

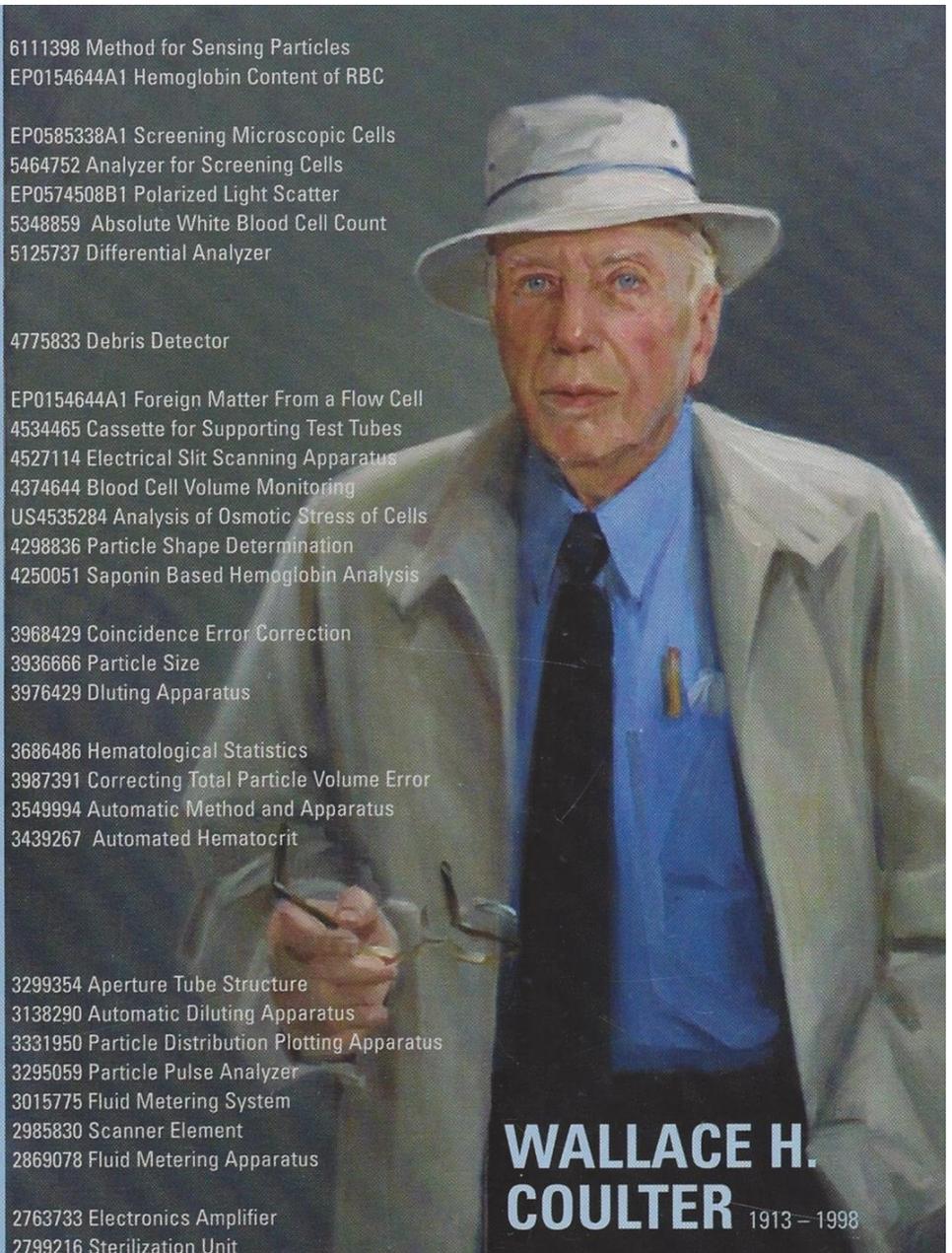


Exploration Plaquettaire

Bernard CHATELAIN

17-mars 2016

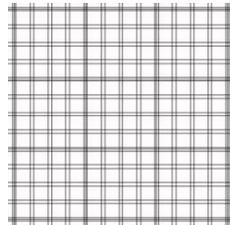
Eight Color Flow Cytometry (Roederer)	1997	6111398 Method for Sensing Particles
	1996	EP0154644A1 Hemoglobin Content of RBC
Five Color Flow Cytometry (Roederer)	1995	
	1994	EP0585338A1 Screening Microscopic Cells
	1993	5464752 Analyzer for Screening Cells
	1991	EP0574508B1 Polarized Light Scatter 5348859 Absolute White Blood Cell Count
Barcoding in Flow Cytometry (Robinson)	1990	5125737 Differential Analyzer
Integration of Video in Flow Cytometry	1987	
Dual beam High Speed Sorting (Gray)	1986	4775833 Debris Detector
Radio Frequency Opacity	1985	
Three Color Flow Cytometry (Parks)	1984	EP0154644A1 Foreign Matter From a Flow Cell
	1983	4534465 Cassette for Supporting Test Tubes
	1982	4527114 Electrical Slit Scanning Apparatus
	1981	4374644 Blood Cell Volume Monitoring US4535284 Analysis of Osmotic Stress of Cells
MAbs to Human Epitopes (Schlossman)	1979	4298836 Particle Shape Determination
	1978	4250051 Saponin Based Hemoglobin Analysis
Two Color Flow Cytometry (Loken)	1977	
MAbs (Köhler & Milstein)	1975	3968429 Coincidence Error Correction
	1974	3936666 Particle Size
	1973	3976429 Diluting Apparatus
Fluorescence Cell Sorter (Herzenberg)	1972	
	1970	3686486 Hematological Statistics
Fluorescence Flow (Göhde/Van Dilla)	1969	3987391 Correcting Total Particle Volume Error
Fluidic Sorter (Kamentsky)	1967	3549994 Automatic Method and Apparatus
Electrostatic Cell Sorter (Fulwyler)	1965	3439267 Automated Hematocrit
Fluidic Analyzer (Kamentsky)	1964	
Ink-Jet Technology (Sweet)	1964	
Image Analysis (Ingram)	1963	
Fluorescent Enzymes (Rotman)	1963	
Character Recognition (Kamentsky)	1962	3299354 Aperture Tube Structure 3138290 Automatic Diluting Apparatus
	1961	3331950 Particle Distribution Plotting Apparatus
	1959	3295059 Particle Pulse Analyzer 3015775 Fluid Metering System
	1958	2985830 Scanner Element
	1956	2869078 Fluid Metering Apparatus
Cytoanalyzer (Tolles)	1956	
Hydrodynamic Focusing (Crosland-Taylor)	1953	
	1952	2763733 Electronics Amplifier
	1951	2799216 Sterilization Unit
	1949	2656508 Particle Counter
Fluorescent Antibodies (Coons)	1942	
Photoelectric Counter (Moldavan)	1934	



WALLACE H. COULTER 1913 - 1998

Numération plaquettaire: principes

- Méthode de référence: hémocytomètre (Burker)



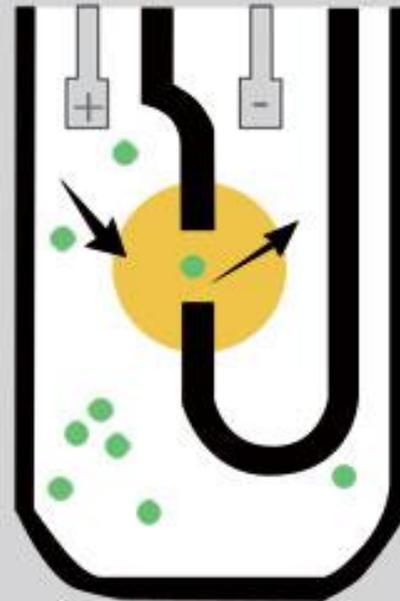
- : précision ↓
spécificité ↓

- Impédance: f(volume)
- Optique: f(surface et indice de réfraction)
- Fluorescence: Sysmex XN : mode étendu
(comptage sur volume plus grand)
- Immunocomptage

1956: Coulter impedance measurement

The Coulter Principle

- Particles suspended in a conductive electrolyte solution are drawn through a small aperture.
- A DC current is applied, creating a "sensing zone". As each particle passes through the aperture, it displaces an amount of saline equivalent to its size, creating impedance resulting in a voltage pulse proportional to the particle volume.



An evaluation of the Celloscope 401 electronic blood cell counter

T. R. J. LAPPIN, A. LAMONT, AND M. G. NELSON

From the Department of Haematology, Royal Victoria Hospital, Belfast

SYNOPSIS For counting erythrocytes the instrument was precise, with a mean coefficient of variation of 1.21%.

Erythrocyte counts showed close agreement with results obtained on a Coulter A electronic counter of proven accuracy.

When the Celloscope 401 was modified by the manufacturers to eliminate electrical interference from other laboratory equipment, satisfactory precision and accuracy for white cell counting was obtained. Using cetrimide diluent the coefficient of variation was 1.6% but when using saponin/saline diluent the coefficient of variation was 3.5%. For leucocyte counting there was close agreement between duplicate tests performed on the Celloscope 401 and the Coulter S.

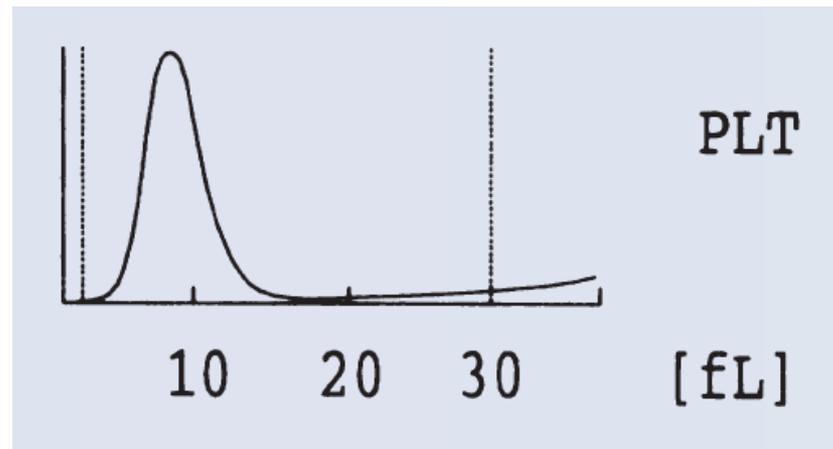
The instrument was capable of satisfactory precision and accuracy in platelet counting, provided that the sedimentation method was used to obtain a platelet-rich plasma. The best results were obtained if a two-step dilution was carried out with a first dilution in 10% EDTA and a second in 2.5 mM cocaine in water. Using this method the precision study indicated a coefficient of variation of 3.11%. Close agreement was obtained between platelet counts on the Celloscope 401 when compared with the results obtained either by phase-contrast microscopy or using another electronic counter.

Allowing for predilution and duplicate counts on each sample, the rate of throughput was approximately 32 samples per hour.

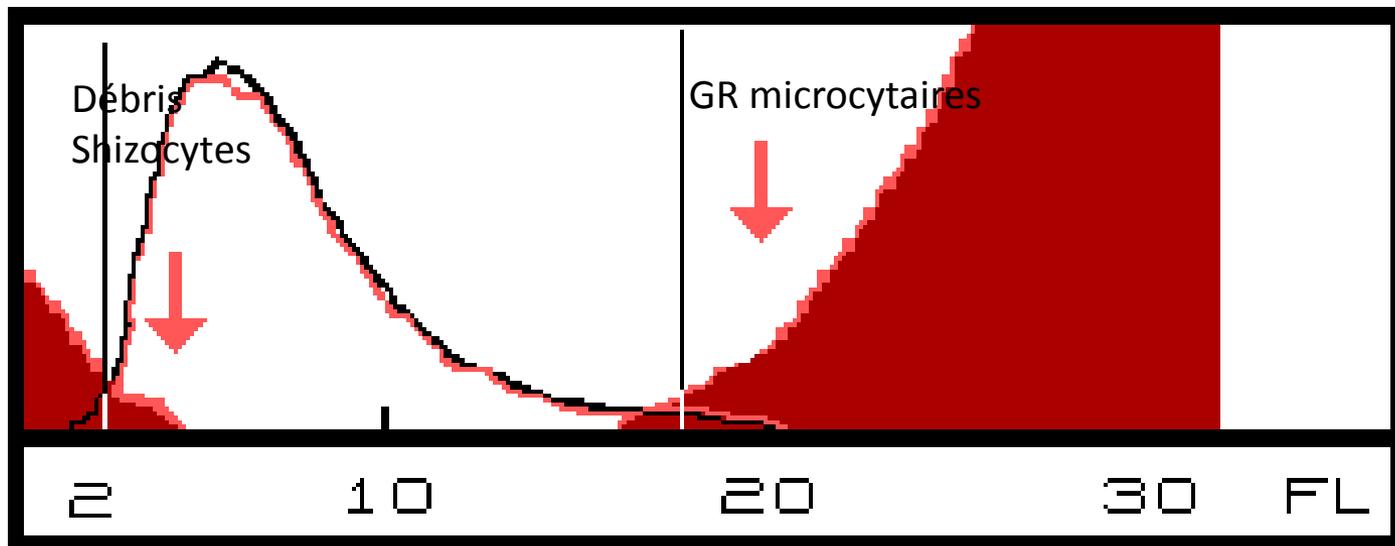
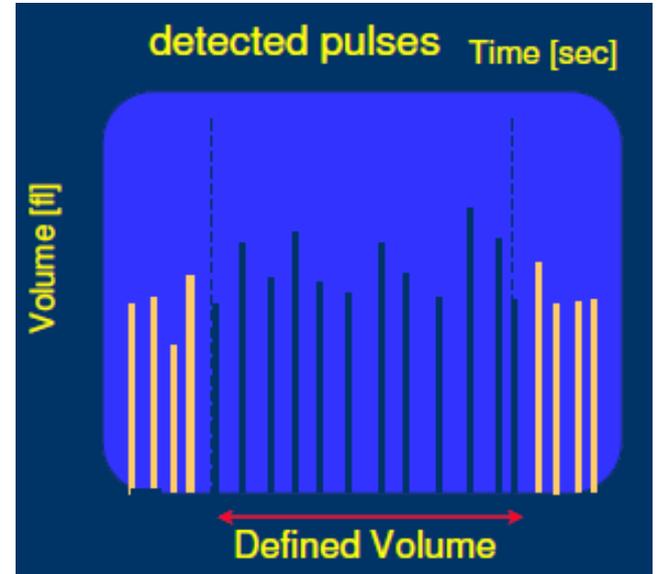
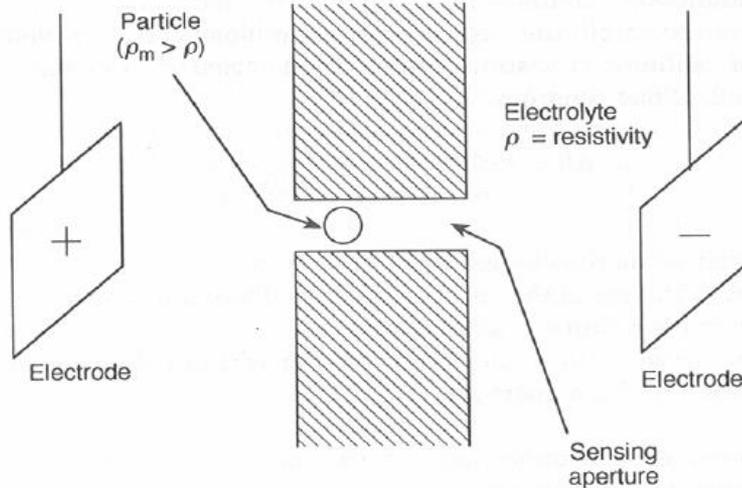
Throughout the test period, the instrument remained electronically and mechanically stable.

1970s: Automated platelet counting

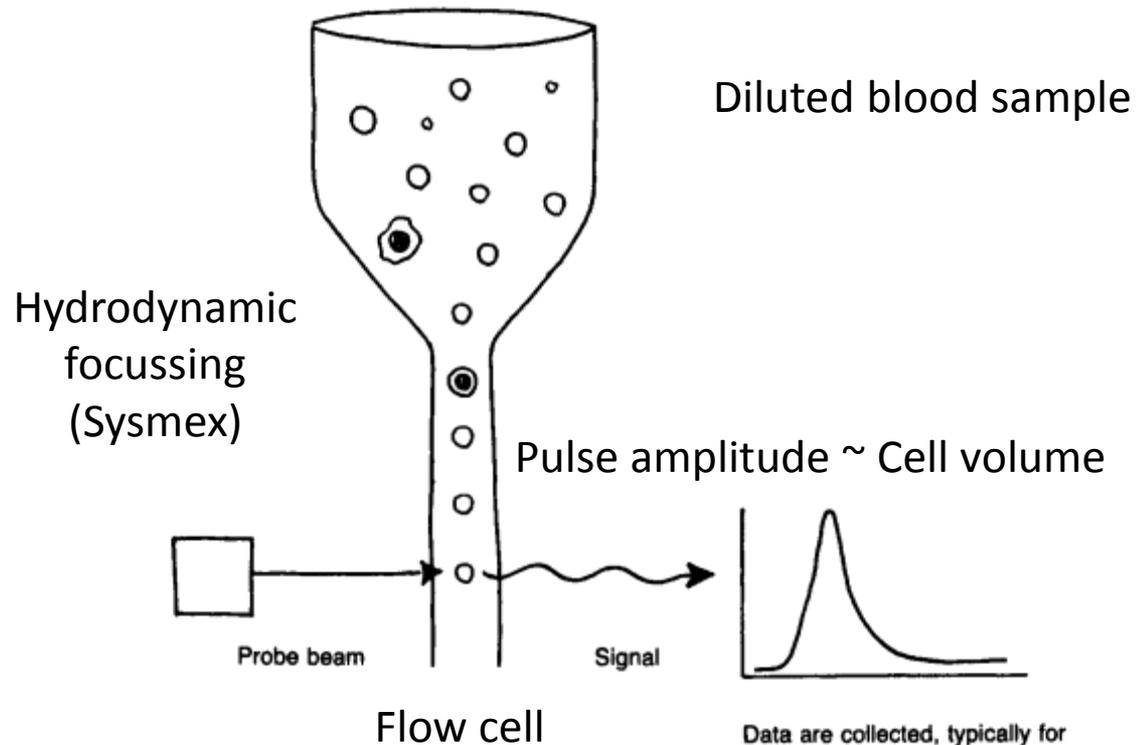
- improvement of the cell counting apertures
 - ⇒ More accurate cell sizing
 - ⇒ Reliable and accurate platelet counts.



Numération plaquettaire par impédance



1970s: Cell counting by light scattering



Data are collected, typically for 10,000 individual cells. The data are displayed and printed in histogram or scattergram form.

