

How to Bring the Work(flow) in the Flow Lab



ESCCA 2025
Montpellier, France
September 18, 2025

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MB&C2026
Leuven, Belgium
February 5, 2026

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Overview

- **Flow in a Multi-Site Laboratory**
- **Technical Workflow**
- **IT Workflow**
- **Perspectives**

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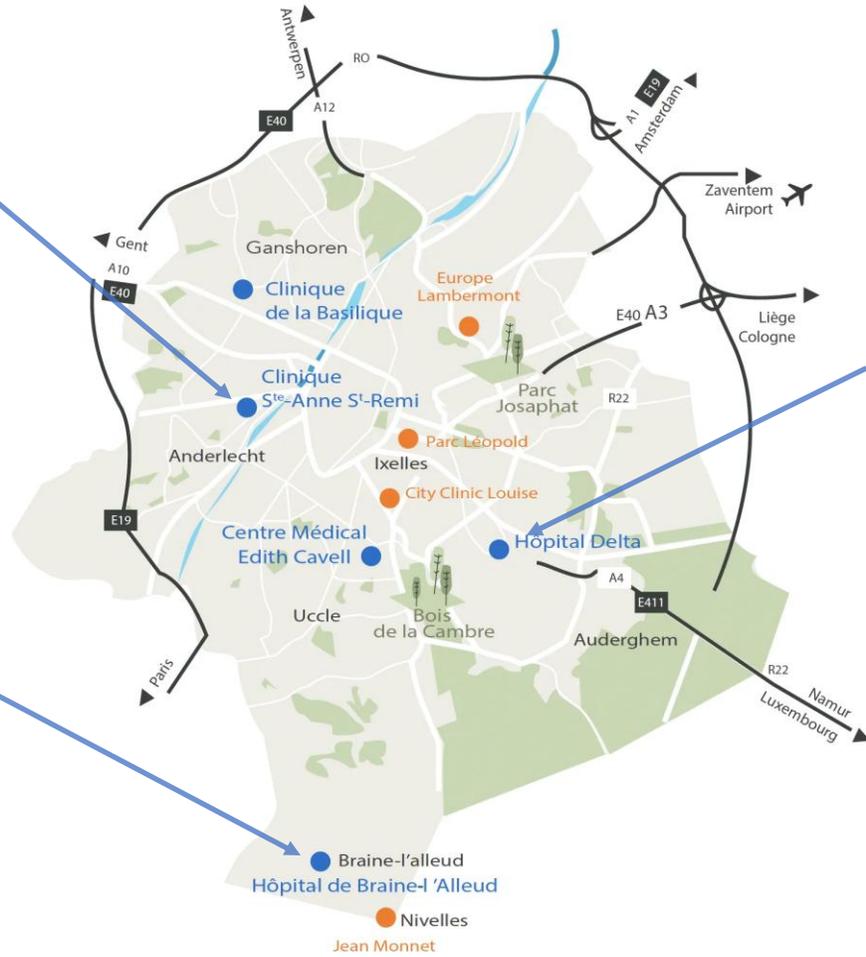
Chirec Hospital Laboratory



