TP+FN)
- Realistic approach: compare patient cohorts with results from morphology, cytogenetics, molecular biology
- May not be possible for rare diseases

Table 5.18 Clinical sensitivity results. Final classification (from NCCLS, 1992 with permission)

		Test method		
		Positive	Negative	
Reference method	Positive	True positive	False negative	TP + FN
	Negative	False positive	True negative	TN + FP
		TP + FP	TN + FN	TP + TN + FP + FN

Imprecision

- Ensure that technical assay performance (instrument, antibodies, data analysis) is reproducible
  - Despite subjective analysis of the data
- 3 replicates of one normal and one abnormal sample with each antibody panel
  - CV of % identified cell populations
    - Target CV<10%
    - Target CV<20% acceptable if population <0,1%

Stability

- Unprocessed specimen: assays at various time points
  - General rule: stable specimen if concordant with baseline (Interpretation)
  - Data analysis must include viability assessment
    - Light scatter
    - Dedicated viability dyes for biopsies and body fluids
- Processed specimen: stained samples assayed at various time points
  - performance criteria: concordance with baseline

Reference range

- General standard used for reference ranges (CLSI C28)
  - 120 subjects, 60 males-60 females
  - If published ranges are used:
    - test a cohort of 20 subjects
    - Less than 10% outside published reference range → OK
- Context-specific reference range
  - Ex: 10 CD34+ cells/µl in mobilized peripheral blood → start apheresis
- Pediatric normal values published for lymphocyte subsets

Other criteria - conclusion

- Linearity, reportable range, reference intervals
  - not applicable

Validation of qualitative assays is intrinsically limited by the diversity of disease-related phenotypes, the rarity of typical samples, the heterogeneity of normal/abnormal subsets in each sample

→ Process validation, not assay validation

→ BCSH guidelines

bjh guideline

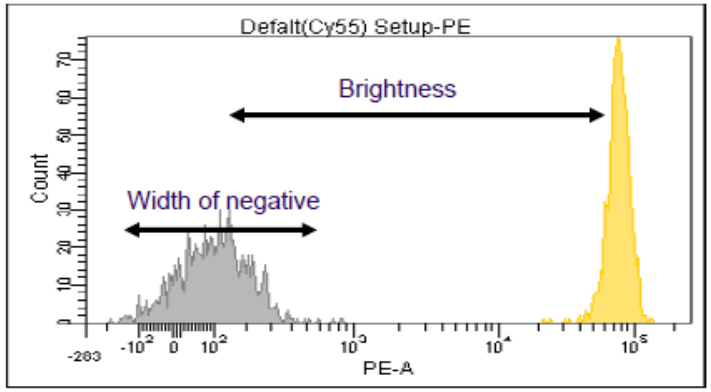
Guidelines on the use of multicolour flow cytometry in the diagnosis of haematological neoplasms

Ulrika Johansson,[1] David Bloxham,[2] Stephen Couzens,[3] Jennifer Jesson,[4] Ricardo Morilla,[5] Wendy Erber,[6] Marion Macey[7] and British Committee for Standards in Haematology

Instrumentation

- Optimize PMT
  - To maximize signal to noise ratio
  - To allow for measuring Ag expression within log ranges
  - Use instrument software

$$\text{Stain Index} = \frac{\text{Brightness}}{\text{Width of Negative}} = \frac{\text{median}_{\text{positive}} - \text{median}_{\text{negative}}}{2 \times \text{rSD}_{\text{negative}}}$$



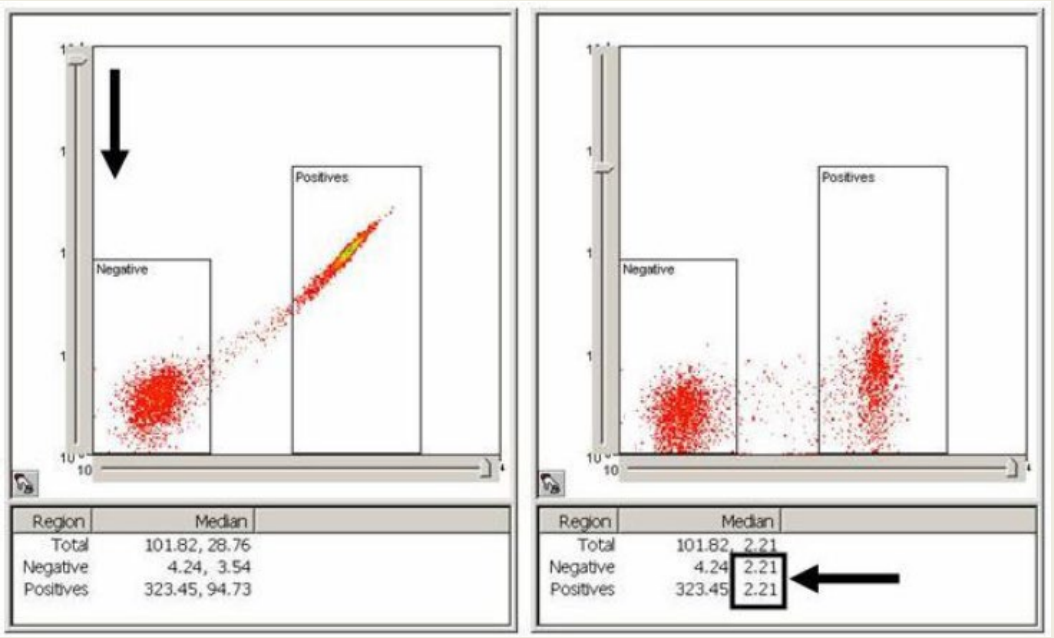
Experiment Name: CD4 Stain Intex (081304)
Tube Name: PE