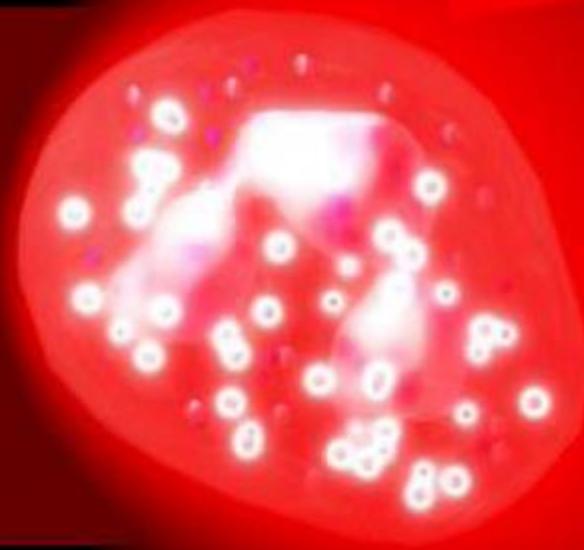
Laser Flowcytometry

Side Fluorescence Light :
RNA/DNA Information

Side Scattered Light :
Intenal Cell Structure

Forward Scattered Light :
Cell Volume Information

Laser Beam
($\lambda = 633\text{nm}$)



SYSMEX XN

CBC

Item	Data	Unit
WBC	3.94	10 ³ /uL
RBC	2.68 -	10 ⁶ /uL
HGB	7.9 -	g/dL
HCT	23.0 -	%
MCV	85.8	fL
MCH	29.5	pg
MCHC	34.3	g/dL
PLT	59 -	10 ³ /uL
RDW-SD	48.8	fL
RDW-CV	15.9	%
PDW	14.9	fL
MPV	10.2	fL
P-LCR	28.4	%
PCT	0.06 -	%
NRBC#	0.00	10 ³ /uL
NRBC%	0.0	%

RET

Item	Data	Unit
RET%		%
RET#		10 ⁹ /L
IRF		%
LFR		%
MFR		%
HFR		%
RET-He		pg

DIFF

Item	Data	Unit
NEUT#	2.17	10 ³ /uL
LYMPH#	1.10	10 ³ /uL
MONO#	0.48	10 ³ /uL
EO#	0.08	10 ³ /uL
BASO#	0.03	10 ³ /uL
NEUT%	55.1	%
LYMPH%	27.9	%
MONO%	12.2	%
EO%	2.0	%
BASO%	0.8	%
IG#	0.08	10 ³ /uL
IG%	2.0	%

PLT-F

Item	Data	Unit
IPF		%

WBC Flag(s)

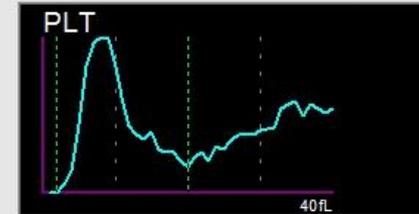
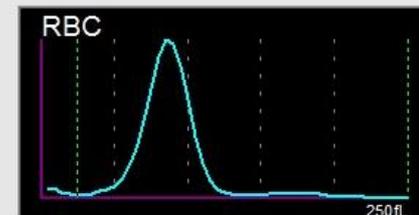
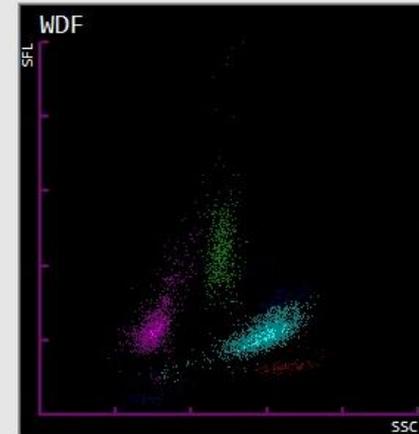
	▲
	▼

RBC Flag(s)

	▲
	▼

PLT Flag(s)

Thrombocytopenia	▲
	▼



SYSMEX XN

CBC

Item	Data	Unit
WBC	8.34	10 ³ /uL
RBC	4.34	10 ⁶ /uL
HGB	10.1	g/dL
HCT	35.3	%
MCV	81.3	- fL
MCH	23.3	- pg
MCHC	28.6	- g/dL
PLT &F	419	+ 10 ³ /uL
RDW-SD	63.7	+ fL
RDW-CV	21.4	+ %
PDW	12.5	fL
MPV	10.9	fL
P-LCR	31.9	%
PCT	0.46	+ %
NRBC#	0.00	10 ³ /uL
NRBC%	0.0	%

RET

Item	Data	Unit
RET%	1.50	%
RET#	65.1	10 ⁹ /L
IRF	28.2	%
LFR	71.8	%
MFR	15.9	%
HFR	12.3	%
RET-He	18.5	pg

DIFF

Item	Data	Unit
NEUT#	6.27	10 ³ /uL
LYMPH#	1.33	10 ³ /uL
MONO#	0.59	10 ³ /uL
EO#	0.08	10 ³ /uL
BASO#	0.03	10 ³ /uL
NEUT%	75.1	+ %
LYMPH%	15.9	- %
MONO%	7.1	%
EO%	1.0	%
BASO%	0.4	%
IG#	0.04	10 ³ /uL
IG%	0.5	%

PLT-F

Item	Data	Unit
IPF	5.3	%

WBC Flag(s)

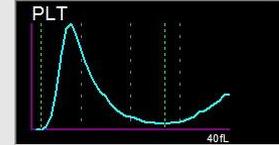
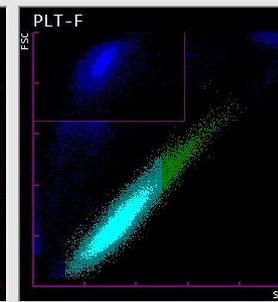
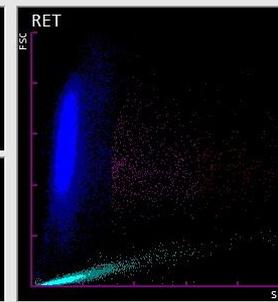
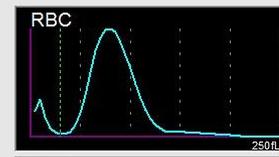
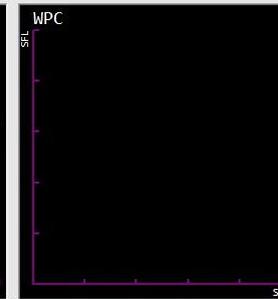
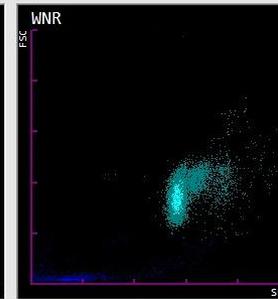
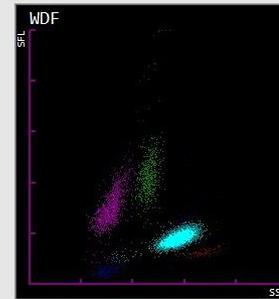
--	--	--

RBC Flag(s)

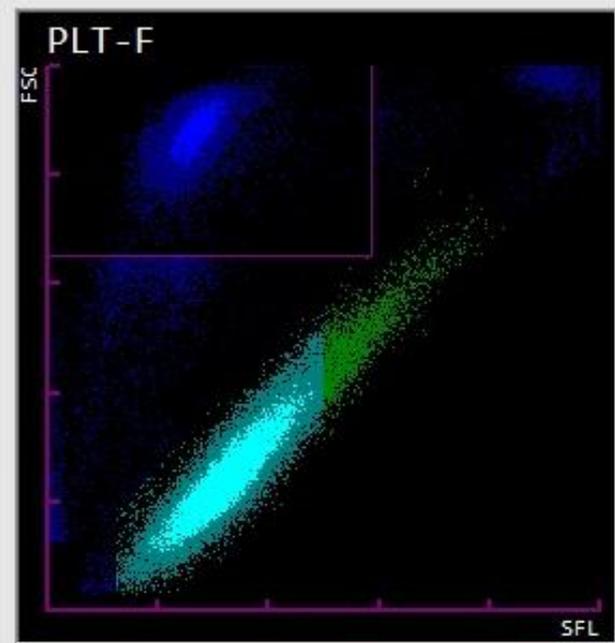
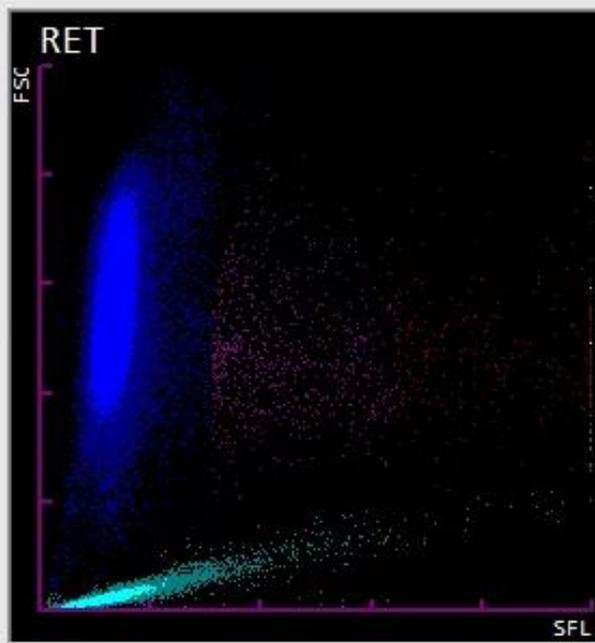
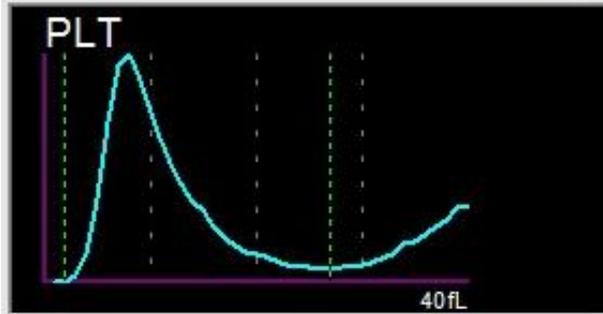
Anisocytosis		
Fragments?		

PLT Flag(s)

--	--	--



SYSMEX XN



SYSMEX XN

CBC

Item	Data	Unit
WBC	8.34	10 ³ /uL
RBC	4.34	10 ⁶ /uL
HGB	10.1	g/dL
HCT	35.3	%
MCV	81.3 -	fL
MCH	23.3 -	pg
MCHC	28.6 -	g/dL
PLT &F	419 +	10 ³ /uL
RDW-SD	63.7 +	fL
RDW-CV	21.4 +	%
PDW	12.5	fL
MPV	10.9	fL
P-LCR	31.9	%
PCT	0.46 +	%
NRBC#	0.00	10 ³ /uL
NRBC%	0.0	%

RET

Item	Data	Unit
RET%	1.50	%
RET#	65.1	10 ⁹ /L
IRF	28.2	%
LFR	71.8	%
MFR	15.9	%
HFR	12.3	%
RET-He	18.5	pg

DIFF

Item	Data	Unit
NEUT#	6.27	10 ³ /uL
LYMPH#	1.33	10 ³ /uL
MONO#	0.59	10 ³ /uL
EO#	0.08	10 ³ /uL
BASO#	0.03	10 ³ /uL
NEUT%	75.1 +	%
LYMPH%	15.9 -	%
MONO%	7.1	%
EO%	1.0	%
BASO%	0.4	%
IG#	0.04	10 ³ /uL
IG%	0.5	%

PLT-F

Item	Data	Unit
IPF	5.3	%

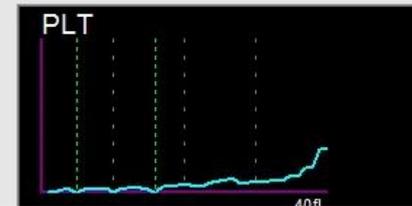
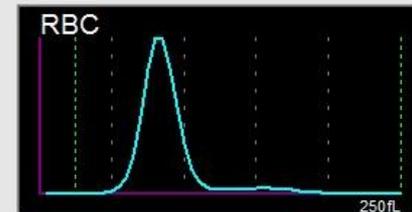
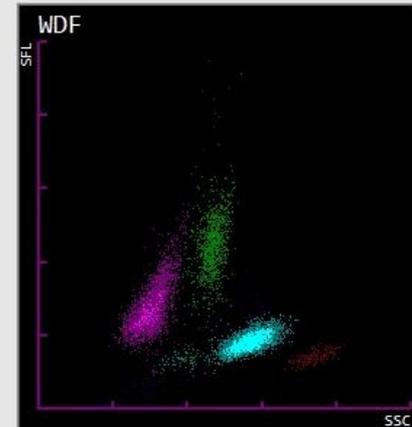
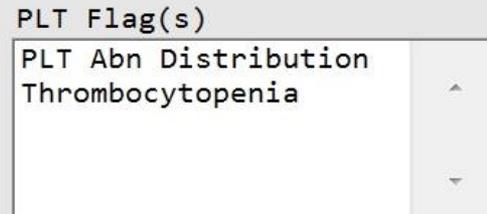
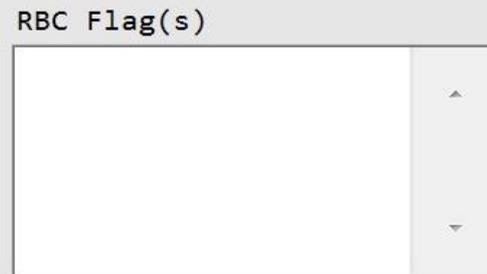
Thrombopénie chez un jeune enfant

Item	Data	Unit
WBC	8.08	10 ³ /uL
RBC	4.57	10 ⁶ /uL
HGB	12.6	g/dL
HCT	37.7	%
MCV	82.5	- fL
MCH	27.6	pg
MCHC	33.4	g/dL
PLT &F	2	- 10 ³ /uL
RDW-SD	37.3	fL
RDW-CV	12.4	%
PDW	----	fL
MPV	----	fL
P-LCR	----	%
PCT	----	%
NRBC#	0.00	10 ³ /uL
NRBC%	0.0	%

Item	Data	Unit
RET%		%
RET#		10 ⁹ /L
IRF		%
LFR		%
MFR		%
HFR		%
RET-He		pg

Item	Data	Unit
NEUT#	4.18	10 ³ /uL
LYMPH#	2.59	10 ³ /uL
MONO#	1.07 +	10 ³ /uL
EO#	0.15	10 ³ /uL
BASO#	0.07	10 ³ /uL
NEUT%	51.7	%
LYMPH%	32.1	%
MONO%	13.2	%
EO%	1.9	%
BASO%	0.9	%
IG#	0.02	10 ³ /uL
IG%	0.2	%

Item	Data	Unit
IPF	20.2 *	%



Thrombopénie chez un jeune enfant

Main Graph Q-Flag Service User Lab. C

Manual XN-9000-2-A

CBC

Item	Data	Unit
WBC	8.09	10 ³ /uL
RBC	4.55	10 ⁶ /uL
HGB	12.3	g/dL
HCT	38.1	%
MCV	83.7	fL
MCH	27.0	pg
MCHC	32.3	g/dL
PLT &F	2	10 ³ /uL
RDW-SD	38.6	fL
RDW-CV	12.6	%
PDW	----	fL
MPV	----	fL
P-LCR	----	%
PCT	----	%
NRBC#	0.00	10 ³ /uL
NRBC%	0.0	%

DIFF

Item	Data	Unit
NEUT#	4.22	10 ³ /uL
LYMPH#	2.61	10 ³ /uL
MONO#	0.99 +	10 ³ /uL
EO#	0.16	10 ³ /uL
BASO#	0.06	10 ³ /uL
NEUT%	52.2	%
LYMPH%	32.3	%
MONO%	12.2	%
EO%	2.0	%
BASO%	0.7	%
IG#	0.05	10 ³ /uL
IG%	0.6	%

RET

Item	Data	Unit
RET%	1.27	%
RET#	57.8	10 ⁹ /L
IRF	6.5	%
LFR	93.5	%
MFR	6.3	%
HFR	0.2	%
RET-He	27.4	pg

PLT-F

Item	Data	Unit
IPF	20.4 *	%

Scattergram

PLT-F

Close

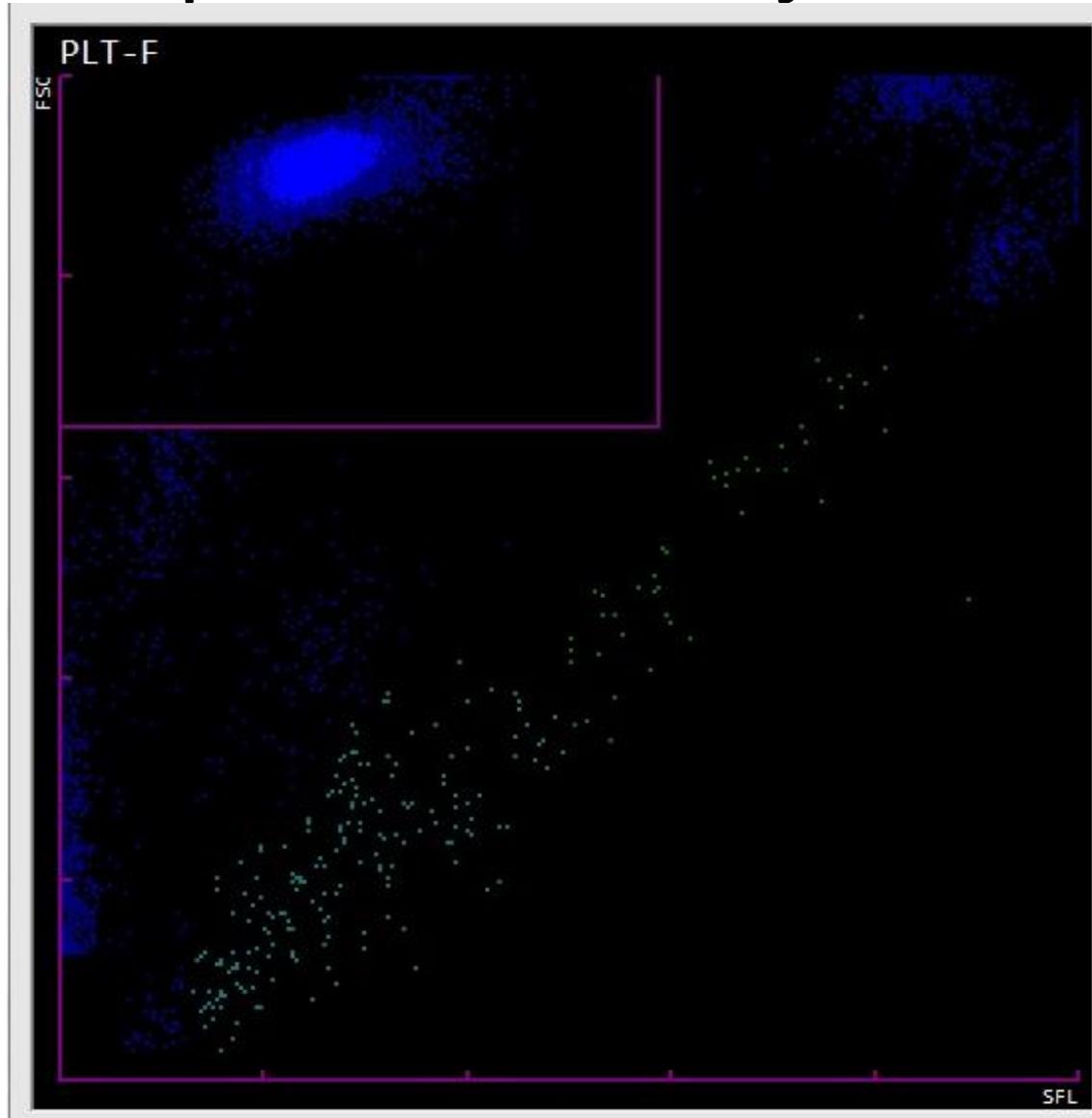
WNR

WPC

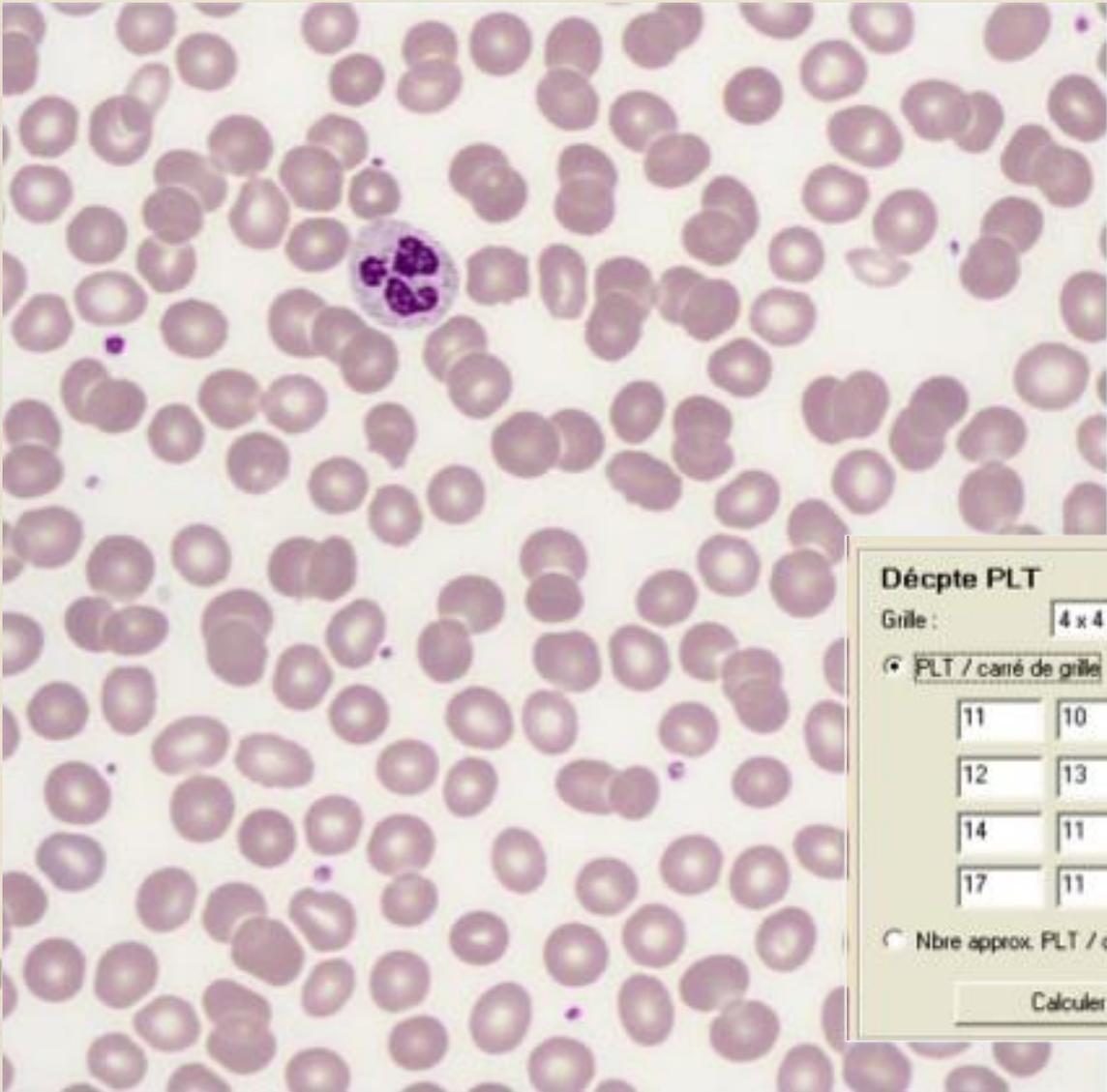
RET

PLT-F

Thrombopénie chez un jeune enfant



Numération Plaquettes sur DM96



Décpte PLT
Grille : 4 x 4 (0,50 HPF/carré)