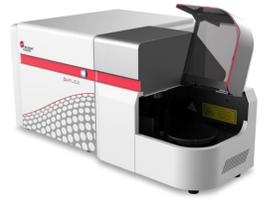
SARE



HBW



DELTA



800 CBCs+ / day
300+ bone marrows / year

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Hematology Workflow



Outpatient Clinics sampling



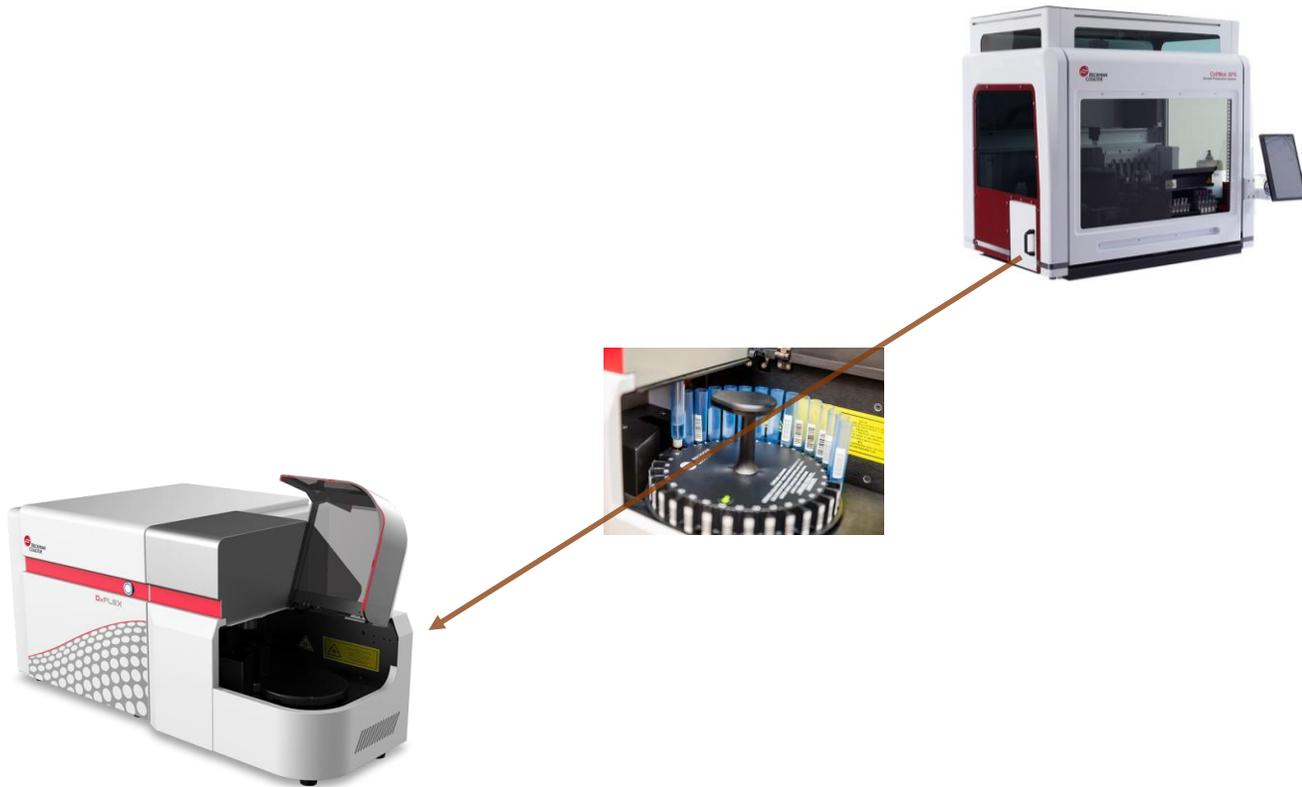
On-site sampling & handling



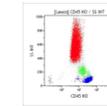
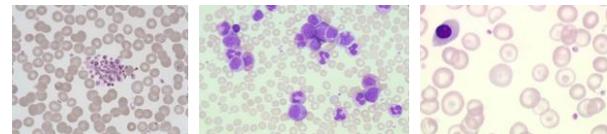
On-site storage
5 days
4°C



Flow Cytometry Workflow

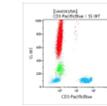


10-20 slides a day / site
(excluding body fluids and
Kleihauer)



CD45-KrO, J33, 100 Tests, CE

Product No: **B36294** **FLOW** Reagent



CD3-PB, UCHT1, 50 Tests, CE

Product No: **B49204** **FLOW** Reagent



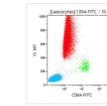
CD19-PC7, J3-119, 100 Tests, CE

Product No: **IM3628** **FLOW** Reagent



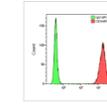
CD16-PC5, 3G8, 100 Tests, CE

Product No: **A07767** **FLOW** Reagent



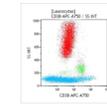
CD64-FITC, 22, 100 Tests, CE

Product No: **B49185** **FLOW** Reagent



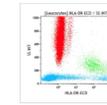
CD34-APC-A700, 581, 50 Tests, CE

Product No: **B92417** **FLOW** Reagent



CD38-APC-A750, LS198-4-3, 50 Tests, CE

Product No: **B49200** **FLOW** Reagent



HLA-DR-ECD, Immu-357, 100 Tests, CE

Product No: **B92438** **FLOW** Reagent

CDE panel (30-70 a day / 14.000 a year)

*Other automated in-house panels:
TBNK (1000 a year), KL (1500 a year),
SLD (1400 a year), HLA-B27 (740 a year)*