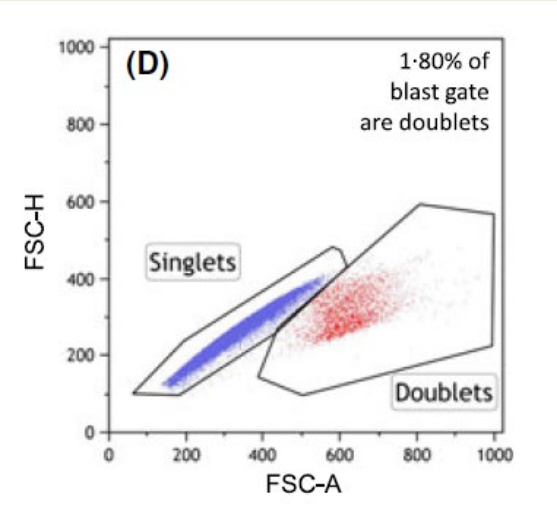
Population	#Events	%Parent	PE-A Median	PE-A rSD
PE-	827	48.9	102	97
PE+	780	46.1	75,652	12,616

$$\text{Stain Index} = \frac{75852 - 102}{2 \times 97} = 390.5$$

Instrumentation

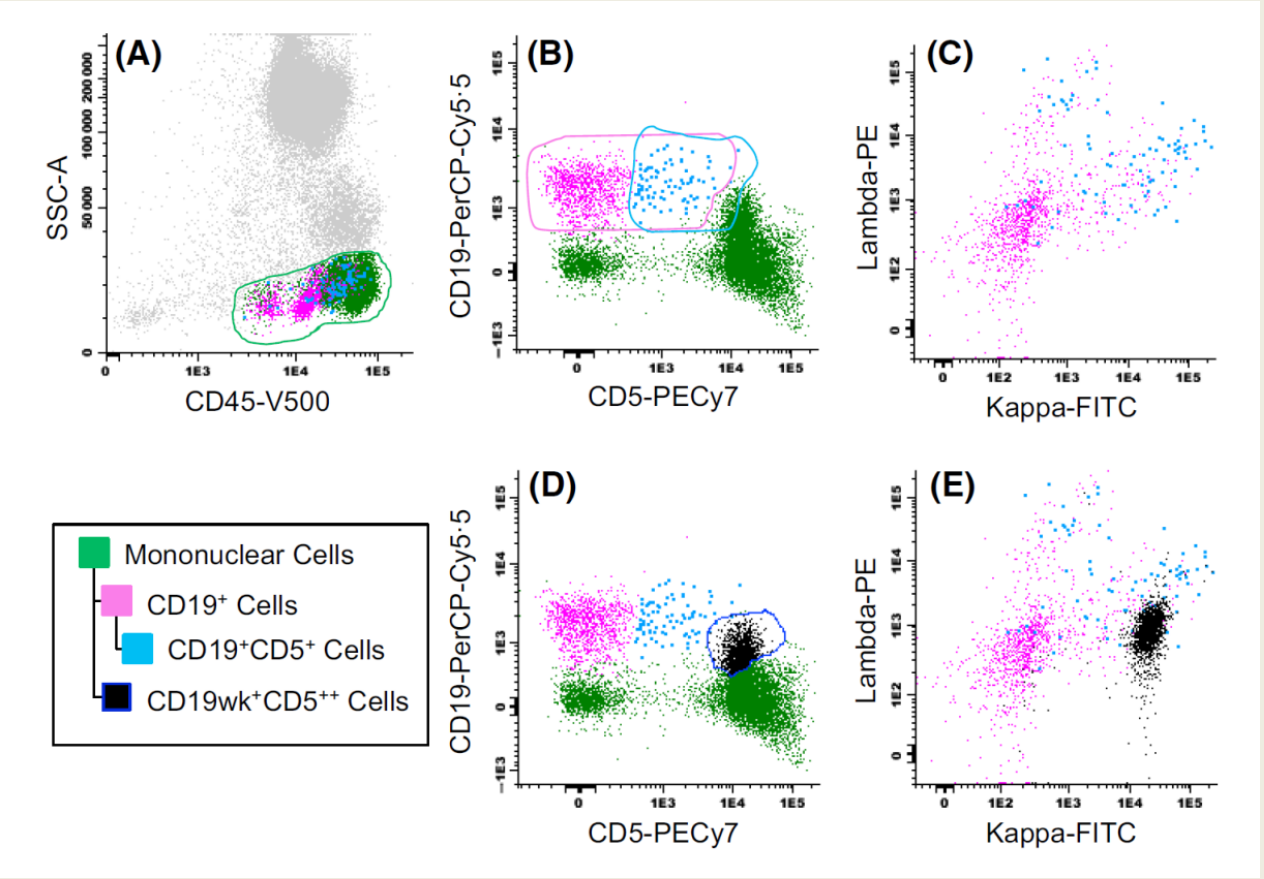
- Ensure stable performance through
  - Daily QC
  - Tracking PMT voltages and correct for drift in MFIs, using manufacturer software
- Use software for compensation setting, and median fluorescence method
- Use light scatter for coincidence monitoring