PLT / carré de grille

Nbre approx. PLT / carré de grille

Calculer résultat PLT

Résultat PLT
Facteur d'évaluation PLT :
Valeur moyenne PLT/HPF :
Concentration plaquettaire : x10⁹/L
 Nombre calculé
 Nombre moyen

Exclure analyse plaquettaire

Remarque plaquettes

Décpte PLT
Grille : 4 x 4 (0,50 HPF/carré)

PLT / carré de grille

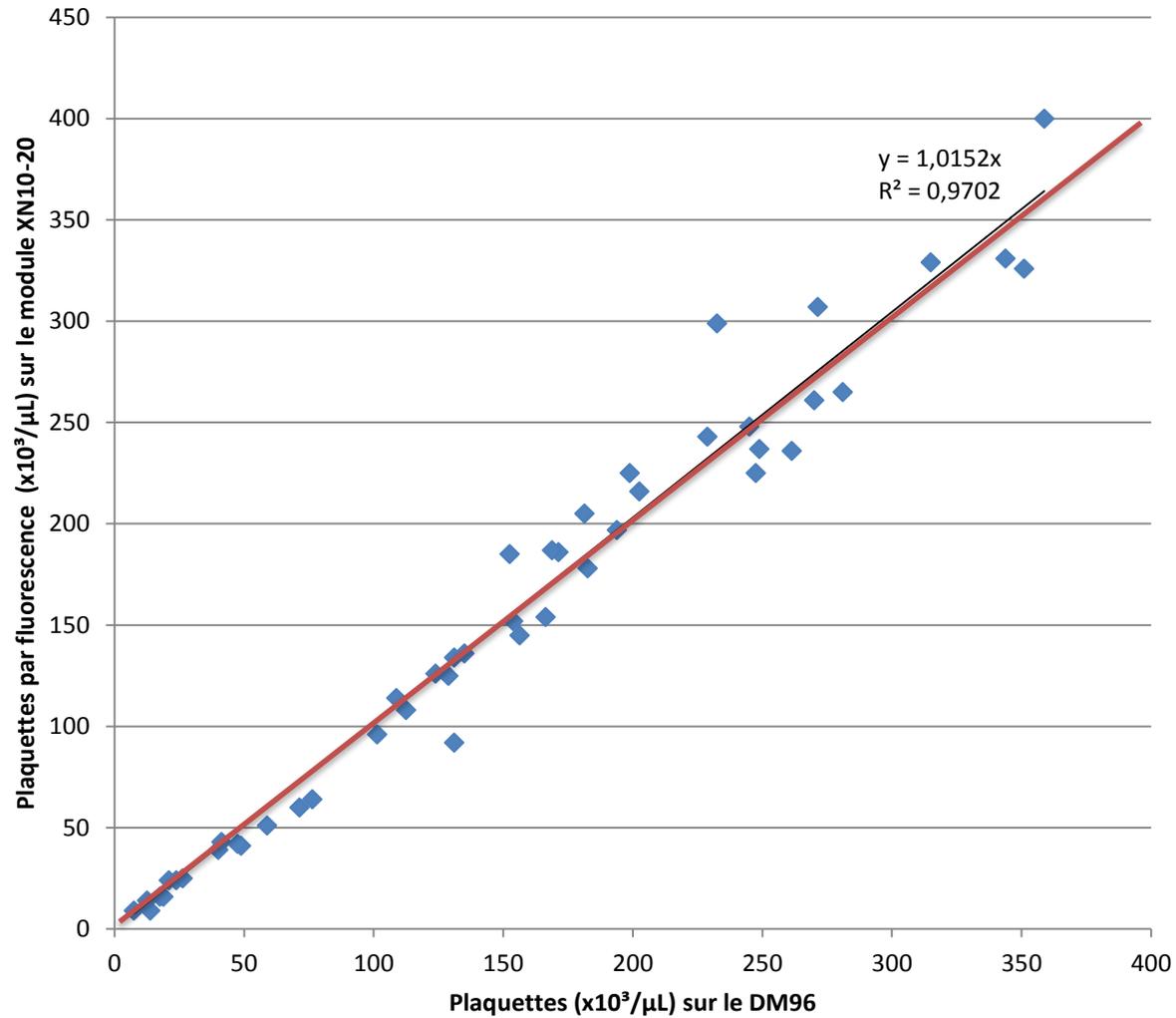
11	10	10	8
12	13	6	5
14	11	12	10
17	11	10	4

Nbre approx. PLT / carré de grille

Calculer résultat PLT

PLT-F vs DM96

PLT-F <400

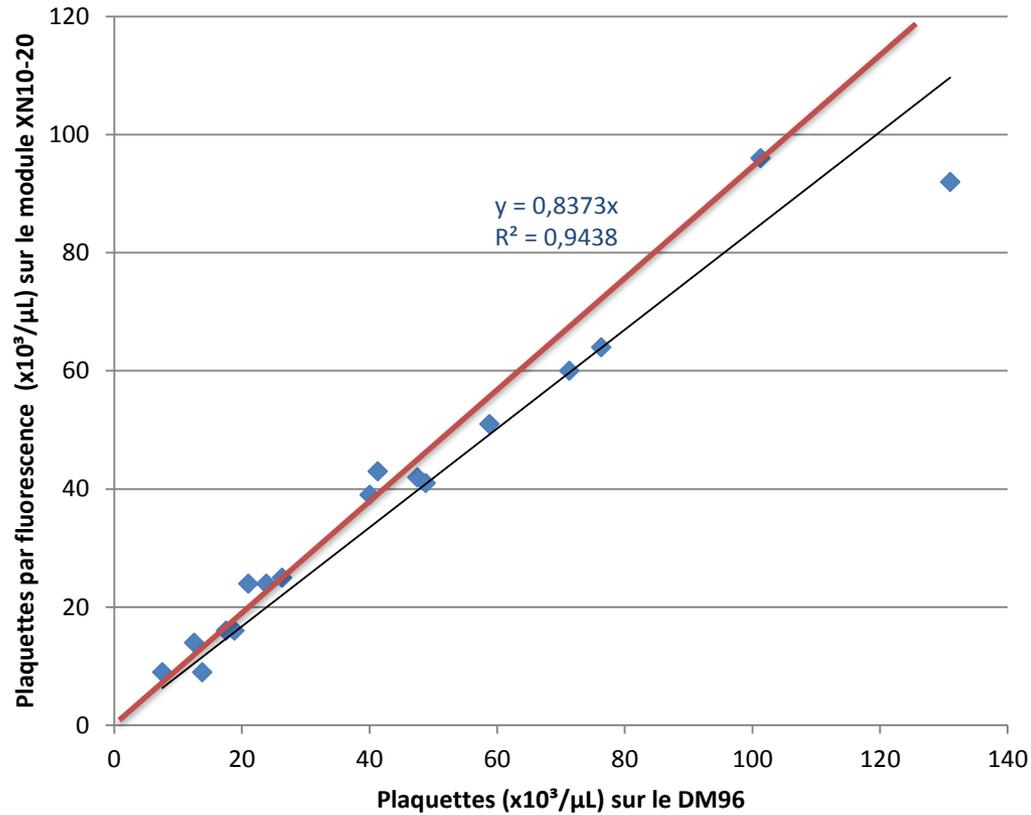


Numération Plaquettes sur DM96

- 17 sujets thrombopéniques pour les 3 méthodes de mesure sur le XN
- Corrélation PLT-F vs PLT-DM96

PLT-F vs DM96

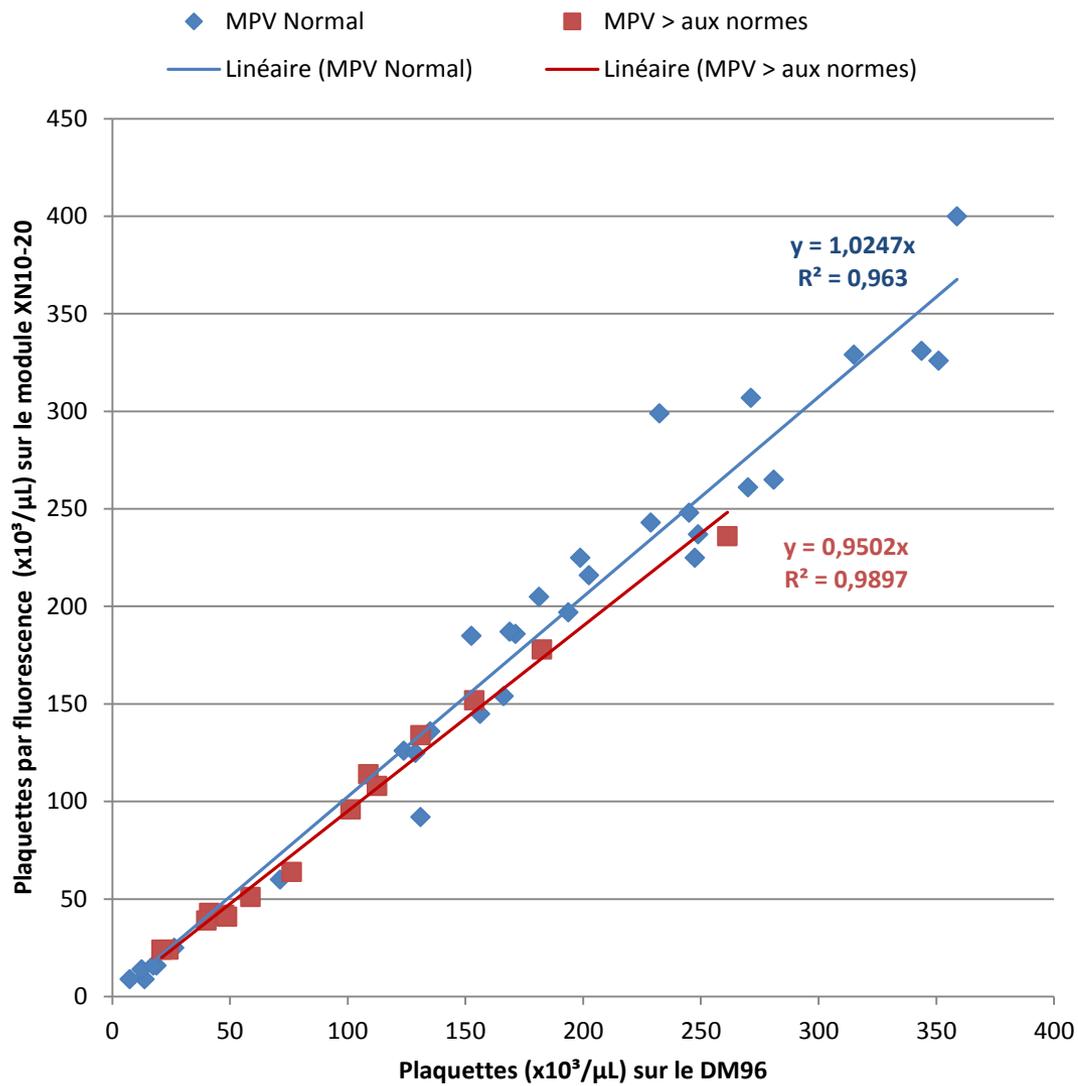
PLT-F <100



PLT et DM96

- Est-ce que les grosses plaquettes influencent la mesure de PLT-F?
- Corrélation DM96 vs PLT-F en isolant les MPV supérieurs à la valeur normale

PLT-F <400



CONCENTRES PLAQUETTAIRES

Item	Data	Unit
WBC	0.00	10 ³ /uL
RBC	0.15	10 ⁶ /uL
HGB	0.0	g/dL
HCT	0.5	%
MCV	33.3	fL
MCH	0.0	pg
MCHC	0.0	g/dL
PLT	2065	10 ³ /uL
RDW-SD	----	fL
RDW-CV	----	%
PDW	13.5	fL
MPV	11.5	fL
P-LCR	38.0	%
PCT	2.38	%
NRBC#	0.01	10 ³ /uL
NRBC%	----	%

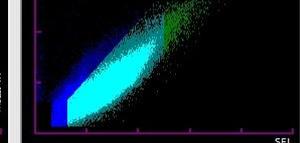
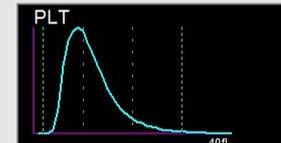
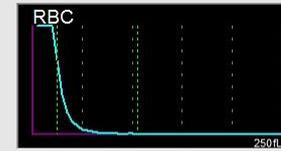
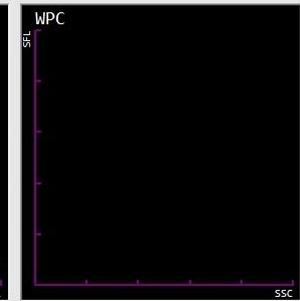
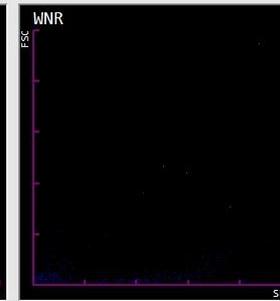
Item	Data	Unit
NEUT#	----	10 ³ /uL
LYMPH#	----	10 ³ /uL
MONO#	----	10 ³ /uL
EO#	----	10 ³ /uL
BASO#	----	10 ³ /uL
NEUT%	----	%
LYMPH%	----	%
MONO%	----	%
EO%	----	%
BASO%	----	%
IG#	----	10 ³ /uL
IG%	----	%

Item	Data	Unit
IPF	1.2	%

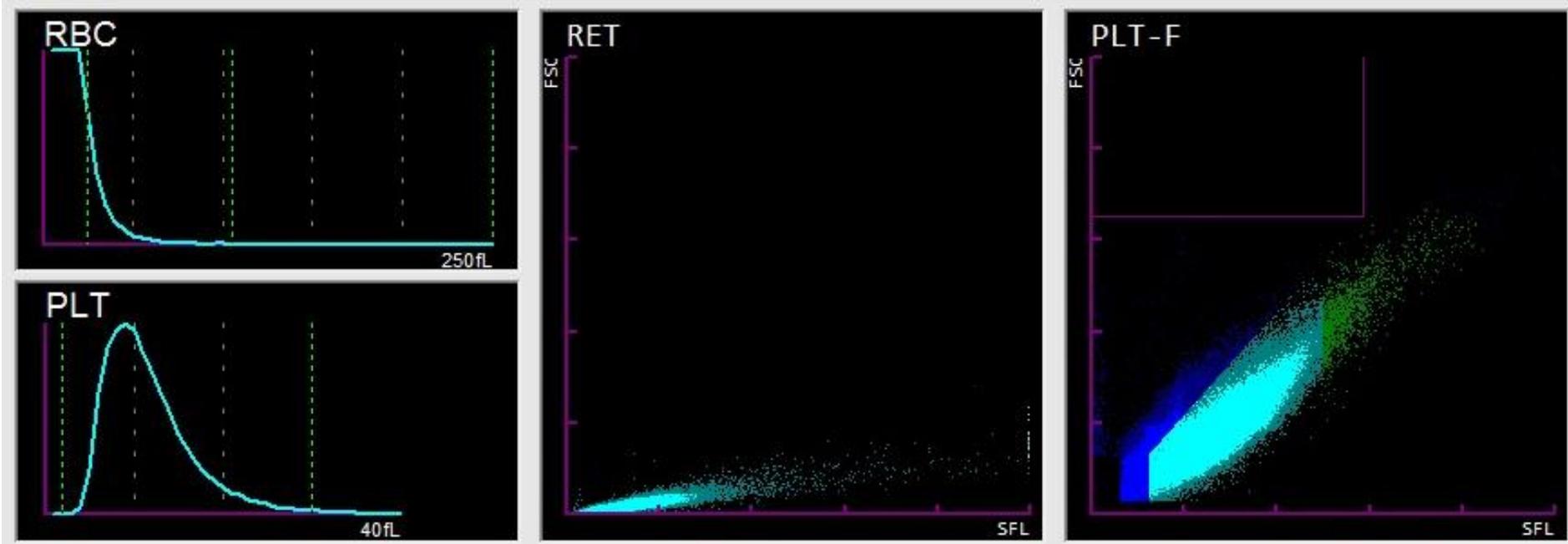
WBC Flag(s)
Leukocytopenia
Blasts/Abn Lympho?

RBC Flag(s)
RBC Abn Distribution

PLT Flag(s)
Thrombocytosis
PLT Clumps?