*Flow
7 days
a week*

CDE preparation and sensitivity

Random access loading of samples

whole blood EDTA
+
CDE Mix of Antibodies



Incubation

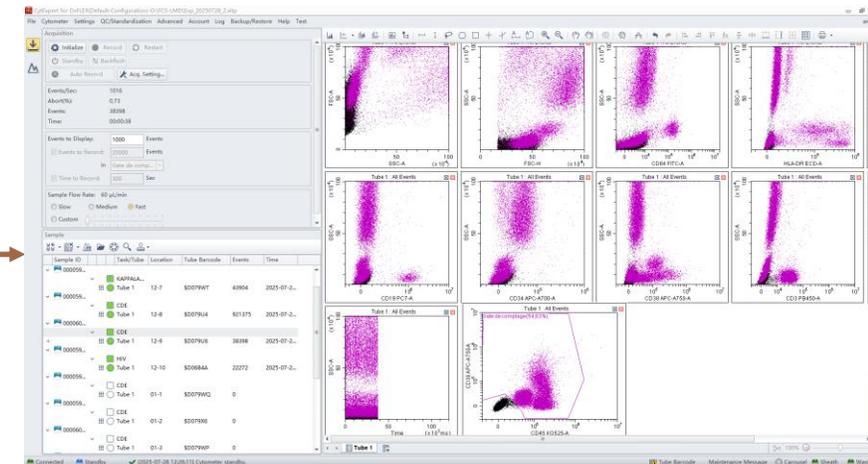
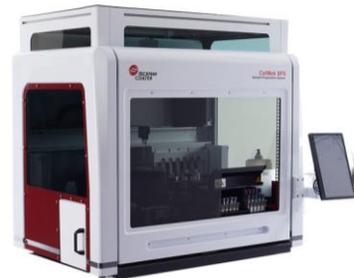
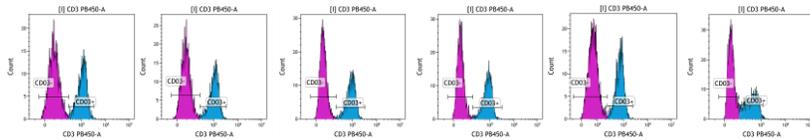
VersaLyse

Incubation

CytExpert
Software



Automated acquisition
Stopping gate: 20.000 leucocytes
Maximum time : 5 minutes per tube



CDE precision



Table II

95%-PREDICTION INTERVALS (IN %) for the results of a very large number of differential counts in smears from one sample of blood.

t	n = 100	n = 200	n = 500	n = 1000
1	0- 3	0 - 2.5	0.2- 2.0	0.4- 1.7
2	0- 5	0.5- 4	0.8- 3.4	1.2- 2.9
3	0- 7	1 - 5.5	1.6- 4.6	2.0- 4.1
4	1- 8	1.5- 7	2.4- 5.8	2.8- 5.3
5	1-10	2 - 8	3.2- 7.0	3.7- 6.4
6	2-11	3 - 9.5	4.0- 8.2	4.6- 7.5
7	2-12	3.5-10.5	4.8- 9.4	5.5- 8.6
8	3-14	4.5-12	5.8-10.4	6.4- 9.7
9	4-15	5 -13	6.6-11.6	7.3-10.8
10	5-16	6 -14.5	7.4-12.8	8.2-11.9
15	8-22	10 -20	12.0-18.2	12.8-17.2
20	12-28	14.5-25.5	16.6-23.6	17.6-22.5
25	17-34	19 -31	21.2-28.8	22.3-27.7
30	21-39	24 -36.5	26.0-34.0	27.2-32.9
35	26-44	28.5-41.5	30.8-39.2	32.1-38.0
40	31-50	33.5-47	35.8-44.4	37.0-43.0
45	35-55	38 -52	40.6-49.4	41.9-48.1
50	40-60	43 -57	45.6-54.4	46.0-53.1

n is the total number of cells counted; t is the real percentage of cells of a given type. The cited limits are included in the 95%-prediction interval.

Ruemke CL. The statistically expected variability in differential counting. In: Koepke JA: Differential leukocyte counting. College of American Pathologists, Sokie, Illinois. 1979

Table 1 95% confidence limits for the actual number of cells per 100 WBC. A = observed cells per 100 WBC.

A	Actual number of cells per 100 WBC				
	n = 100	n = 200	n = 500	n = 1,000	n = 10,000
0	0-3.6	0-1.8	0-0.7	0-0.4	0-0.1
1	0.0-5.4	0.1-3.6	0.3-2.3	0.5-1.8	0.8-1.3
5	1.6-11.3	2.4-9.0	3.3-7.3	3.7-6.5	4.5-5.5
10	4.9-17.6	6.2-15.0	7.5-13.0	8.2-12.0	9.4-10.7
15	8.6-23.5	10.4-20.7	12.0-18.4	12.8-17.4	14.3-15.8
20	12.7-29.2	14.7-26.2	16.6-23.8	17.6-22.6	19.2-20.8
30	21.2-40.0	23.7-36.9	26.0-34.2	27.2-32.9	29.1-31.0
40	30.3-50.3	33.2-47.1	35.7-44.4	36.9-43.1	39.0-41.0
50	39.8-60.2	42.9-57.1	45.5-54.5	46.9-53.1	49.0-51.0
70	60.0-78.8	63.1-76.3	65.8-74.0	67.1-72.8	69.0-70.9
80	70.8-87.3	73.8-85.3	76.2-83.4	77.4-82.4	79.2-80.8
90	82.4-95.1	85.0-93.8	87.0-92.5	88.0-91.8	89.3-90.6
100	96.4-100	98.2-100	99.3-100	99.6-100	99.9-100

Systemex educational enhancement and development, SEED Haematology, April 2016

CDE precision and specificity

Table 1 Coefficients of variation (CV), and mean percentages of cells (\bar{x}), corresponding to B-E imprecision in differential leukocyte counting