Tude design and validation

- Select antigens to be investigated on published evidence
- Select fluorochrome to match antigen intensity; avoid spillover of bright fluorochromes in channels with dim signals
- Run FMO controls for all new combinations, to check for artifacts
- Check for steric hindrance: resolution from each color of a multicolour combination should be equivalent to single color staining (intensity not reduced by >1/3 log)



Reagent handling

- Use antibody cocktails!
- Complete audit trail of cocktail preparation
  - Records for lot numbers, date, operator
  - Validate each new cocktail with parallel staining on a known representative sample
- Validate cocktail shelf-life

Reagent audit trail

LPD Screen cocktail checklist sheet

Use a total vol of 49 µl from the cocktail for each test

Date:

Antibody	Kappa FITC	CD4 FITC	Lambda PE	CD8 PE	CD19 PerCP-Cy5.5	CD2 PECy7	CD20 APC	CD3 APCH7	CD56 V450	CD45 V500
Batch number										
Volume per test (µl)	2	5	2	5	10	5	5	5	5	5
Volume for 20 tests (µl)	40	100	40	100	200	100	100	100	100	100
Antibody added (tick)										

Cocktail prepared by: No of uses:

Stick Patient labels

Fig 4. Antibody cocktail audit trail. Example of a controlled document for an in-house made antibody cocktail enabling a complete audit trail.

Pre-analytical variables: specimen

- Sample age and quality must be assessed
  - Live/dead stain on biopsies
- PB/BM: EDTA recommended as anticoagulant
  - Storage for 48h at RT
- CSF: use Transfix or culture media, at 4°c, for 48h
- Other fluids: 4°c, 24h
- Tissue preparations: culture media, at 4°c, for 48h

Reporting

- Patient information: indication, previous FCM studies, other lab results (WBC, differential)
- Sample information: sample type, anticoagulant, date collected/received
- Sample preparation: **antibodies used**, cell viability
- Data analysis:
  - Overall information on normal cells (B/T cells, CD4:CD8 ratio, NK, monocytes, granulocytes)
  - If present, % abnormal cells compared to a defined population (total leucocytes, total lymphocytes…)
  - Marker distribution on abnormal cells: +, –, partial; fluorescence intensity if relevant (dim, bright, heterogeneous, homogeneous)
- Interpretation:
  - Differential diagnosis according to WHO defined subtypes
  - A definite diagnosis requires integration with relevant pathology/molecular biology/cytogenetic data

Bethesda Consensus Conference, Wood et al., Cytometry, 2007, 72B-S14

Specific reporting recommandations may be applicable: eg PNH

FLOW CYTOMETRY REPORT – PNH EVALUATION

SAMPLE REPORT

Name: PNH, Positive
DOB: 7/31/1973 Sex: M MR #: 123456789
Facility: Ordering Facility
Dept: Outpatient

Pathology Number: F-07-20349
Date of Procedure: 6/15/2007
Date of Accession: 6/15/2007

Physician: Ordering Provider, M.D.
Ordering Facility
Street Name
City, State Zip code
(999) 123-4567

Copies to: Other providers/clinicians

TISSUE/SPECIMEN: Peripheral Blood in Heparin

DIAGNOSIS: PNH CLONE IDENTIFIED IN BOTH WBC AND RBC

Comment: Flow cytometric analysis shows a PNH clone within the granulocytes (60.9%), monocytes (61.5%) and RBC's (9.4%). These findings are consistent with a diagnosis of paroxysmal nocturnal hemoglobinuria (PNH). Any potential difference in clone size between the white blood cells and the red blood cells may be due to hemolysis and/or recent transfusion. The PNH clone in the monocytes and granulocytes showed a bimodal distribution, indicating Type II and Type III cells. The clinical significance of this finding is still under investigation.