CONCENTRES PLAQUETTAIRES



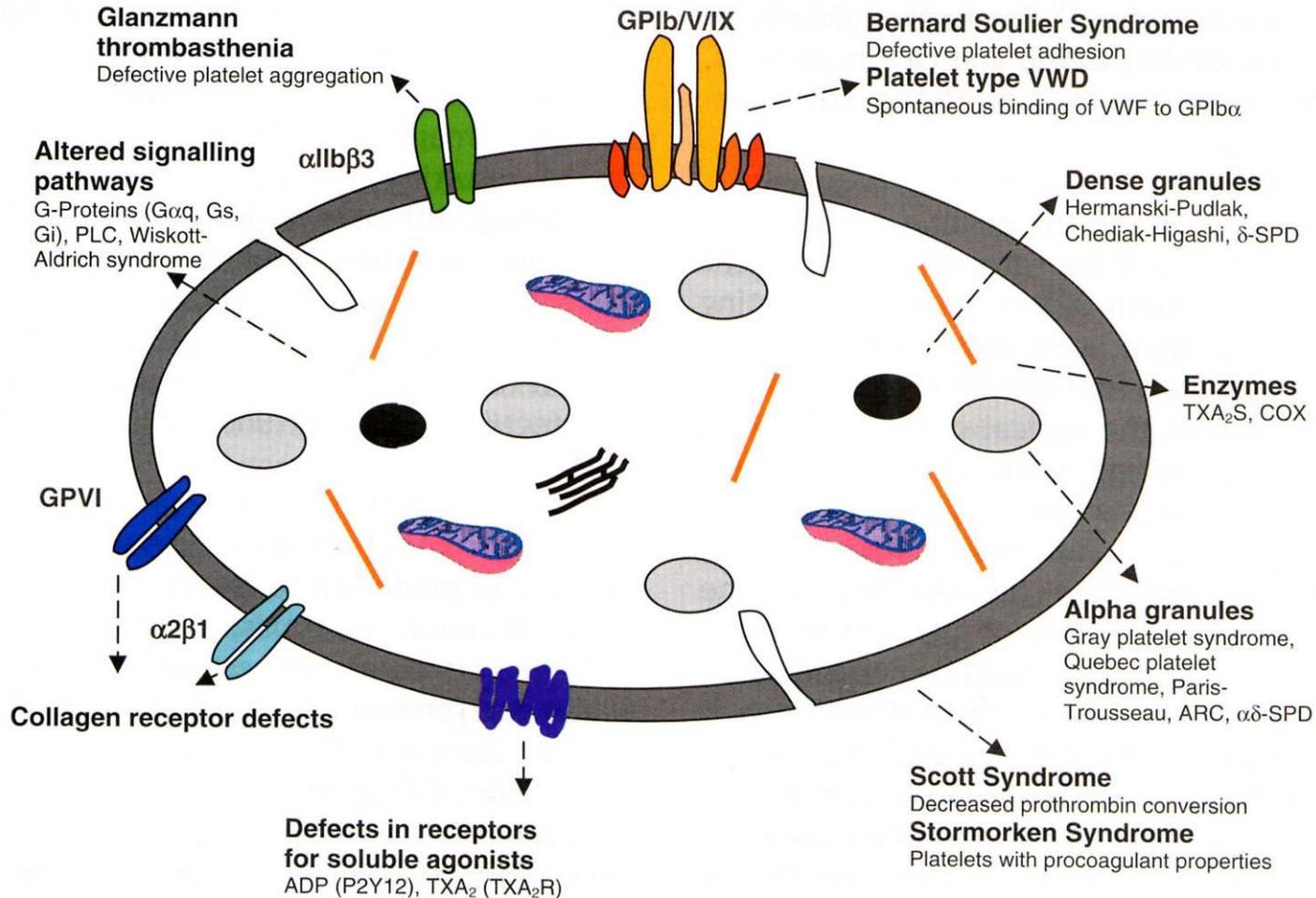
CONCENTRES PLAQUETTAIRES

- Numérations instrument dépendantes (Différences parfois de plus de 50%!!!)
- Haute concentration n'est pas un réel problème (linéarité à des valeurs très élevées)
- Différence d'anticoagulant (Citrates): modification de l'indice de réfraction
 - toujours remettre dans EDTA K2
- Absence de Globules rouges
 - Différence de viscosité
 - Correction de coïncidence (PLT-RBC) non asservie à la concentration en Globules rouges
 - Différence de position du seuil mobile pour numération impédance (Sysmex)
- Absence de technique de référence pour calibration
- Absence de contrôle adapté

- Conclusion : immuno**hématologie** transfusionnelle

Immunocomptage

Thrombopathies



Anticorps utilisés

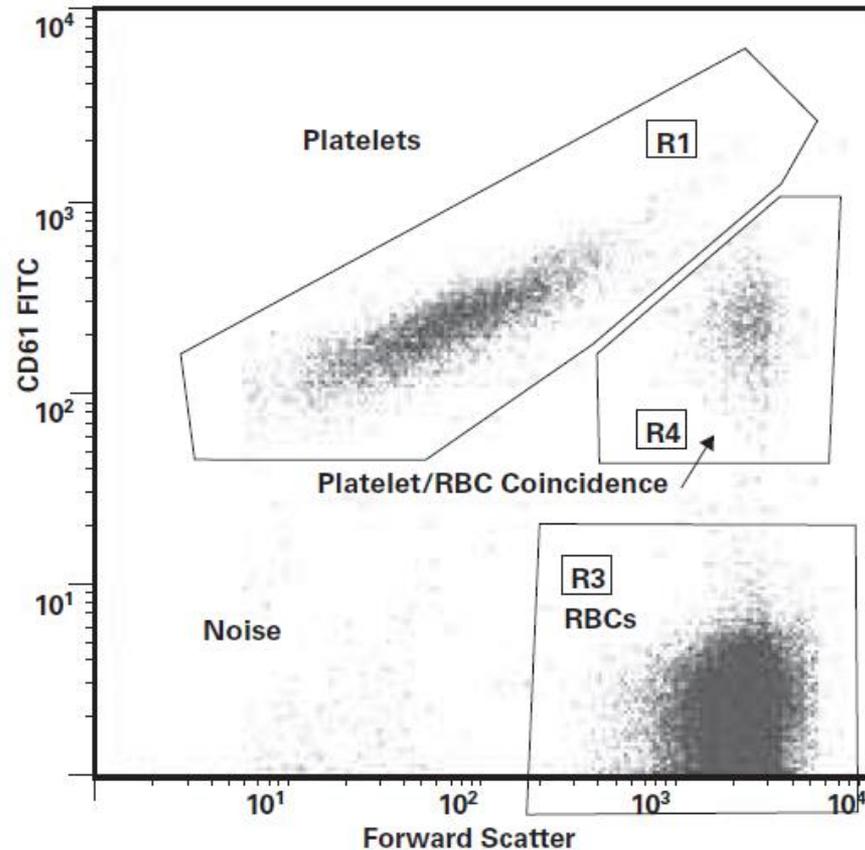
Glycoprotéine	CD	Clone
Gpla	CD49b	Gi9
Ib α	CD42b	SZ2
		MB45 (mutant BSS Bolzano)
IIb	CD41	P2 (IIb dans complexe intact avec IIIa)
IIIa	CD61	4F8 (strictement IIIa)
		SZ21
		Lyp18 (IIIa complexée à la IIb)
V	CD42d	SW16
IX	CD42a	FMC25
		SZ1

Gp Screen, Biocytex, Stago

Immunoplatelet counting: a proposed new reference procedure

PAUL HARRISON,* ALLAN HORTON,† DONNA GRANT,* CAROL BRIGGS* AND SAM MACHIN* *Haemostasis Research, Department of Haematology, 98 Chenies Mews, University College London WC1E 6HX, UK, and †Gulf Coast Pathology, Cellular Analysis Division, Fort Myers, FL, USA

Received 7 October 1999; accepted for publication 11 October 1999



+ : SPECIFICITE ↑ ↑ ↑

Immunocomptage plaquettaire

- Double plateforme: [PLT] (plt/ μ l)=

$$\frac{\text{Nombre de PLT du cytomètre + coïncidences (PLT/GR)}}{\text{Nombre de GR du cytomètre + coïncidences (PLT/GR) + doublets GR/GR X 2}} \times \begin{matrix} \text{[GR] de l'analyseur} \\ \text{d'hématologie} \\ \text{(GR/ μ l)} \end{matrix}$$

- Nécessite la concentration en GR
- Non utilisable pour les concentrés plaquettaires et les plasmas riches en plaquettes
- Cumul de deux imprécisions

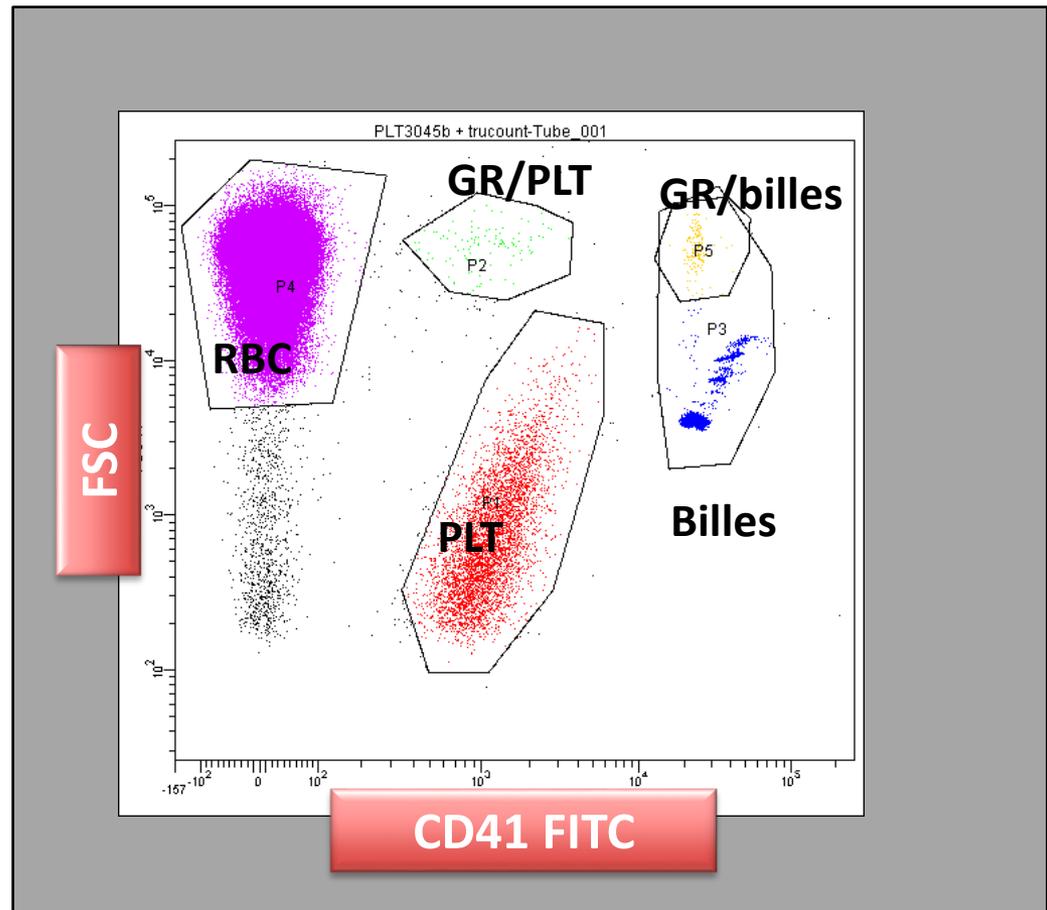
- Simple plateforme:

- billes (standard interne): quantité connue ajoutée à un volume connu de suspension cellulaire
- volumétrique

Immunocomptage plaquettaire: simple et double plateforme

Sur BD Facs Canto II:

- Canto SP (v/s billes)
- Canto DP (v/s Sysmex XE2100)

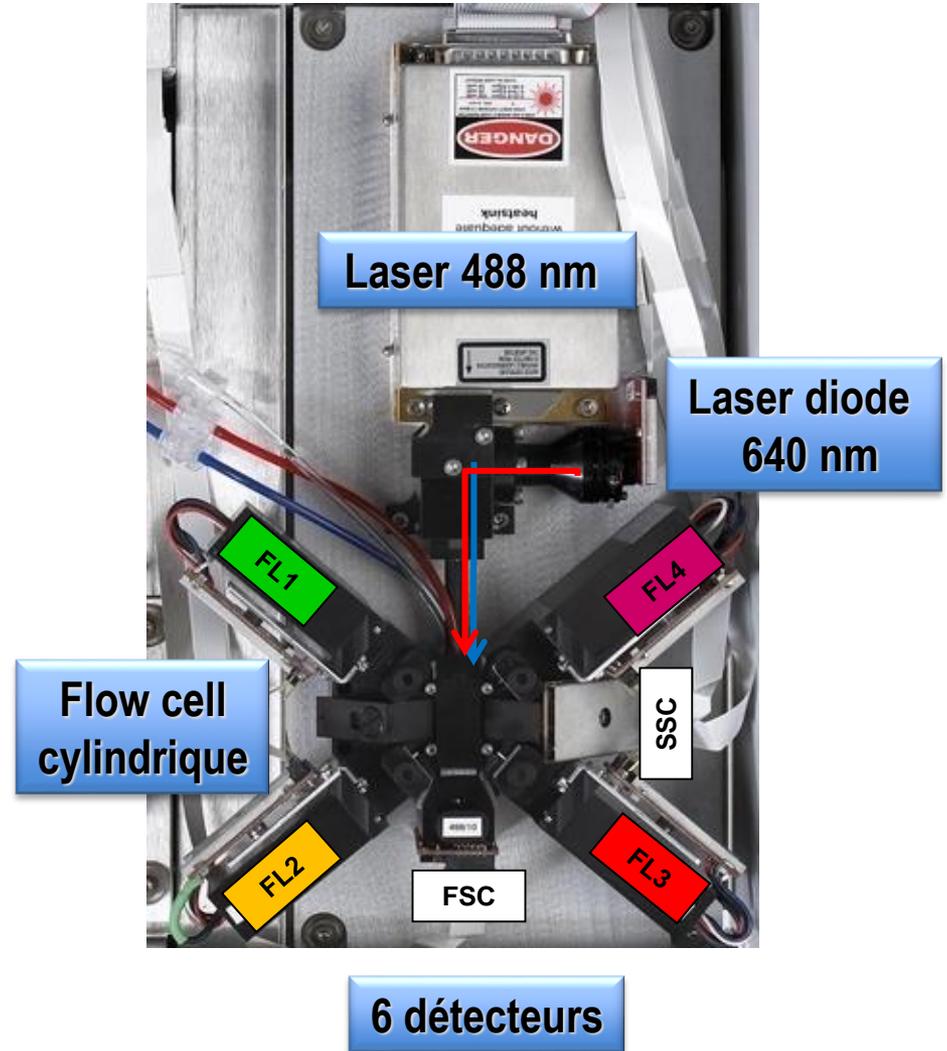


Billes Trucount (BD Biosciences)

Cytomètre en flux Accuri C6 (BD Biosciences)



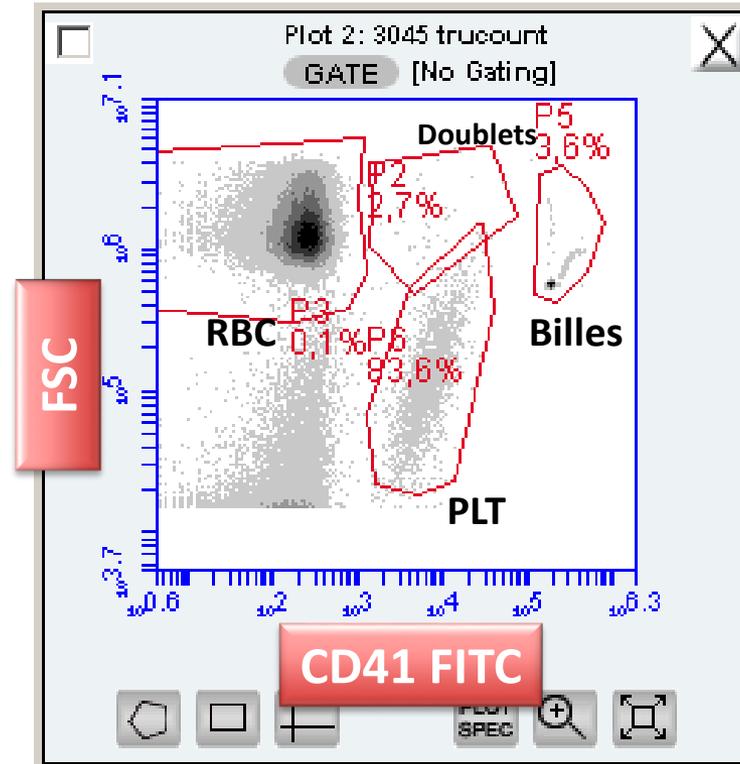
2 pompes péristaltiques (push et pull)



Immunocomptage plaquettaire: simple et double plateforme

Sur BD Accuri C6:

- Accuri SP (v/s billes)
- Accuri DP (v/s Sysmex XE2100)
- Accuri SP (volumétrique)



↳ Cytomètre capable de fournir directement une numération absolue

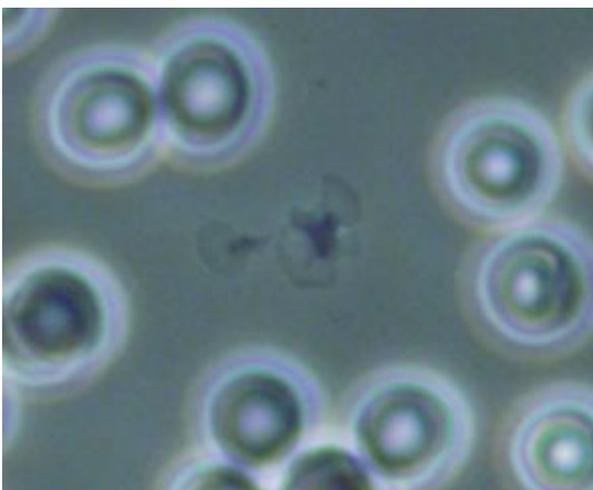
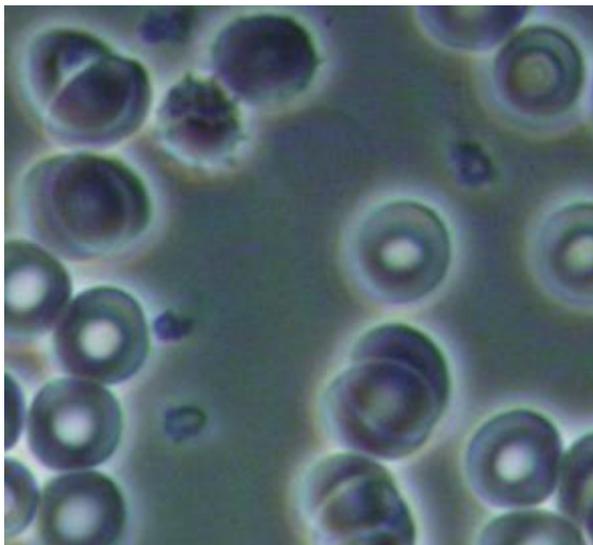
$$[\text{PLT}] (\text{plt}/\mu\text{l}) = \frac{\text{Nombre de PLT du cytomètre}}{\text{Volume de suspension cellulaire aspiré}} \times \text{X facteur de dilution de la préparation}$$

Prélèvement

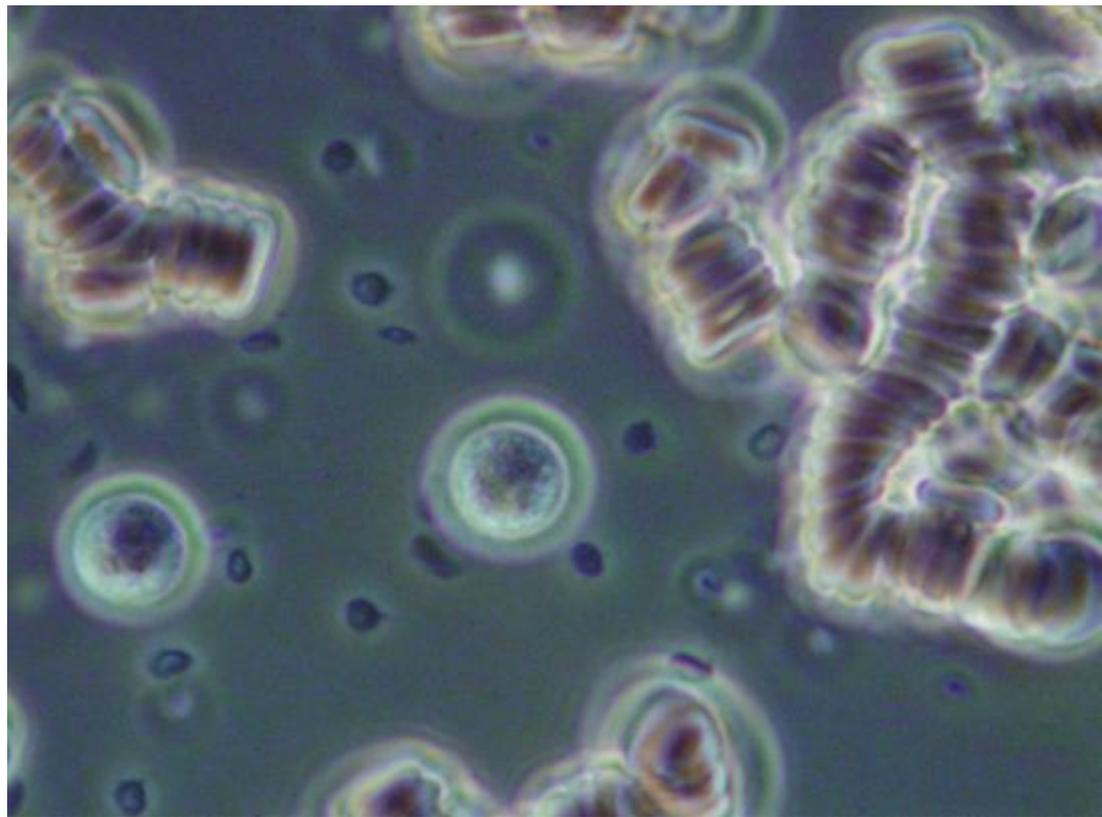
- Qualité
- Anticoagulant
- Acheminement (télétube...)
- Conservation