Quantity	\bar{x} (%)	CV (%)
Lkcs (B)—neutrophilocytes; num. fr.	78.15	6.6
Lkcs (B)—lymphocytes; num. fr.	10.78	32.5
Lkcs (B)—monocytes; num. fr.	4.91	55.0
Lkcs (B)—eosinophilocytes; num. fr.	1.88	68.8
Lkcs (B)—basophilocytes; num. fr.	0.19	263.2
Lkcs (B)—myelocytes; num. fr.	0.83	132.5
Lkcs (B)—metamyelocytes; num. fr.	3.16	69.6

Quantities corresponding to the differential leukocyte counting are described in accordance with the standards EN 1614 [6] and ISO 15189 [7]

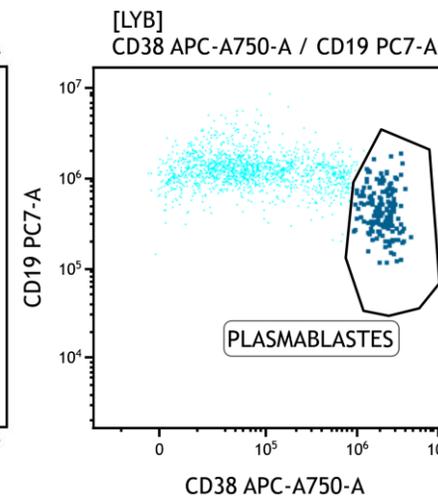
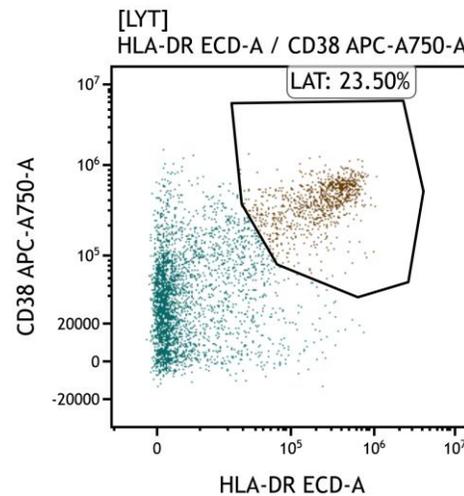
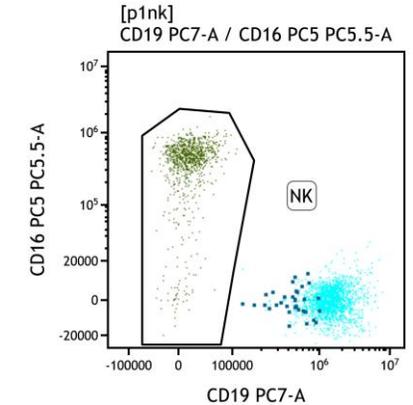
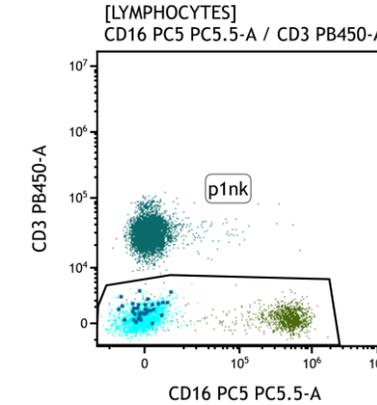
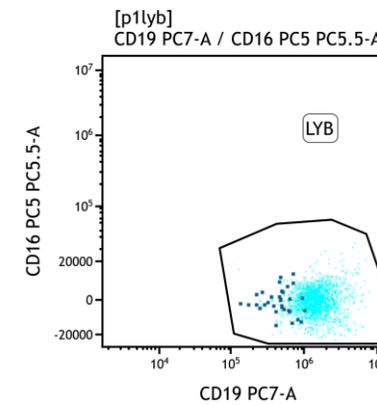
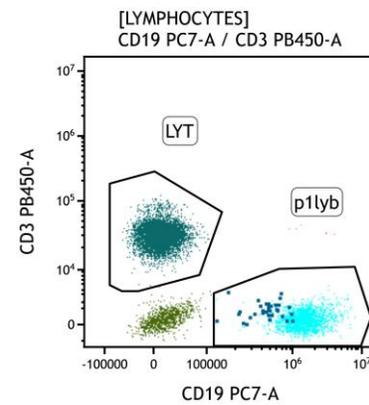
Lkcs, leukocytes; B, blood; num. fr., number fraction

Fuentes-Arderiu, X., García-Panyella, M. & Dot-Bach, D. Between-examiner reproducibility in manual differential leukocyte counting. Accred Qual Assur 2007



Zini, G.