Reference: Richards et al: Diagnosis and Management of PNH, Blood 2005, 106 (12)

Flow Results: ***Immunophenotypic analysis*** was performed using gating antibodies CD45, CD15, CD33, CD64, GPI-linked antibodies CD59, CD14, CD24, as well as fluorescent Aerolysin (FLAER).

Cell Type	Deficiency	Result
RBC	Type II (partial CD59 deficiency)	5.3%
	Type III (complete CD59 deficiency)	4.1%
	PNH Clone size (Type II and Type III combined)	9.4%
WBC - Monocytes	FLAER/CD14 Deficiency	61.5% (57.2% Type III + 4.3% Type II)
WBC - Granulocytes	FLAER/CD24 Deficiency	60.9 (54.7% Type III + 6.2% Type II)

Borowitz et al., Cytometry Part B (Clinical Cytometry) 78B:211–230 (2010)

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Training

- Senior staff
  - Training in an experienced FC laboratory
  - Participation to external courses and EQA programs
  - Exposure to 100 new clinical cases/year
- Technologists
  - Clearly defined levels of responsibility based on documented specific criteria
  - Individual competencies reviewed on annual basis

	MLA	HCS	MFC specialist HCS	MFC & Haematology-Oncology specialist HCS
Basic flow cytometry principles		X	X	X
In-house instrument training	X	X	X	X
Manufacturer's training courses			X	X
Advanced cytometry and software training			X	X
Competent in range of analysis software			X	X
Instrument QC	X	X	X	X
Responsibility for instrument optimization/QC monitoring/compensation			X	X
Protocol and panel setup			X	X
Responsibility and supervizing of protocol development and R&D				X
Reagent preparation/QC	X	X	X	X
Specimen handling	X	X	X	X
Specimen set-up	X	X	X	X
Responsibility for panel selection			X	X
Acquisition		X	X	X
Data analysis- basic		X	X	X
Data analysis- complex			X	X
Data analysis portfolio		X	X	X
CPD portfolio/courses		X	X	X
Prepare diagnostic report			X	X
Authorize and clinical interpretation of flow report				X
Morphology review			X	X

MLA, Medical Laboratory Assistant; HSC, Health Care Scientist; MFC, multicolour flow cytometry; QC, quality control; R&D, Research and Development; CPD, Continuing Professional Development.

Each section would have more specific set criteria for training relevant to specific laboratory and case load. This would include reference to all department Standard Operating Procedures and health and safety procedures.

See complete guideline in Bethesda conference, Greig et al., Cytometry Part B (Clinical Cytometry) 72B:S23–S33 (2007)

Main topics for audit

- Cytometer:
  - documentation of daily maintenance
  - manual or automated tracking of MFI
  - error and maintenance log
- Reagents:
  - Records for titration and compensation monitoring
  - Complete trail of reagents lots used for every sample
- Panels:
  - Records of validation
  - Gating strategy / documentation of normal, reactive, regenerating and abnormal sample dot plots
  - Published evidence accessible
- Data storage:
  - Archive of LMD with compensation matrices
- Training/Competency
  - Competency annual reviews
  - Monitoring of agreement <> clinical data, morphology, cytogenetics, molecular biology, IHC; archives of multidisciplinary oncology meetings

Conclusion

What is specific in accreditation of flow cytometry?

HARD WORK….. not a lack of guidelines

- Medical competence – case load monitoring
- Documentation/check of reagent lots/cocktails
- Training documentation for each step of a FC experiment
- Documentation of tube patterns in normal / reactive / abnormal cases (casebook)

Further readings

- Flow cytometry method validation protocols, Selliah et al, current protocols in cytometry e53, volume 87
  - More on acceptance criteria for different parameters of validation
- CLSI – H62: validation of assays perfomed by flow cytometry (2021)
  - More on:
    - Assay development and optimization
    - Instrument qualification and standardization (incl. cross-site)
- Euroflow.org
  - Leukemia/lymphoma, Immunodeficiency
    - Panels and SOPs
    - Reference ranges PB leucocytes (incl. children)

Thank you !