Anticoagulant

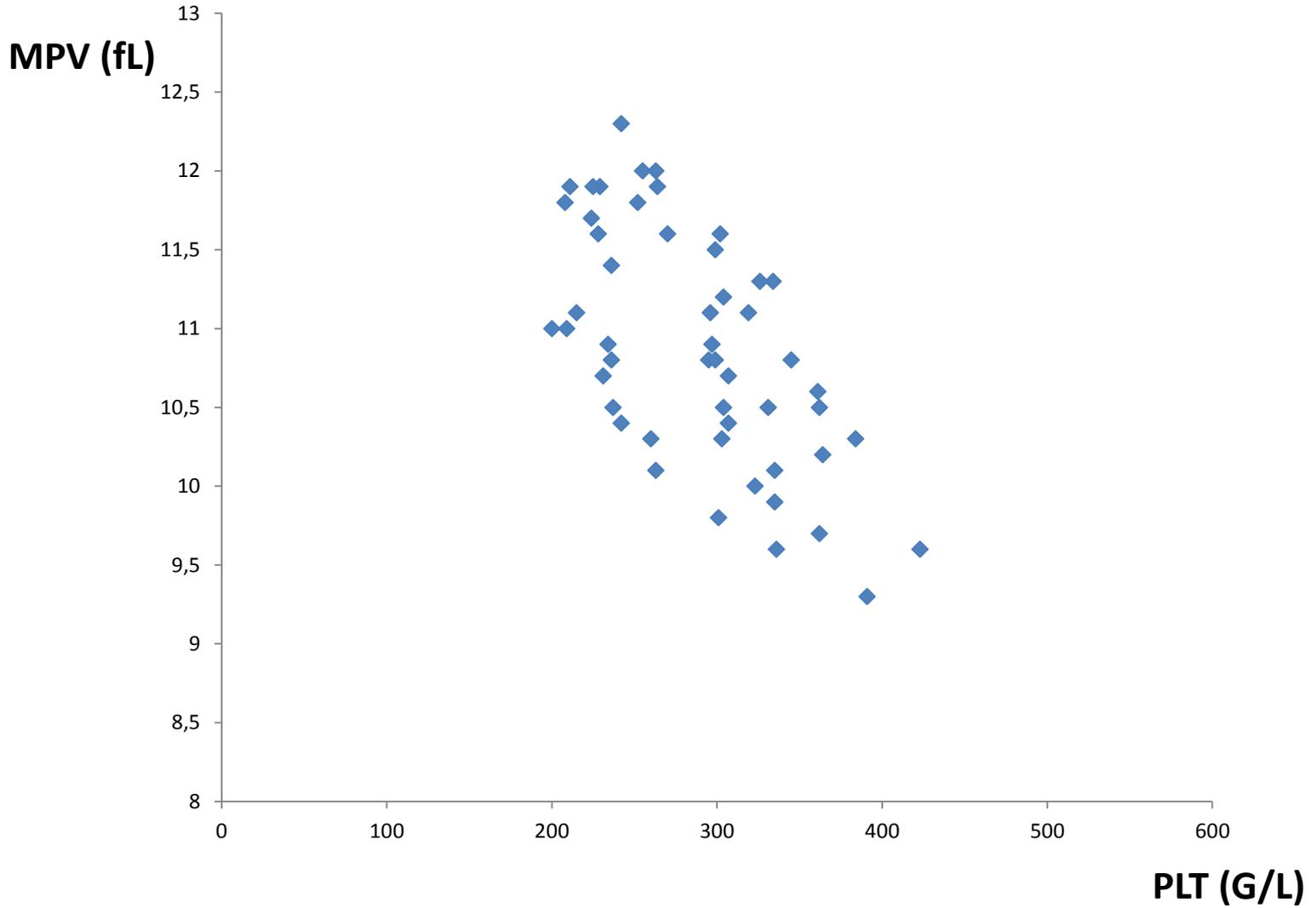
EDTA K2



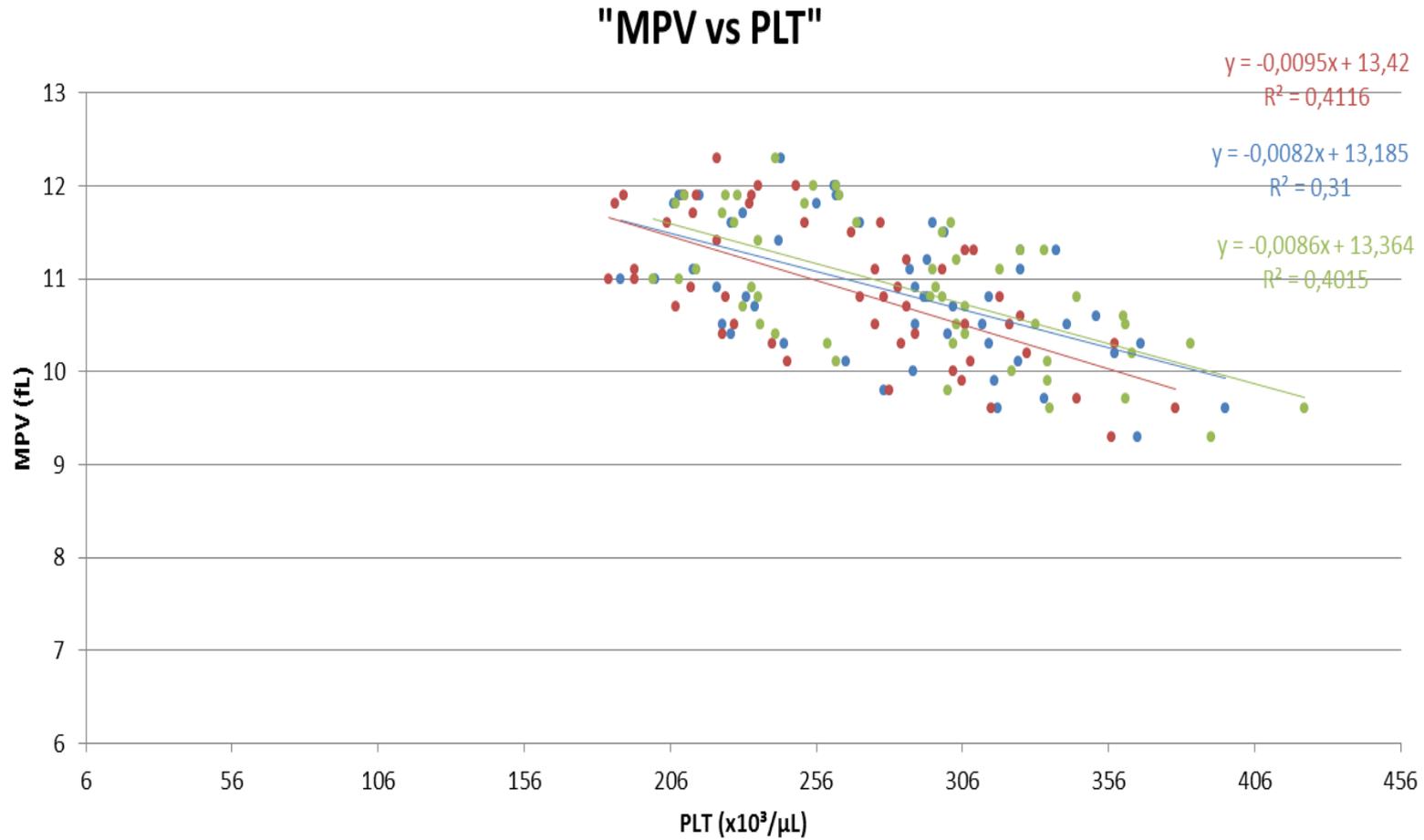
CTAD



Relation MPV- Conc. Plaquettaire



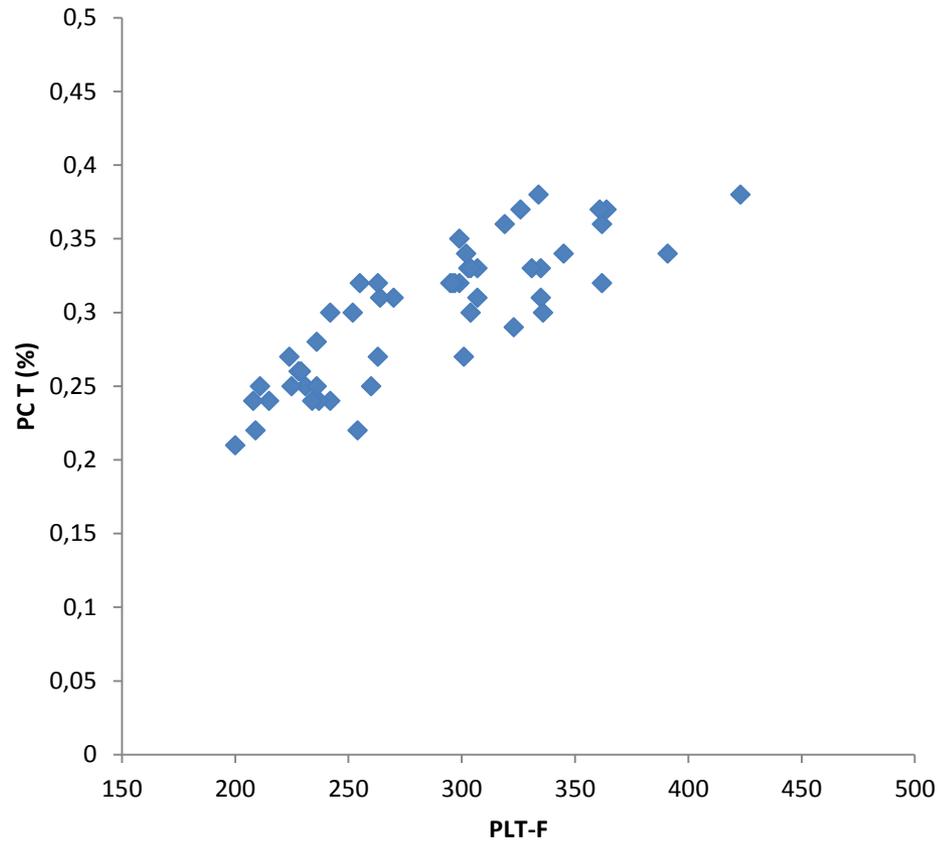
Relation MPV- Conc. Plaquettaire



- MPV vs PLT-I
- MPV vs PLT-O
- "MPV vs PLT-F"
- Linéaire (MPV vs PLT-I)
- Linéaire (MPV vs PLT-O)
- Linéaire ("MPV vs PLT-F")

Thrombocytocrite

PLT-F vs PCT



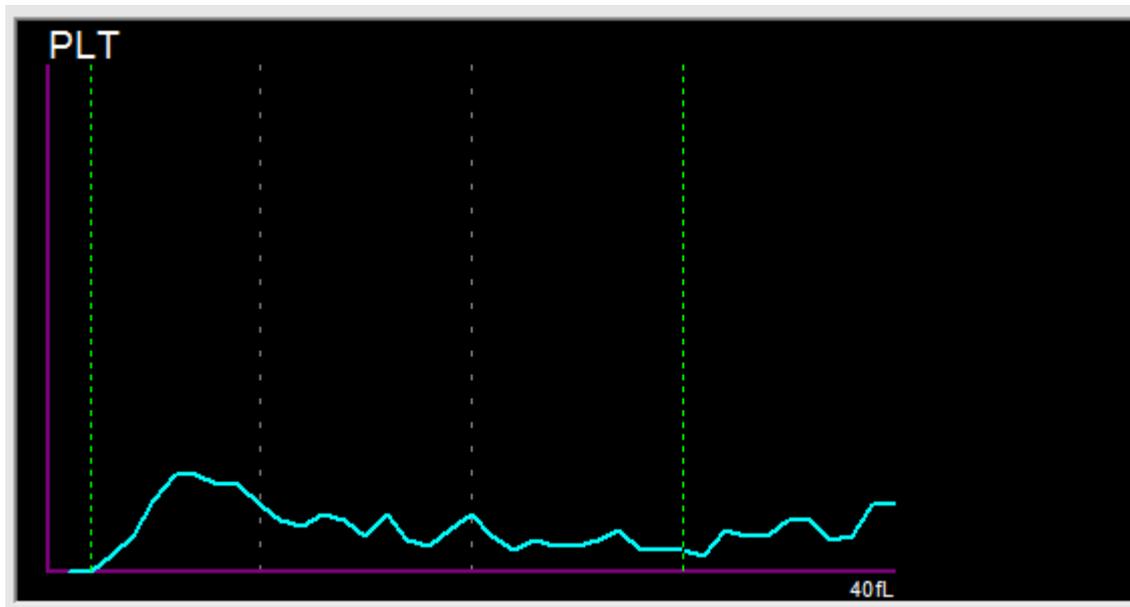
MFPV

- Sur le Sysmex, il s'agit du paramètre P-MFV
 - *P-MFV is the peak of the distribution curve for the platelets. It is a useful parameter because unlike the median value, it is available even when there is severe thrombocytopenia.*
- Paramètre de recherche
 - Reproductibilité?

Reproductibilité du MFPV

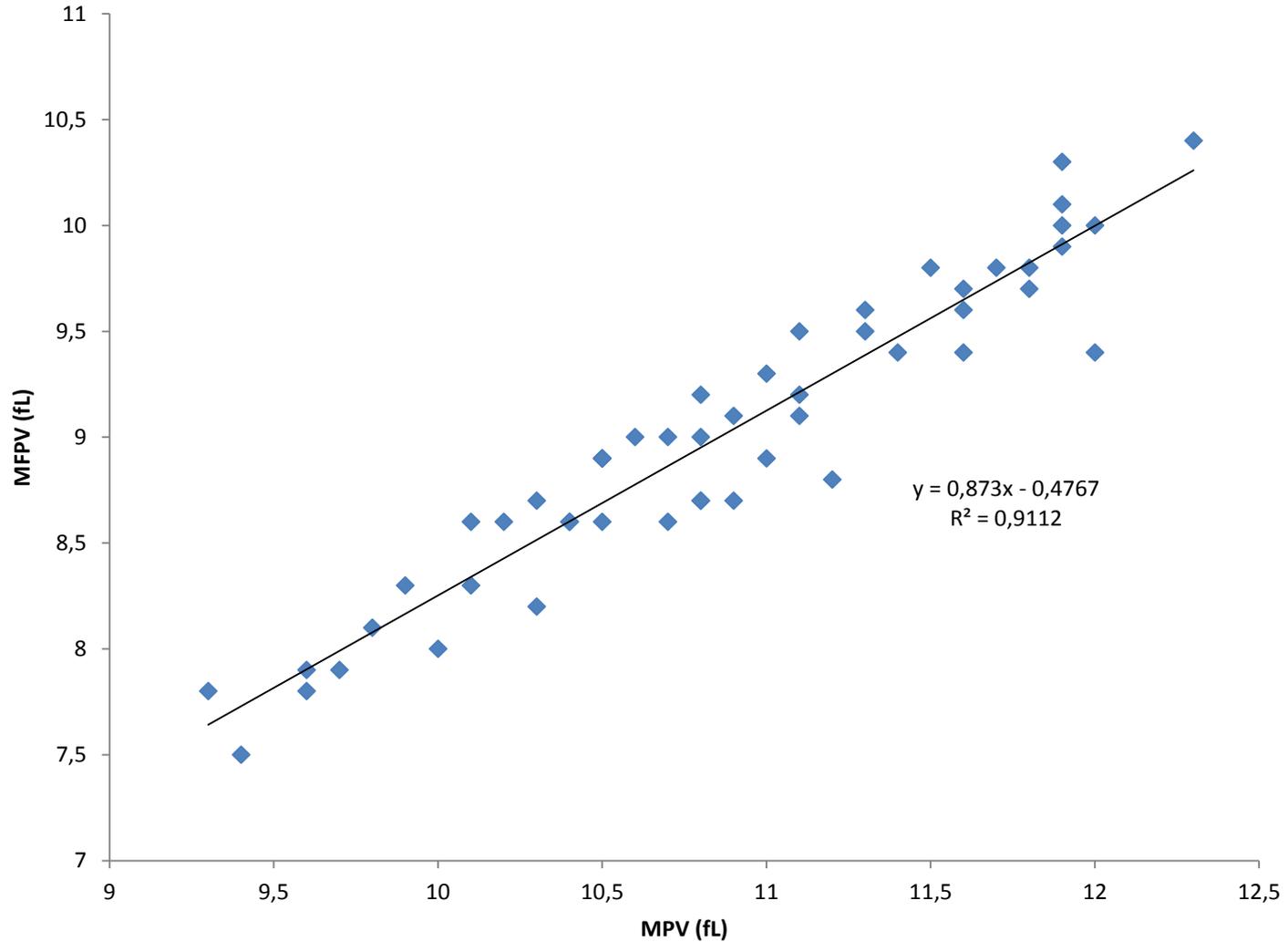
- Chez un patient thrombopénique, réalisation de 6 runs :

RUN	MPV	MPFV	IPF
Run 1	13,00	9,00	14,80
Run 2	11,40	8,00	13,70
Run 3	13,10	8,70	12,70
Run 4	11,10	7,80	13,40
Run 5	13,20	7,30	12,40
Run 6	ND	7,60	14,40
CV (%)	8,26	8,14	6,89