Hematological cytormorphology: Where we are IJLH2024



GB	GLOBULES BLANCS	5	1	11.7	1000/mm ³
NEU	NEUTROPHILES	5	↓	1	22.5
NEUVA	NEUTROPHILES (VA)	5	1	2.63	1000/mm ³
EOS	EOSINOPHILES	5	↓	1	0.7
EOSA	EOSINOPHILES (VA)	5	1	0.08	1000/mm ³
BAS	BASOPHILES	5	1	0.1	%
BASOVA	BASOPHILES (VA)	5	1	0.02	1000/mm ³
MON	MONOCYTES	5	1	1.1	%
MONOVA	MONOCYTES (VA)	5	1	0.13	1000/mm ³
LYM	LYMPHOCYTES	5	↓	1	30.4
LYMVA	LYMPHOCYTES (VA)	5	↓	1	3.55
LYTCD	LYMPHOCYTES T	5	1	2.370	1000/mm ³
LYBCD	LYMPHOCYTES B	5	1	0.990	1000/mm ³
NKCD	LYMPHOCYTES NK	5	1	0.180	1000/mm ³
LYAT	LYMPHOCYTES ATYPIQUE	5	1	0.2	%
PLBLCD	PLASMABLASTES	5	1	0.0	/μL
BLA	BLASTES	5	↑	1	42.64
BLAVA	BLASTES VA	5	↑	1	4.990
GRIM	GRANULOCYTES IMMATUR	5	1	2.3	%

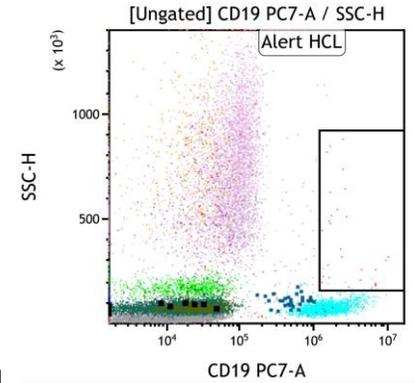
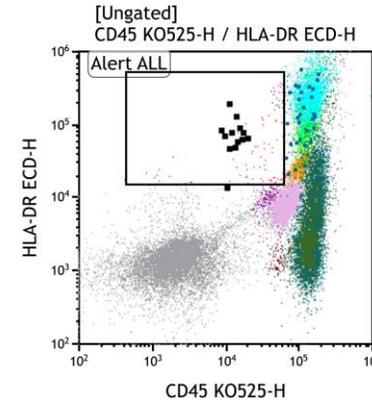
CDE gating



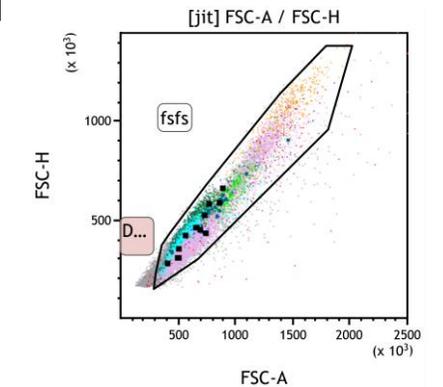
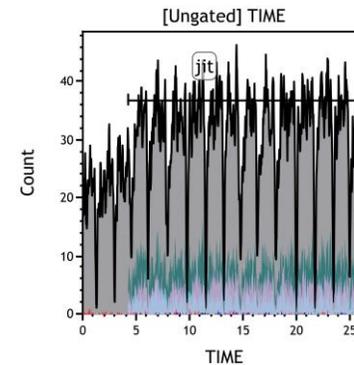
10 Technicians (7.6 FTE)



KALUZA
Software



GAP 0.11



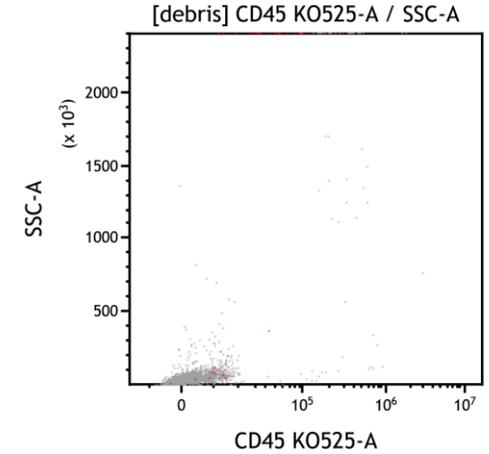
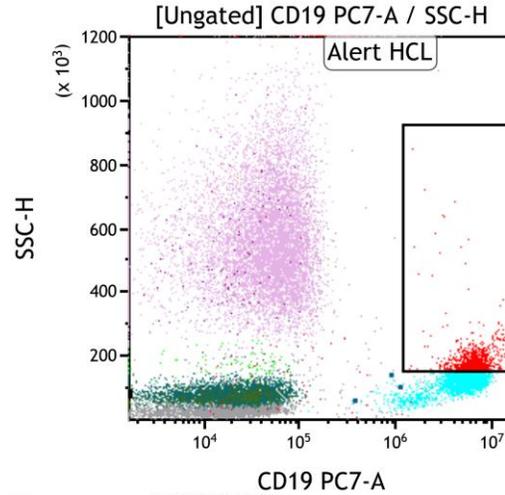
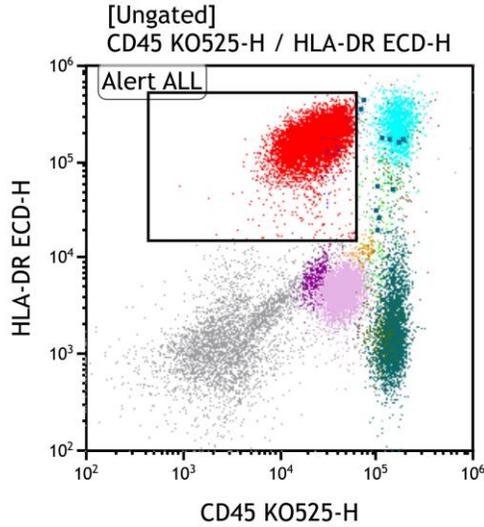
> 50% of CDE
analyses
< 1 minute

- **Lymphosum**=(COUNT("LYT", 1)+COUNT("LYB", 1)+COUNT(NK, 1))/COUNT("LYMPHOCYTES", 1)*100
- **Leucosum**=(COUNT("MYELOIDES", 1)+COUNT("LYMPHOCYTES", 1)+COUNT(MONOCYTES, 1)+COUNT(EOSINOPHILES, 1)+COUNT("BASOPHILES", 1)+COUNT("BLASTES", 1))/COUNT(CELLULES, 1)*100
- **Gap**=(COUNT(CELLULES, 1)-COUNT("MYELOIDES", 1)-COUNT("LYMPHOCYTES", 1)-COUNT(MONOCYTES, 1)-COUNT(EOSINOPHILES, 1)-COUNT("BASOPHILES", 1)-COUNT("BLASTES", 1))/COUNT(CELLULES, 1)*100

CDE gating

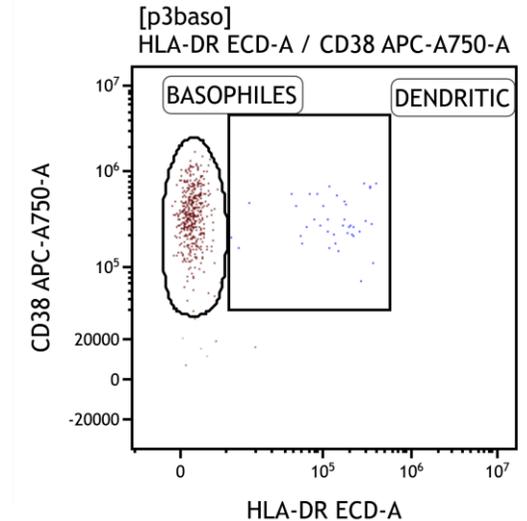
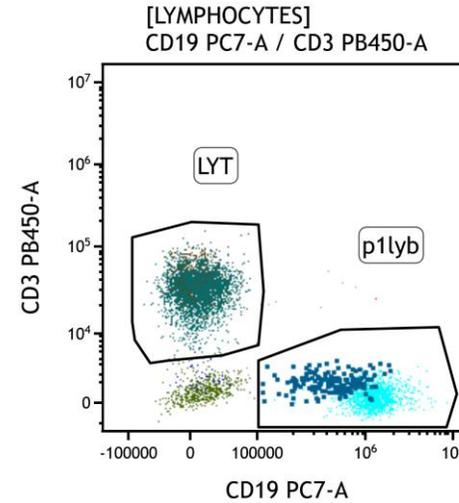
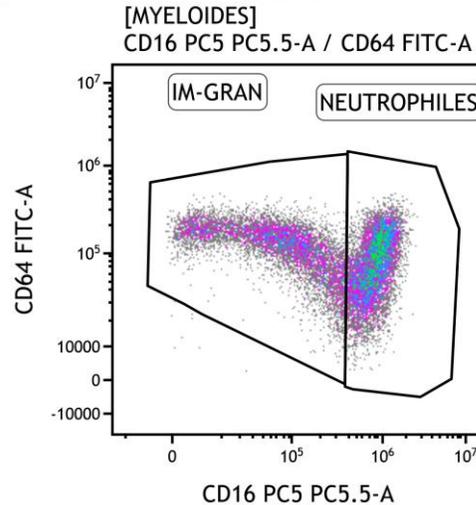
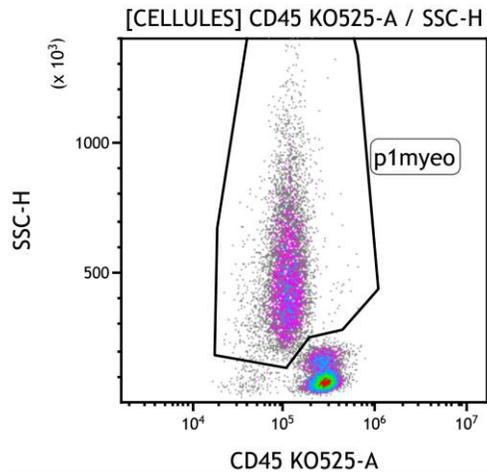