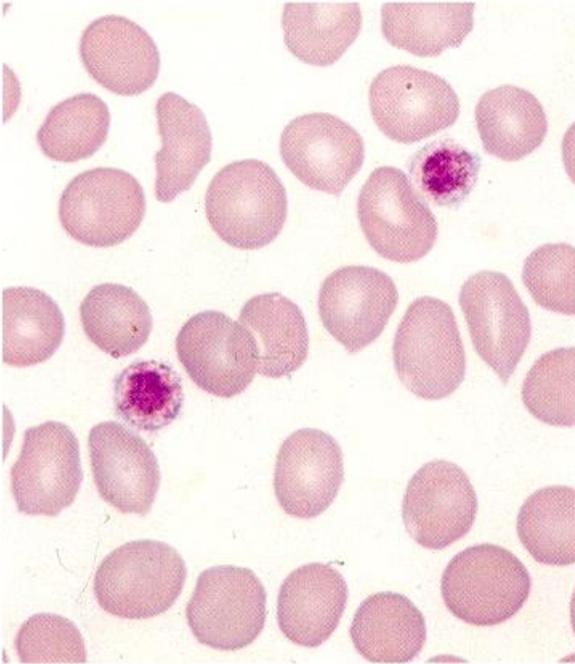


Moyenne ou Mode

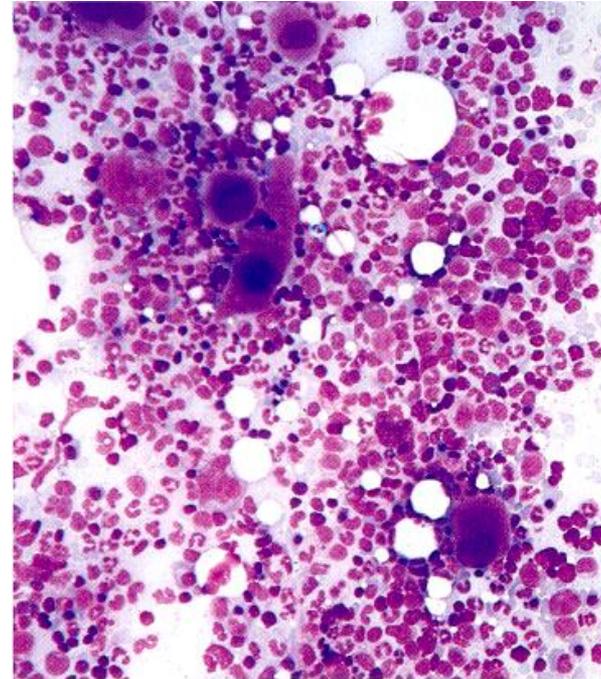
"MPV vs MFPV"



PLAQUETTES RÉTICULÉES



Par analogie aux réticulocytes
Plaquettes plus jeunes
Riches en acides nucléiques
De grande taille

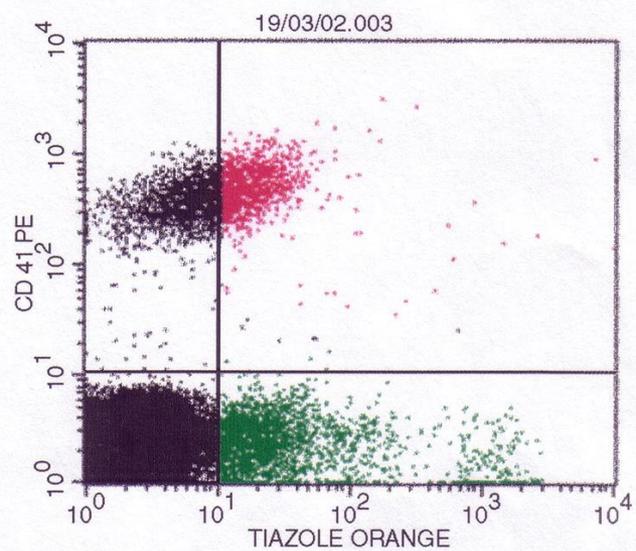
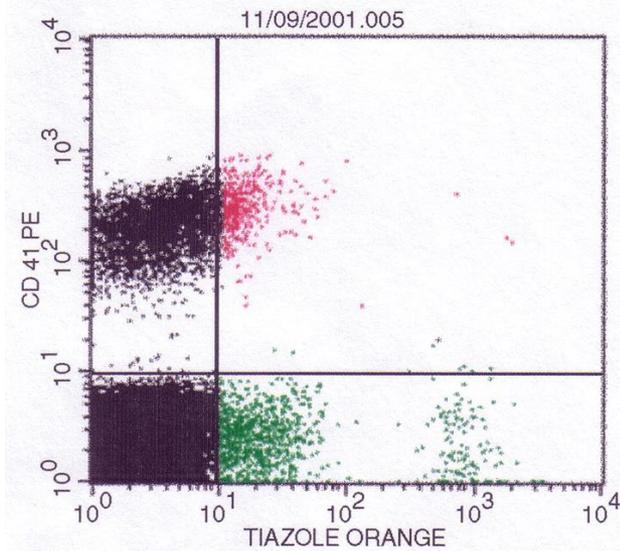
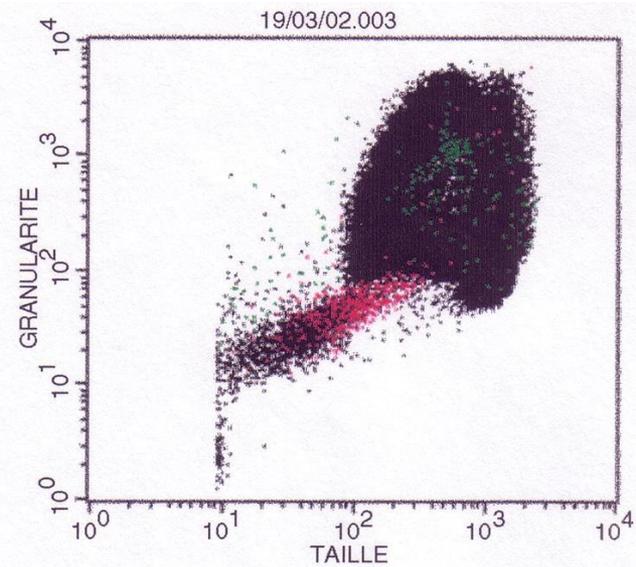
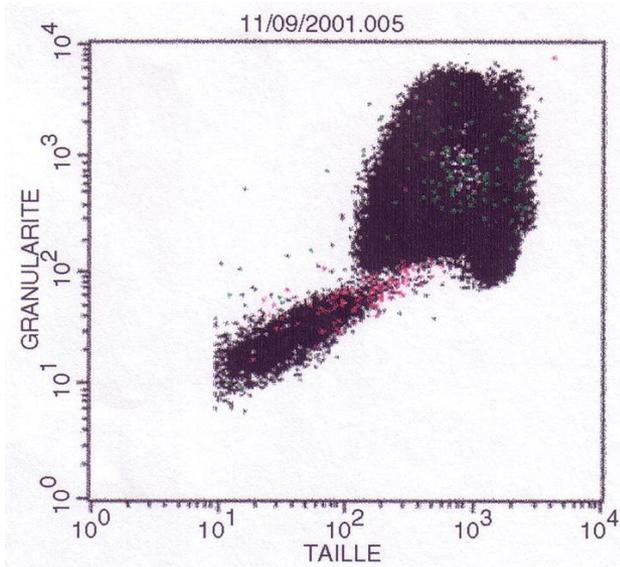


Atteinte centrale ou
périphérique?
Indice de régénération
plaquettaire?

DONNEES DE LA LITTERATURE



- 1969 découverte des PR en microscopie électronique (*Ingram et al*)
- 1986 marquage ARN par le TO (*Lee et al*)
- 1990 intérêt dans le Dg des thrombopénies (*Kienast et Schmidt*)
- 1992 PR = index de thrombopoïèse (*Ault et al*)
- 1993-1998 : 10 études avec protocoles différents
 - PTI, SMP, insuffisance Médullaire, post-chimiothérapie*
- Absence de consensus méthodologique

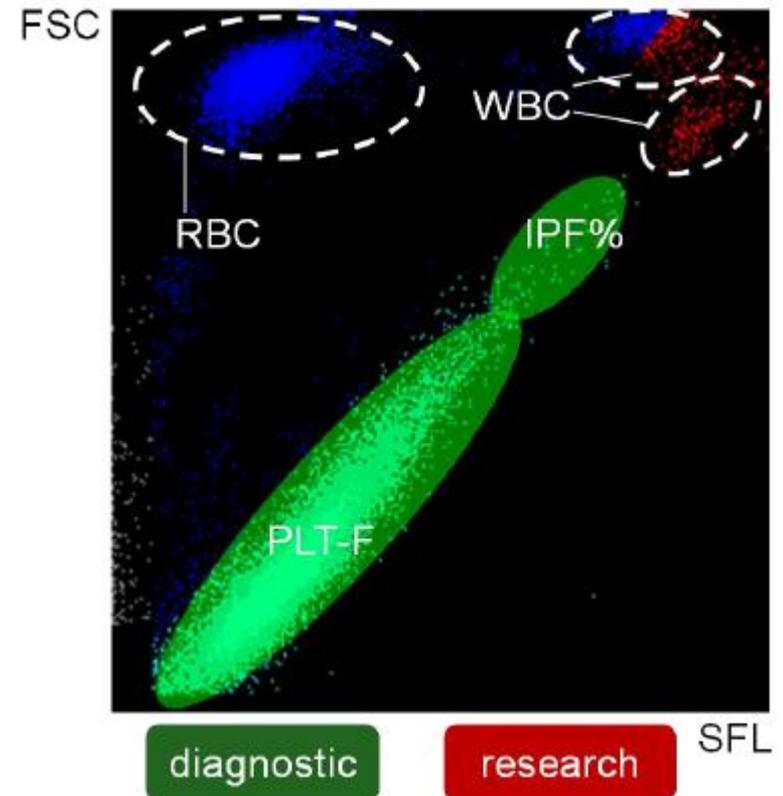


Tpénie centrale PR 6%

Tpénie périphérique PR 43%

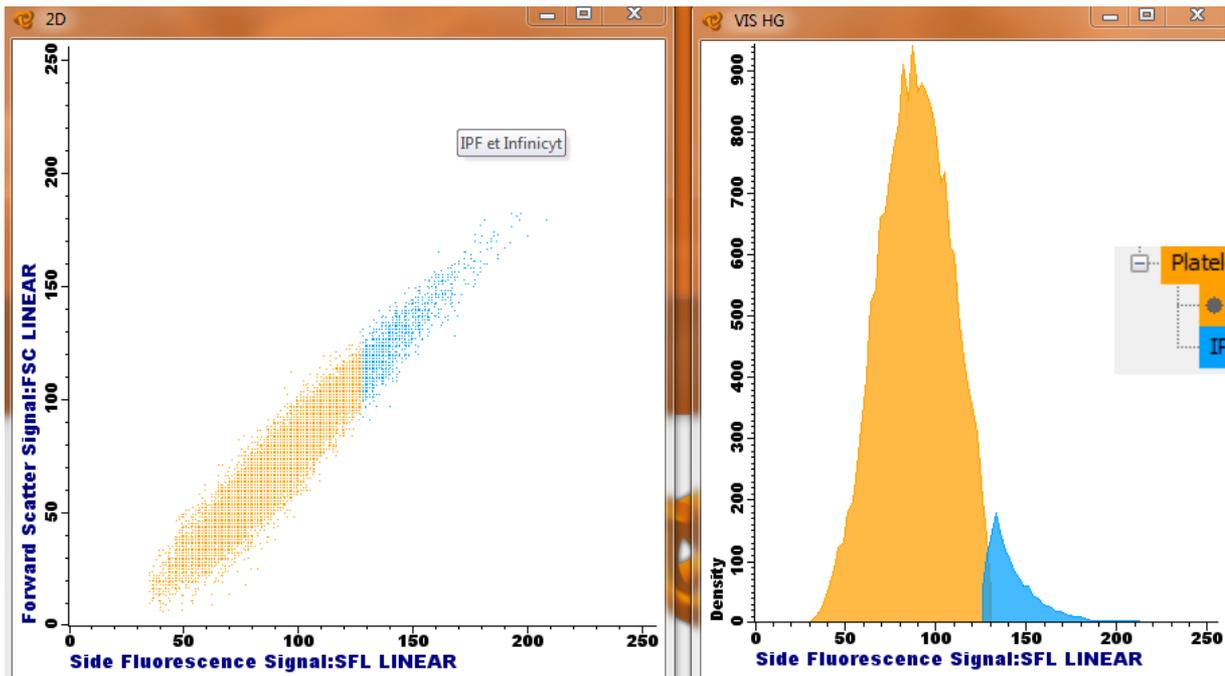
La mesure de la fraction immature des plaquettes (IPF)

- L'IPF est obtenu sur le canal Plaquette Fluorescence
 - FSC: Diffusion axiale
 - SFL: Fluorescence après Oxazine
- Sur un FSC vs SFL , IPF= la portion supérieure en FSC et SFL de la population des plaquettes

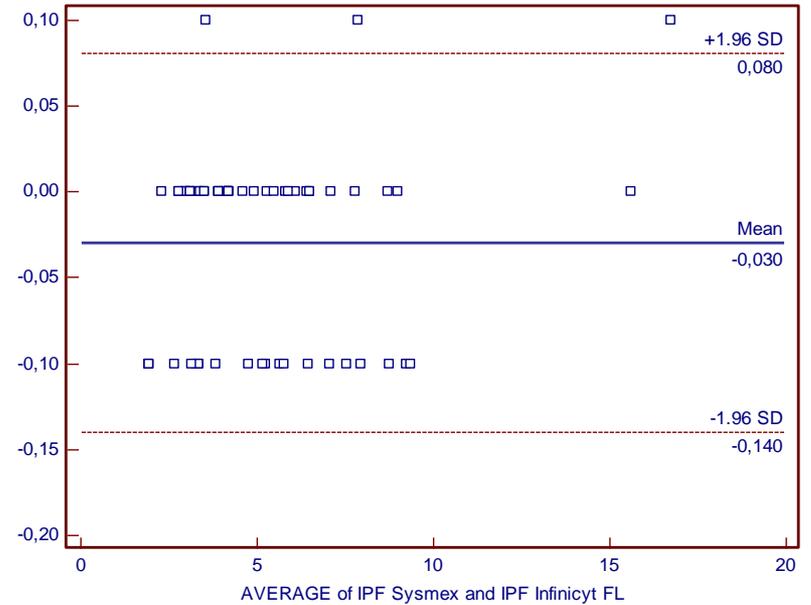
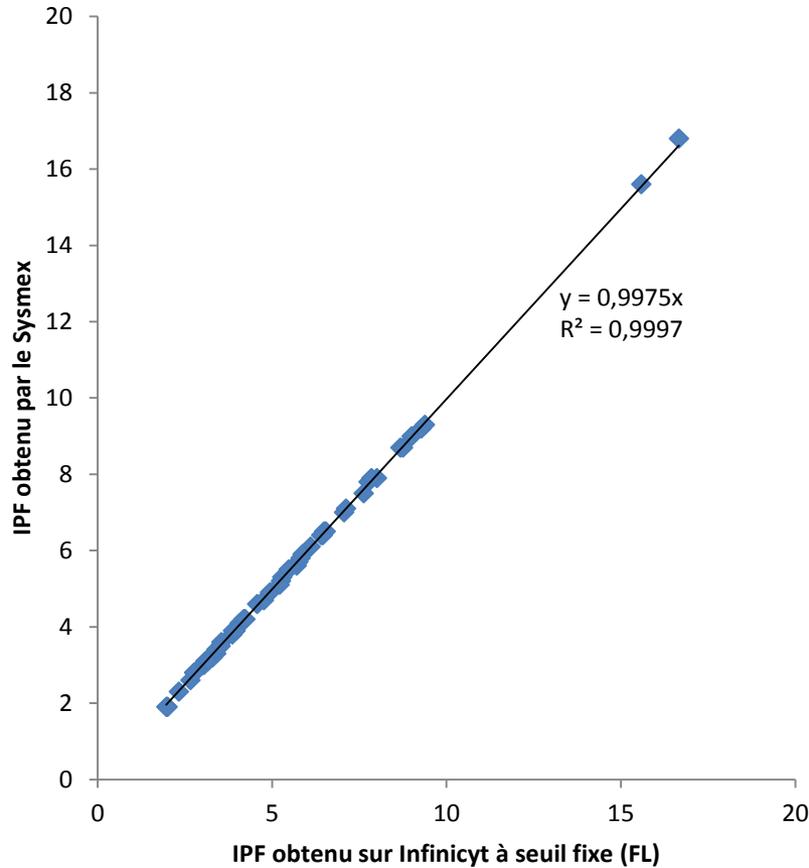


IPF

- seuil IPF fixe sur SFL.

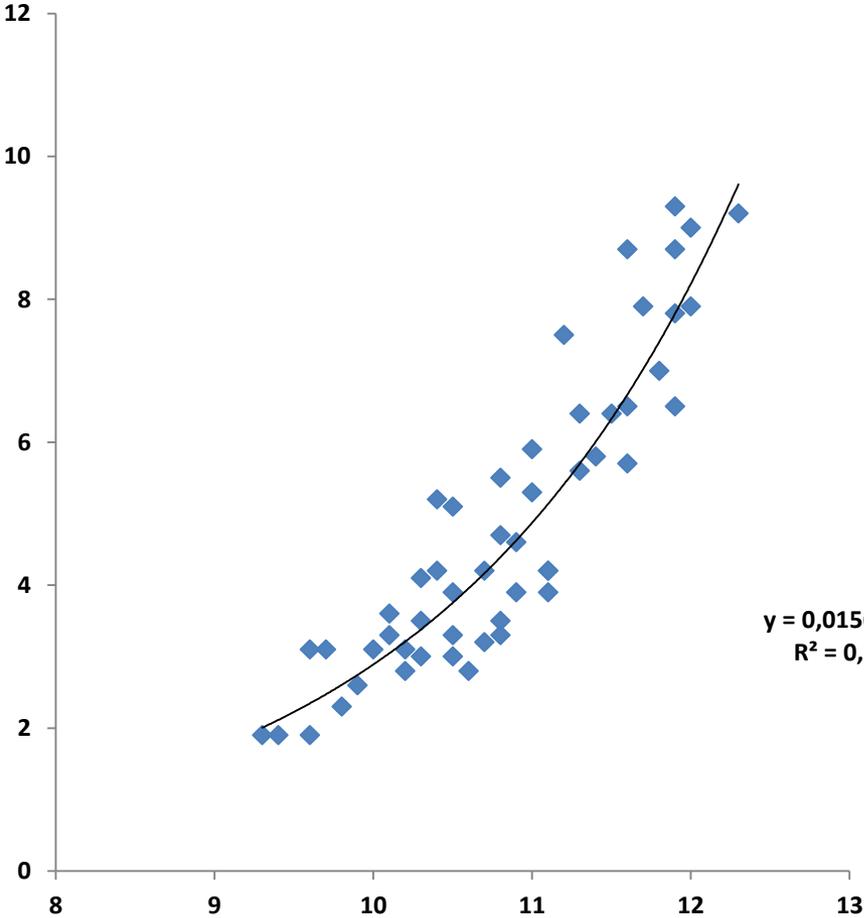


IPF SYSMEX vs Infinicyt (seuil FL)



IPF vs MPV

IPF (%)



$y = 0,0156e^{0,5223x}$
 $R^2 = 0,8359$

MPV (fL)

Relationship between size and thiazole orange fluorescence of platelets in patients undergoing high-dose chemotherapy

C. L. BALDUINI,¹ P. NORIS,¹ P. SPEDINI,¹ S. BELLETTI,¹ A. ZAMBELLI² AND G. A. DA PRADA²

¹*Institute of Internal Medicine and Medical Oncology, IRCCS Policlinico San Matteo, Pavia, and*