'Alert Gates'



**Final 'Debris Gate'
Boolean Gate =
'NOT CELLS'**

Cell Population Allocation Gates that might require adjustments



Gap < 0.8 → Send CDE results

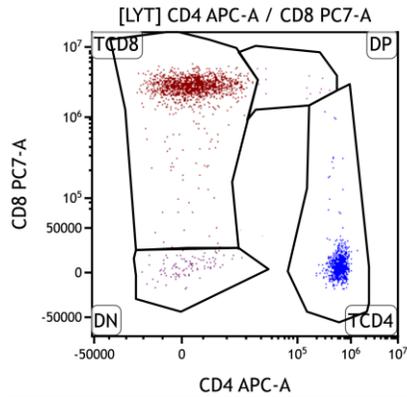
Gap ≥ 0.8 → more thorough analysis

Other automated panels - As prescribed -



TBNK

(Lyse No Wash)



TBNK (1000 a year)

KL (1500 a year)

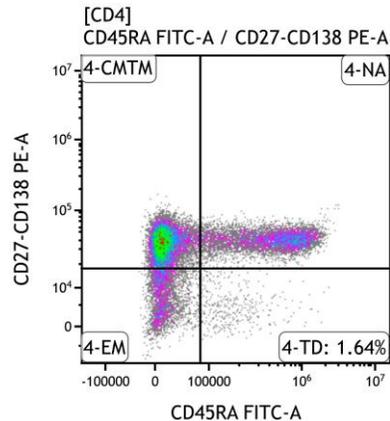


SLD (1400 a year)

HLA-B27 (740 a year)

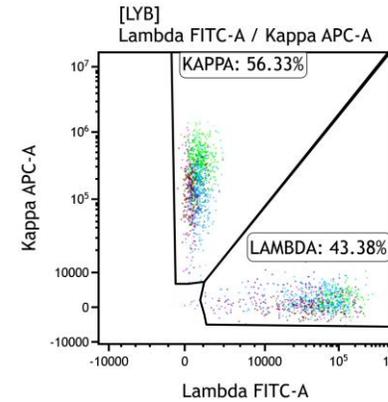
SLD

(Lyse No Wash)



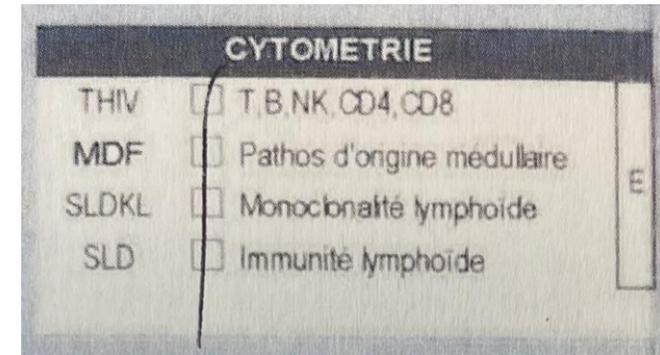
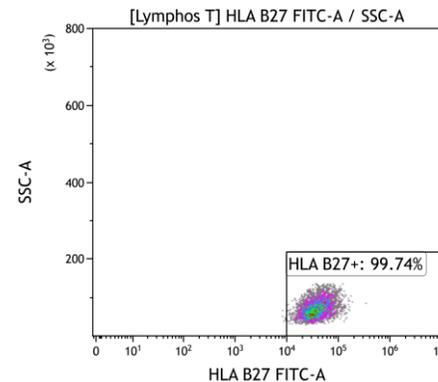
K/L

(Lyse Wash)



HLA-B27

(Lyse No Wash)