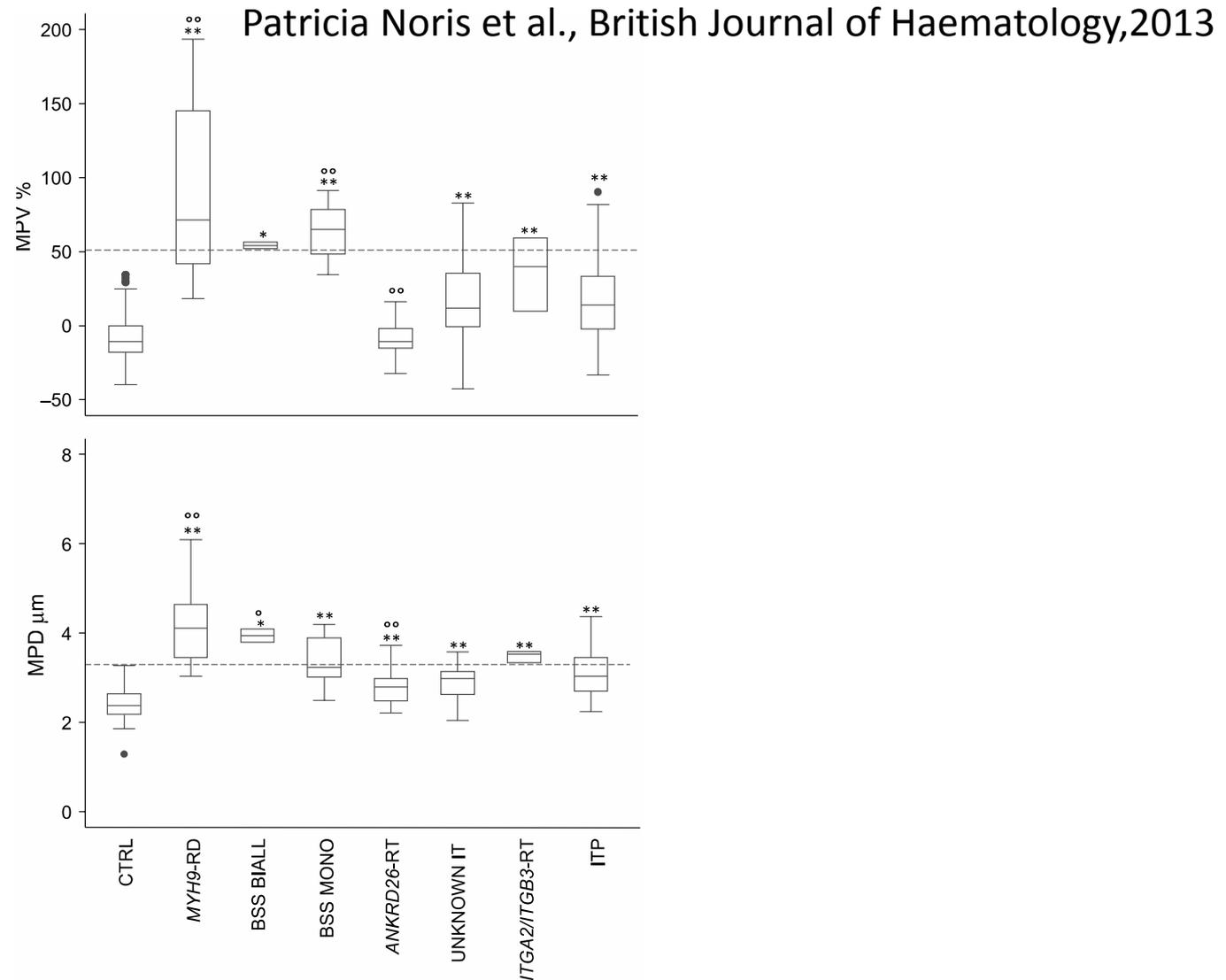
²*Division of Medical Oncology, IRCCS Fondazione S. Maugeri, Pavia, Italy*

Thiazole Orange

	Normal platelets	Old platelets	Young platelets
TO (arbitrary units)	2'96 0'4	3'06 0'3	3'36 0'4
TO post-TRAP (arbitrary units)	2'06 0'3**	1'96 0'4**	2'06 0'4**
TO post-RNase (arbitrary units)	1'76 0'4**	1'96 0'2**	2'16 0'5**
TO post-TRAP‡ RNase (arbitrary units)	1'26 0'2**	1'36 0'3**	1'36 0'3**

** P < 0'01 with respect to TO.

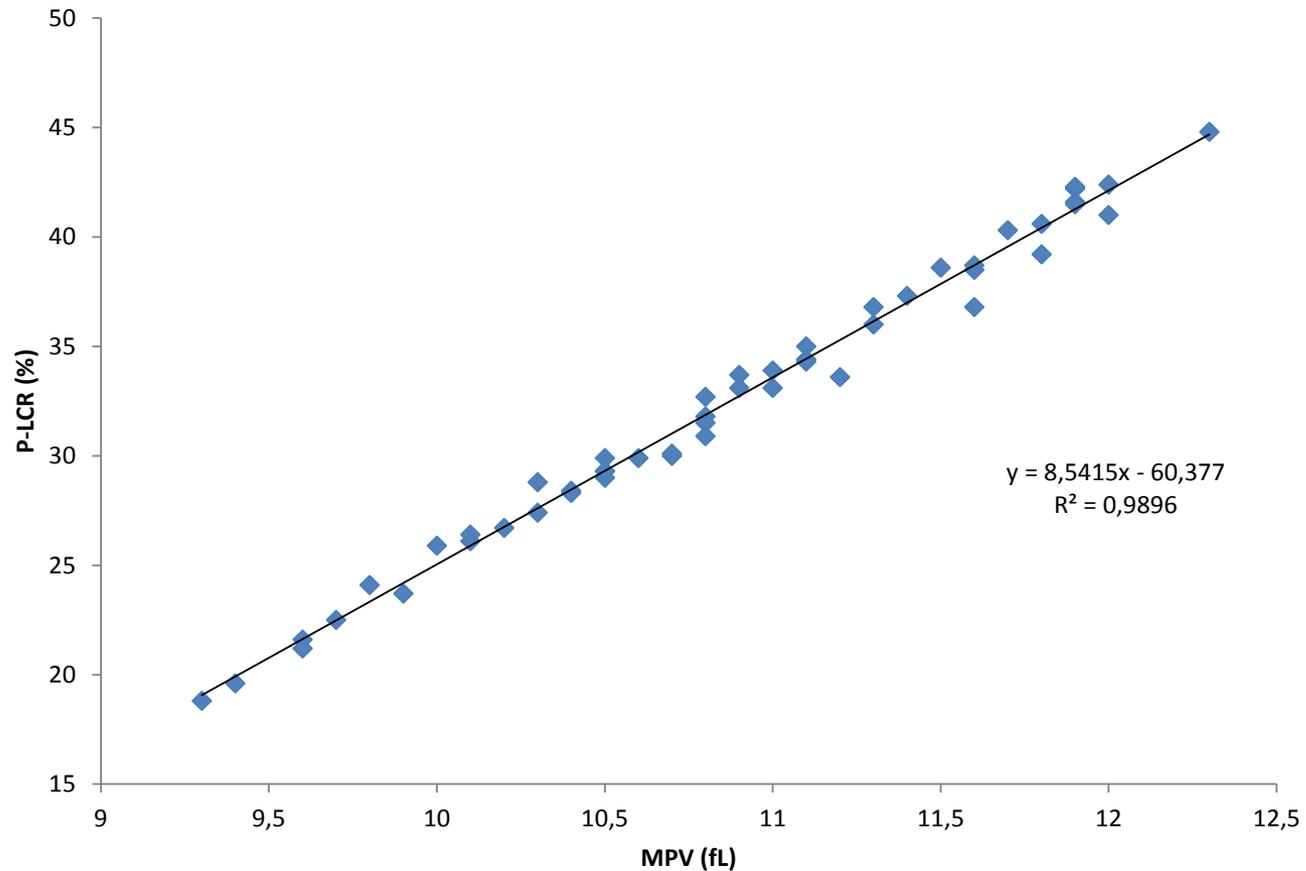
Platelet size for distinguishing between inherited thrombocytopenias and immune thrombocytopenia: a multicentric, real life study



Paramètre redondant

Relation MPV vs P-LCR (fraction des plaquettes >12 fL)

"MPV vs P-LCR"



CASE REPORT

Dr. Amandine Magnette

Positive

Morph. Count

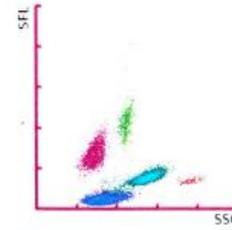
WBC	7.52	[10 ³ /uL]		
RBC	4.81	[10 ⁶ /uL]		
HGB	14.4	[g/dL]		
HCT	44.2	[%]		
MCV	91.9	[fL]		
MCH	29.9	[pg]		
MCHC	32.6	[g/dL]		
PLT &F	62	[10 ³ /uL]		
RDW-SD	43.8	[fL]		
RDW-CV	13.0	[%]		
PDW	----	[fL]		
MPV	----	[fL]		
P-LCR	----	[%]		
PCT	----	[%]		
NRBC	0.00	[10 ³ /uL]	0.0	[%]
NEUT	4.44	[10 ³ /uL]	59.0	[%]
LYMPH	2.19	[10 ³ /uL]	29.1	[%]
MONO	0.67	[10 ³ /uL]	8.9	[%]
EO	0.15	[10 ³ /uL]	2.0	[%]
BASO	0.05	[10 ³ /uL]	0.7	[%]
IG	0.02	[10 ³ /uL]	0.3	[%]
RET	1.26	[%]	60.6	[10 ⁹ /L]
IRF	7.3	[%]		
LFR	92.7	[%]		
MFR	6.9	[%]		
HFR	0.4	[%]		
RET-He	33.0	[pg]		
IPF	70.2	[%]		

WBC-BF	[/uL]		
RBC-BF	[10 ³ /uL]		
MN	[/uL]		[%]
PMN	[/uL]		[%]
TC-BF#	[/uL]		