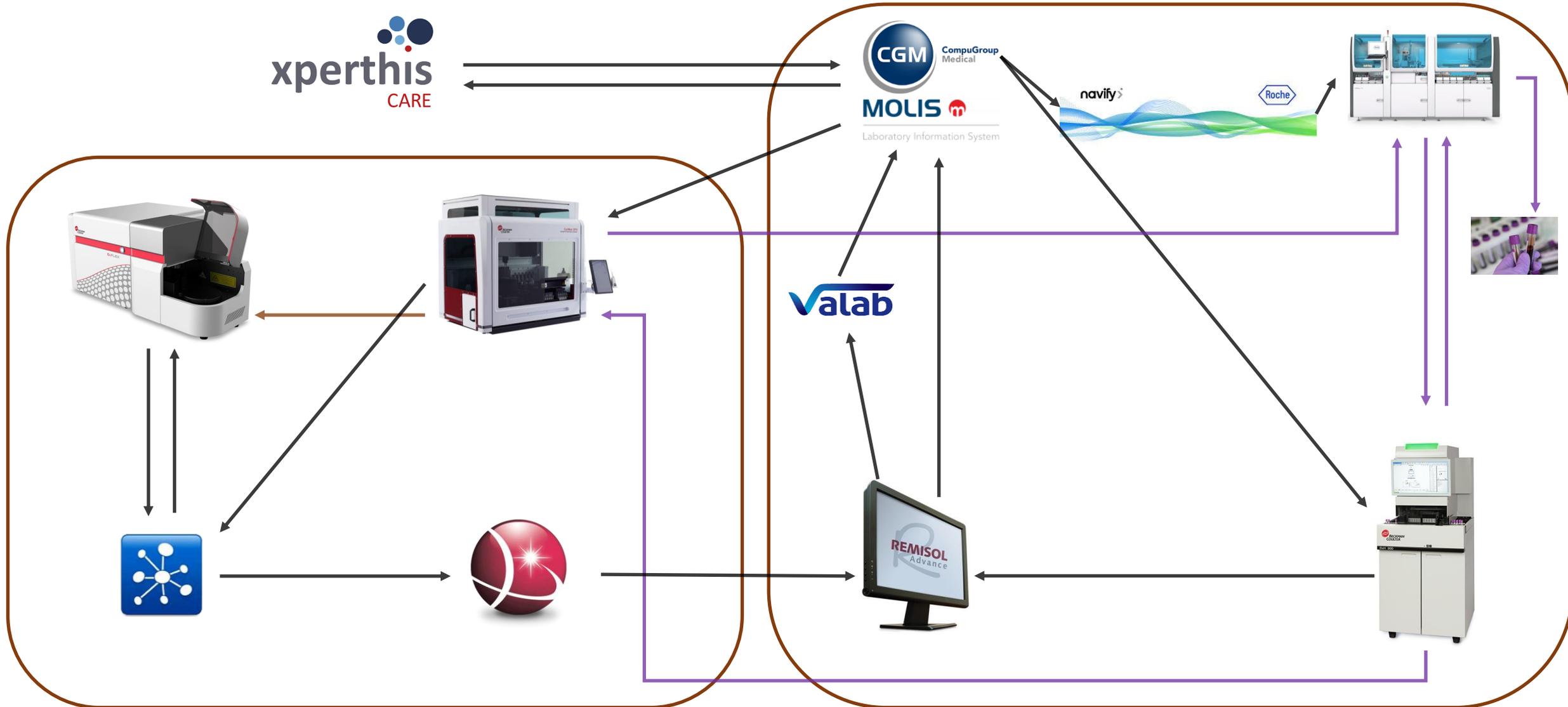
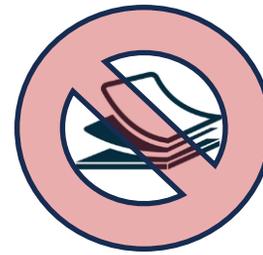
TLEUCO	TYPAGE LYMPHOCYTAIRE	0
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IT connections for Flow



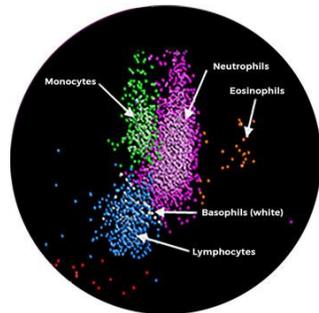
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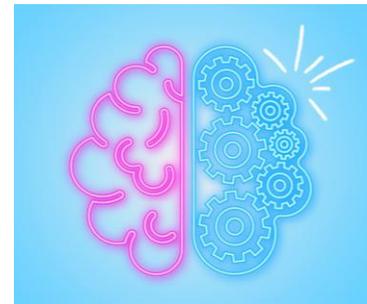
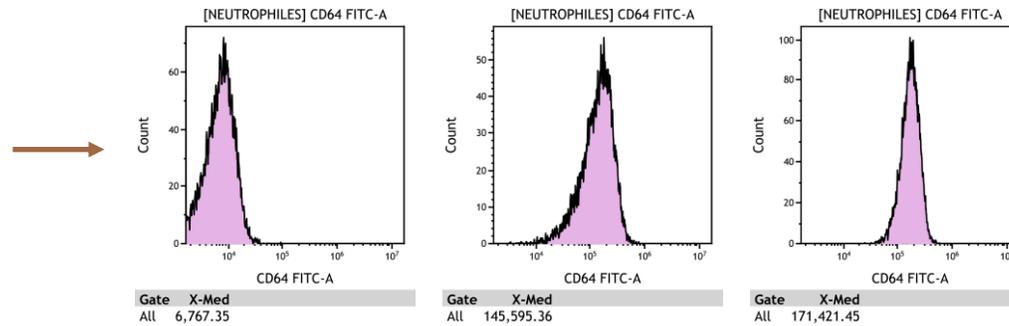
Perspectives



Activated Monocytes



Septic
MDW=28.6



Acknowledgments



Olivier Pradier MD PhD