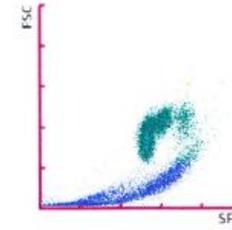
WBC IP Message

RBC IP Message

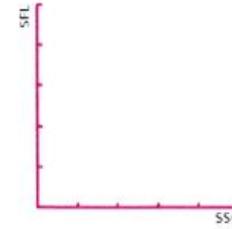
WDF



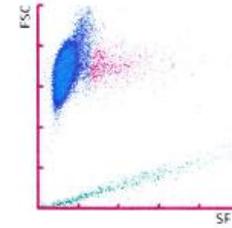
WNR



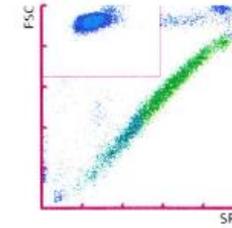
WPC



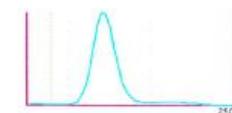
RET



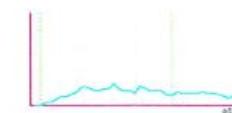
PLT-F



RBC

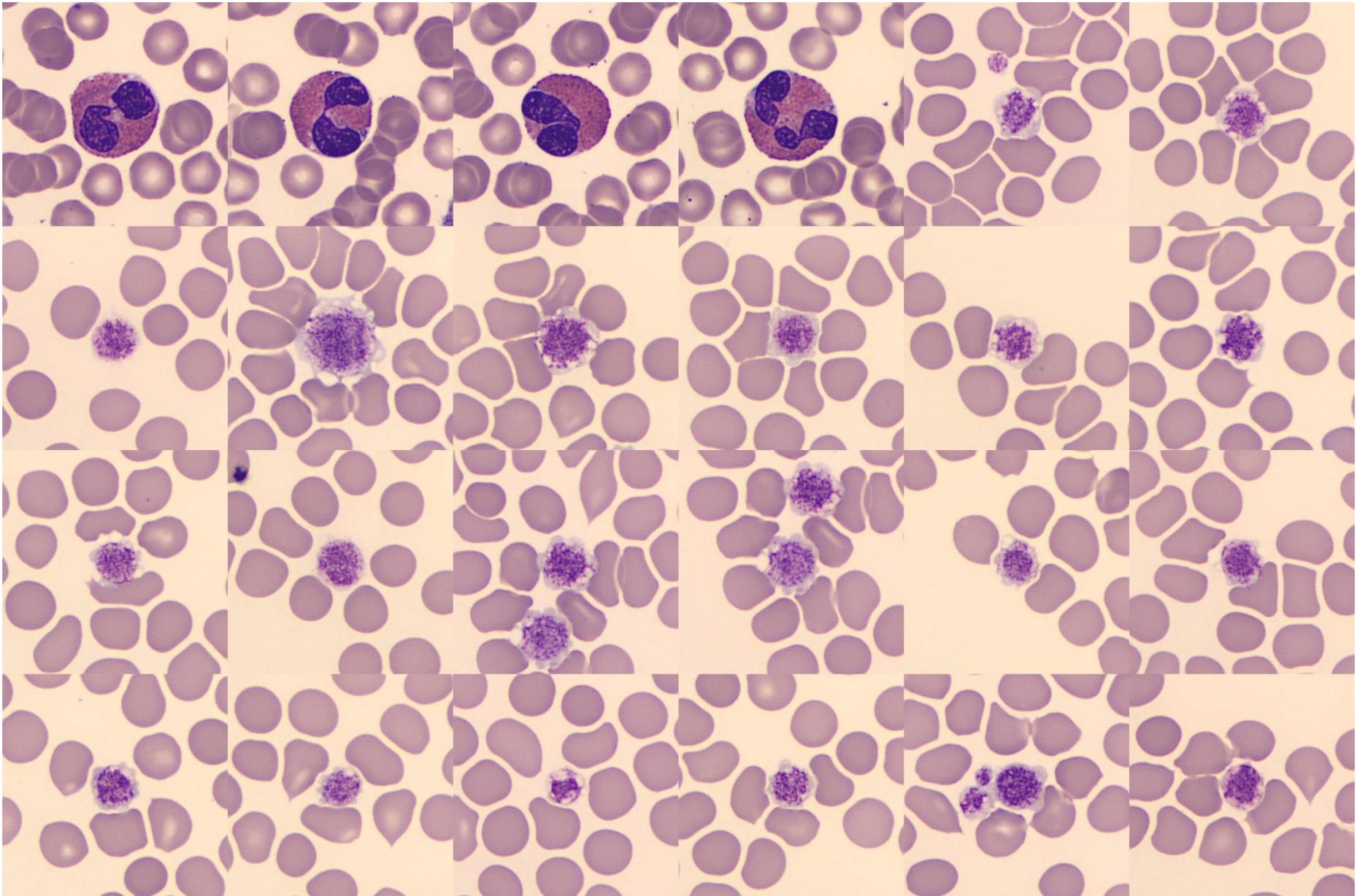


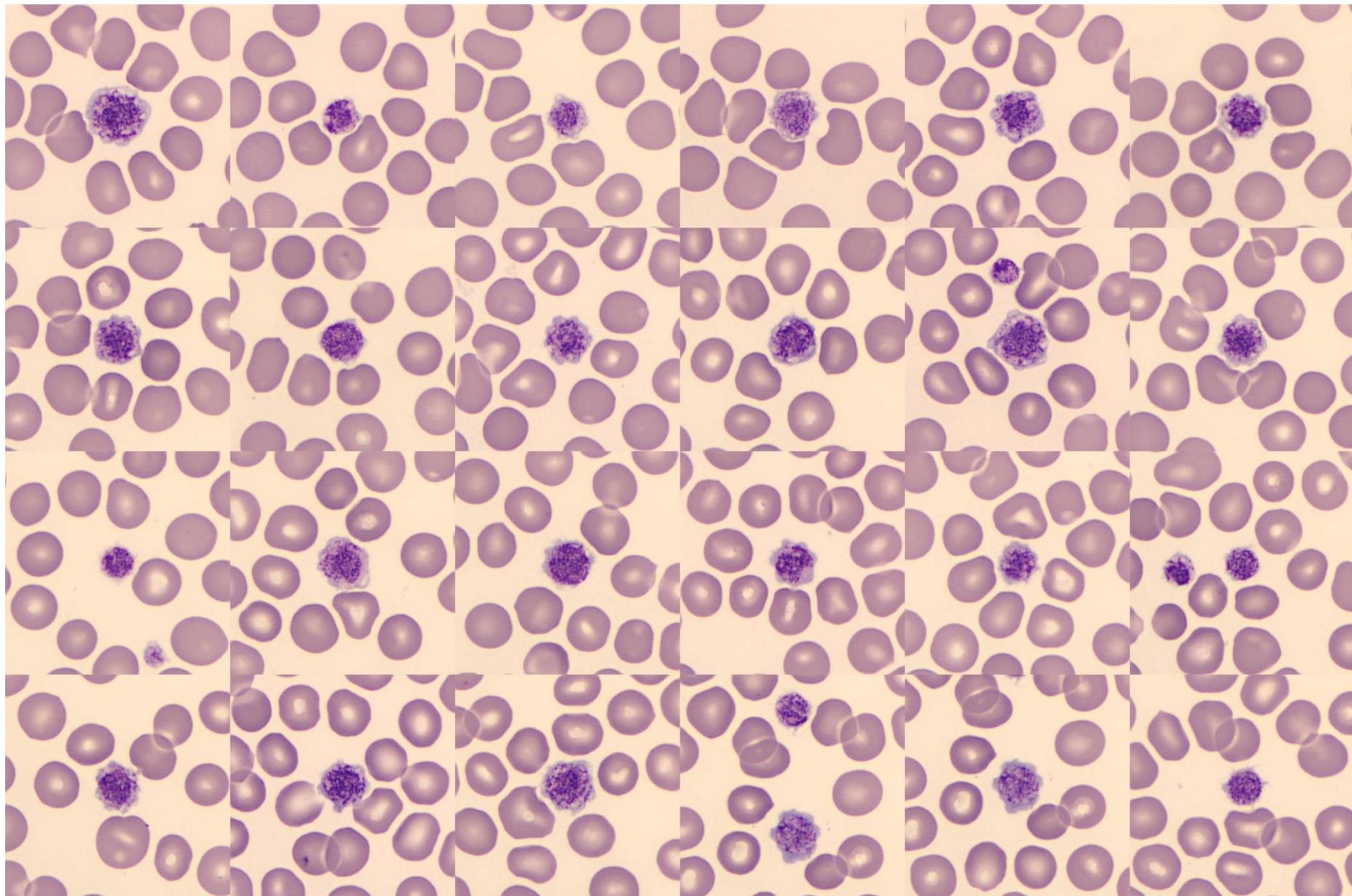
PLT

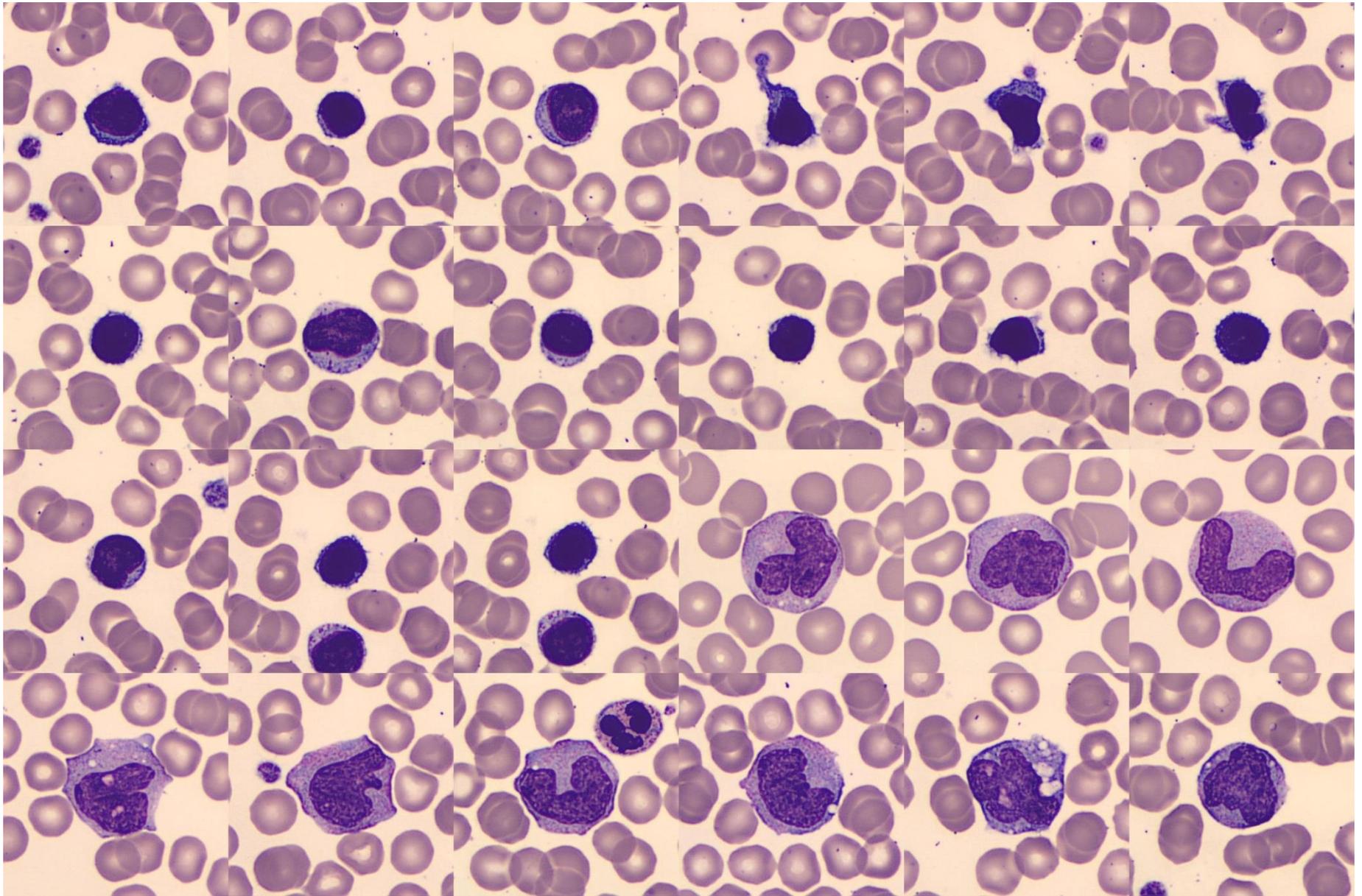


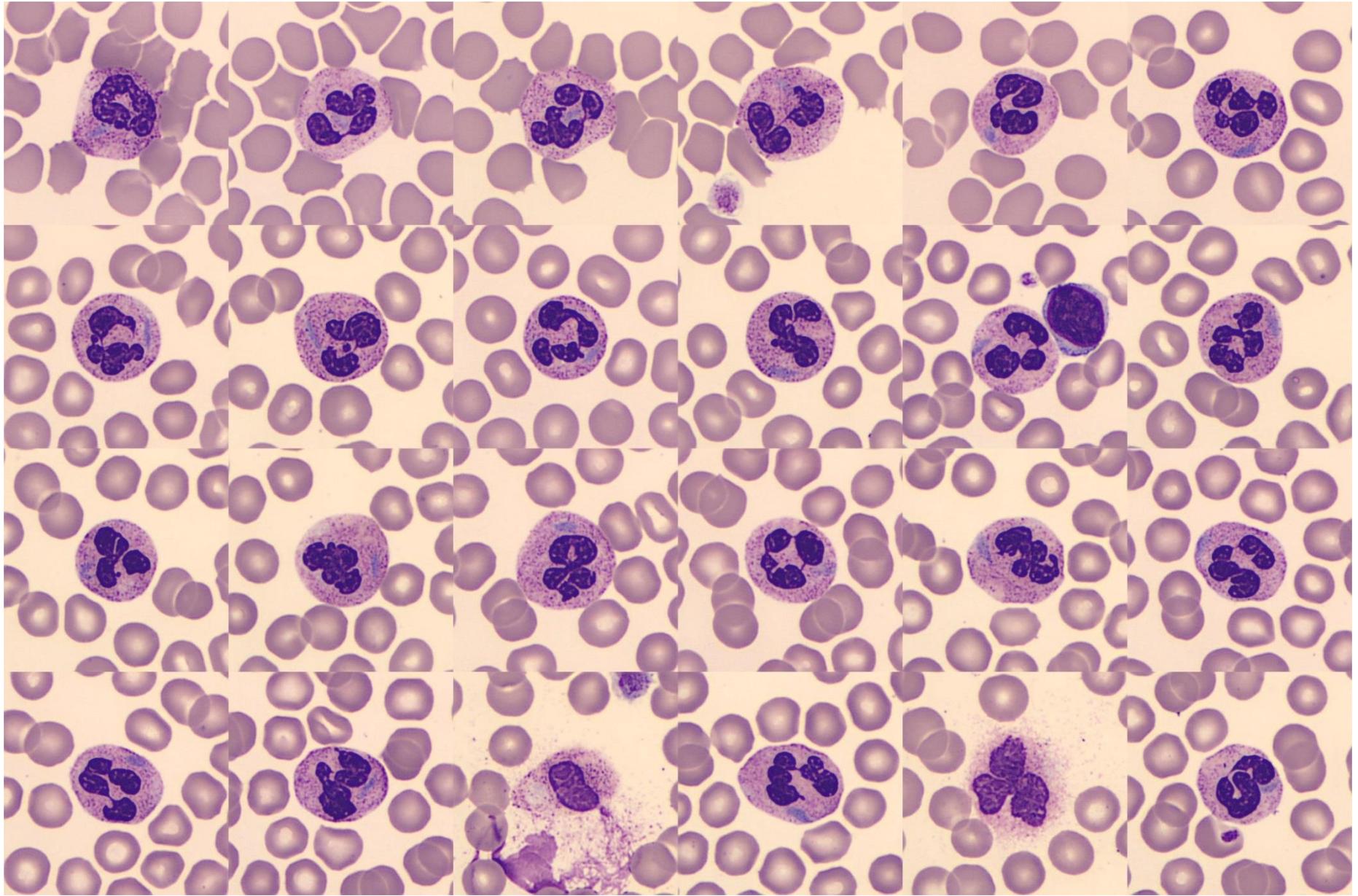
PLT IP Message
 PLT Abn Distribution
 Thrombocytopenia

HF-BF	[/uL]	[/100WBC]	PLT-I	28 * [10 ³ /uL]
NE-BF	[/uL]	[%]	PLT-O	52 [10 ³ /uL]
LY-BF	[/uL]	[%]	PLT-F	62 [10 ³ /uL]







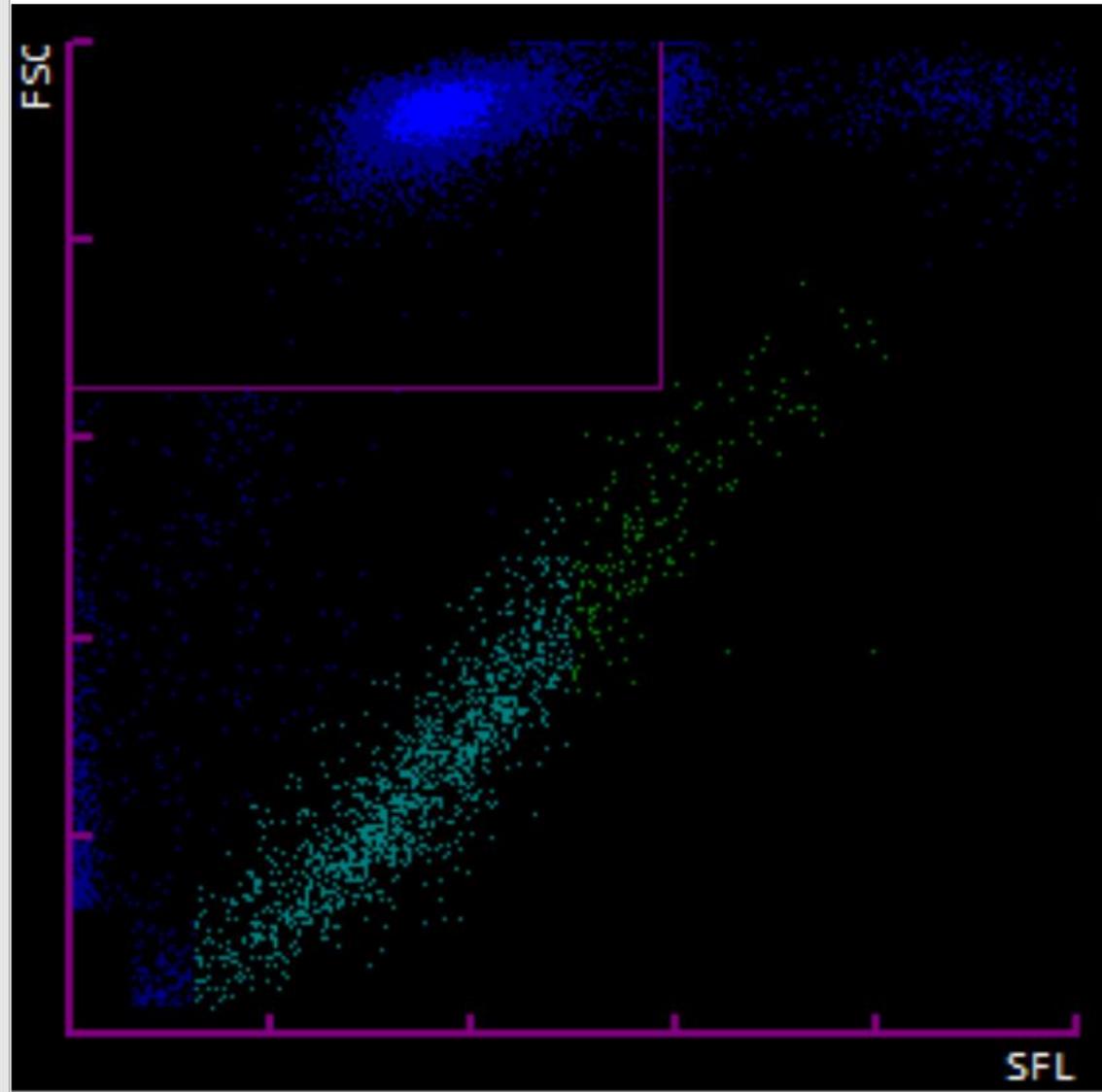


Hémogramme

- Taux de plaquettes
 - PLT-I = 28
 - PLT-O = 52
 - PLT-F = 62
- PDW = _____
- MPV = _____
- P-LCR = _____
- PCT = _____
- MFPV = $17\mu^3$
- En partant de la corrélation MPV vs MFPV ou:
 - MPV = x
 - MFPV = y
 - $Y = 0,8759x - 0,5114$
 - $x = 19,99\mu^3$
- Ce qui est supérieur aux VNs définies

PCT

- $PCT = MPV \times PLT$ (VR 0,2 – 0,4%)
 - $PLT-I = (19,99 \times 28)/10000 = 0,056 \%$
 - $PLT-O = (19,99 \times 52)/10000 = 0,104 \%$
 - $PLT-F = (19,99 \times 62)/10000 = 0,124 \%$



Fermer

Valeurs de référence

- IPFPLT : 0.7 – 9.0 %
- PCT : 0.2 – 0.4 %
- MPV : 9.3 – 12.4 fL

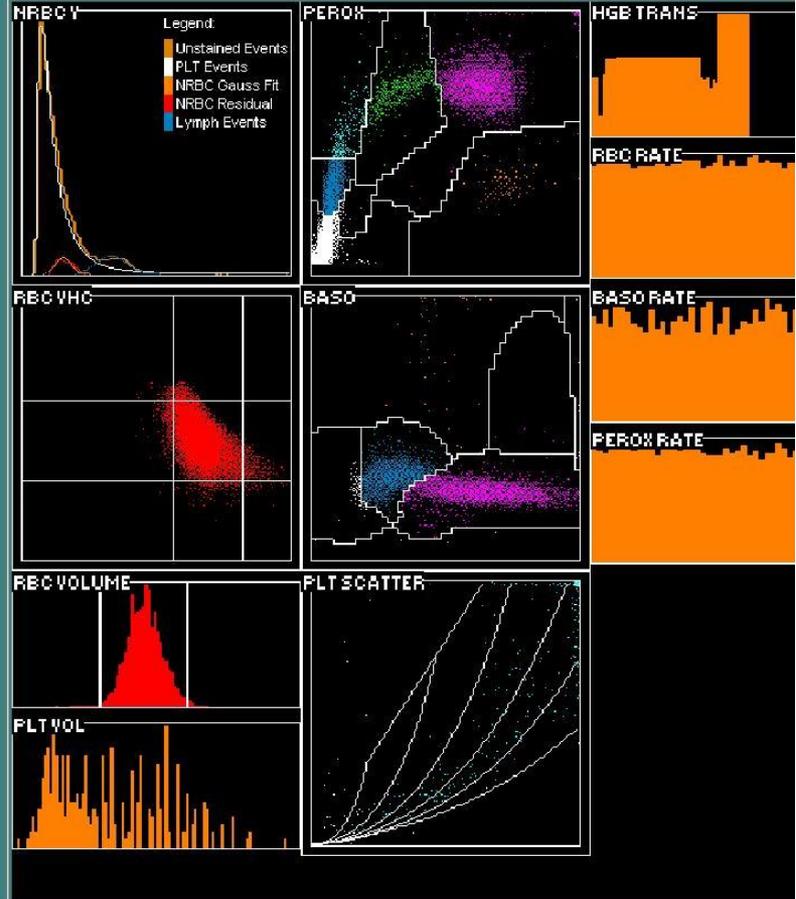
Siemens Advia

1 / 1 14120592814
 System/RackPos ADVIA Baud ADV120 01 017-04
 Date/Time 02.02.2016 10:14:49

Snd-
Text Pat-
Pr

02.02.2016-02.02.2016 ADVIA Baud
 Selection totale

I	D	F	Analysis	Result	H	T	Q	PrevRes	Flags
			WBC	7.03				6.65 19.01.2016	WC
			RBC	4.79				4.42 19.01.2016	
			HGB	14.4				13.2 19.01.2016	
			HCT	44.1				40.2 19.01.2016	
			MCV	92.0				90.9 19.01.2016	
			MCH	30.1				29.8 19.01.2016	
			MCHC	32.7				32.8 19.01.2016	
			CHCM	33.0				33.8 19.01.2016	
			PLT	38				35 19.01.2016	
			MPV	20.0				22.8 19.01.2016	
			RDW	13.0				12.8 19.01.2016	
			%NEUT	60.0				45.8 19.01.2016	VX...
			%LYMPH	25.3				44.9 19.01.2016	VX...
			%MONO	7.5				4.8 19.01.2016	VX...
			%EOS	1.6				1.3 19.01.2016	VX...
			%BASO	0.7				0.5 19.01.2016	NC
			%LUC	4.9				2.6 19.01.2016	VX...
			#NEUT	4.22				3.05 19.01.2016	VX...
			#LYMPH	1.78				2.99 19.01.2016	VX...
			#MONO	0.53				0.32 19.01.2016	VX...
			#EOS	0.12				0.09 19.01.2016	VX...
			#BASO	0.05				0.03 19.01.2016	NC
			#LUC	0.34				0.17 19.01.2016	VX...
			%NRBC		S	F		S 19.01.2016	NC
			#NRBC		S	F		S 19.01.2016	NC



GRA: q:\h1-gra\20160202\
 2016020214120592814

ATYP: +

LPLT: +++

WBCP: 9.82

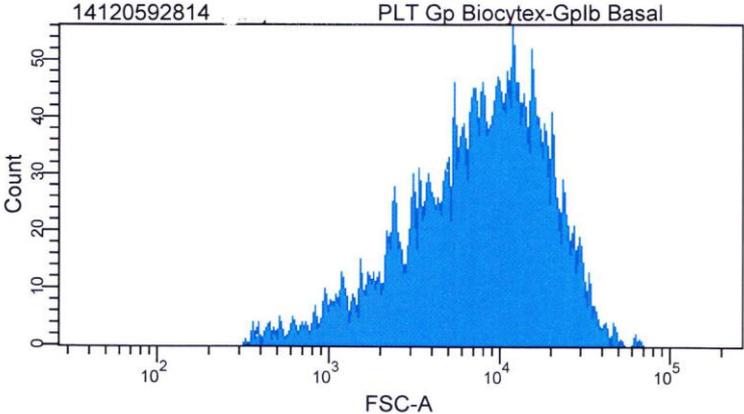
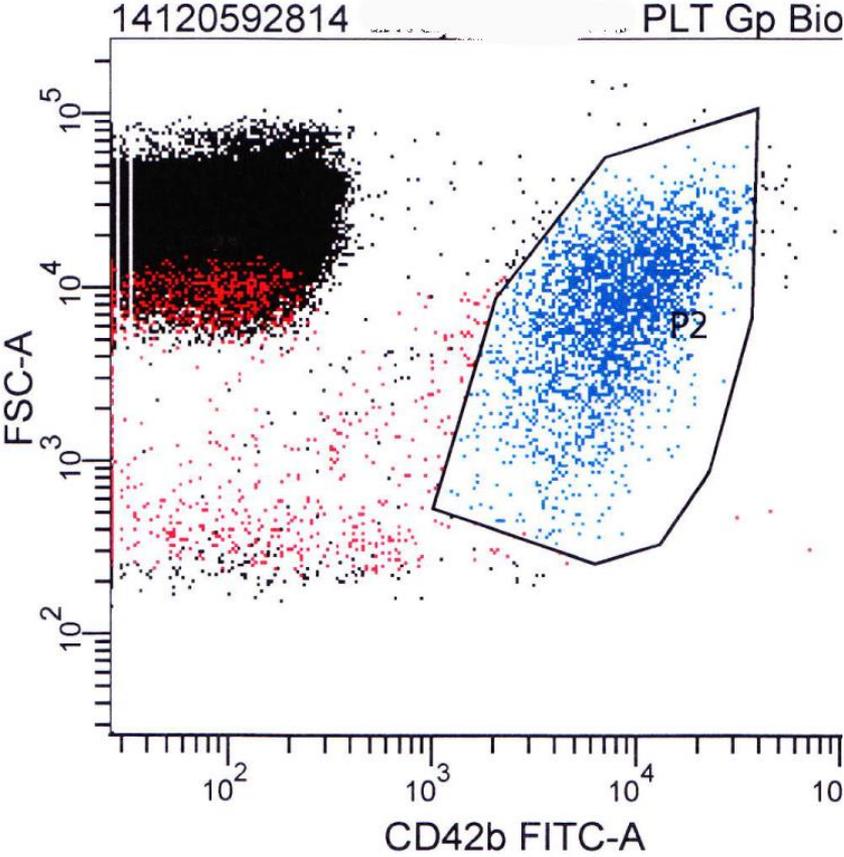
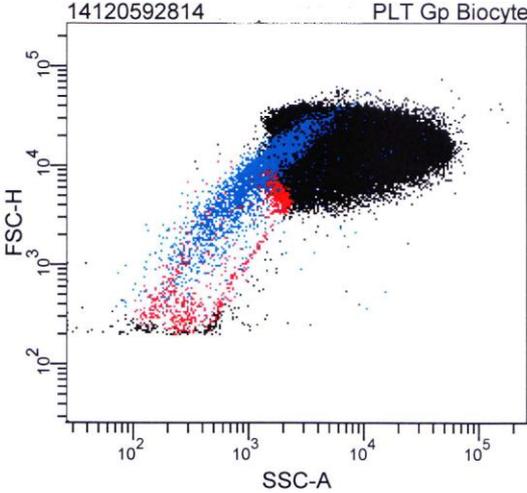
MPXI: -4.4
 WBCB: 7.03

NEUTX: 60.79
 MNX: 14.8

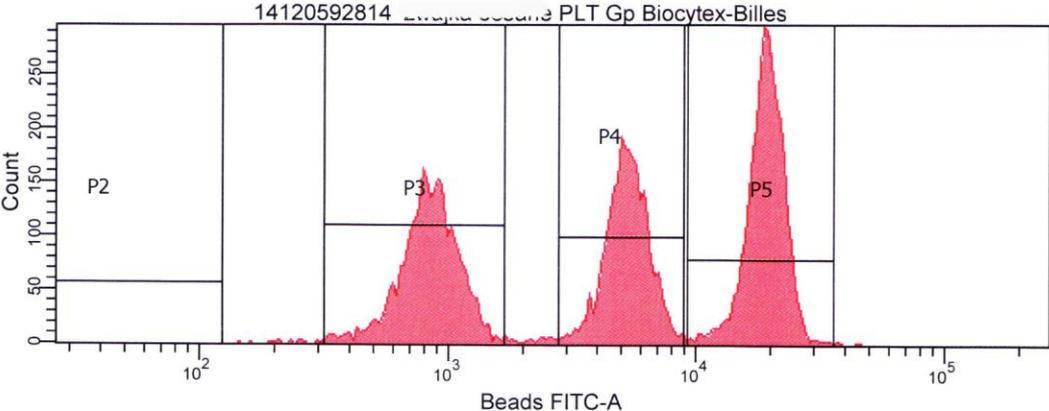
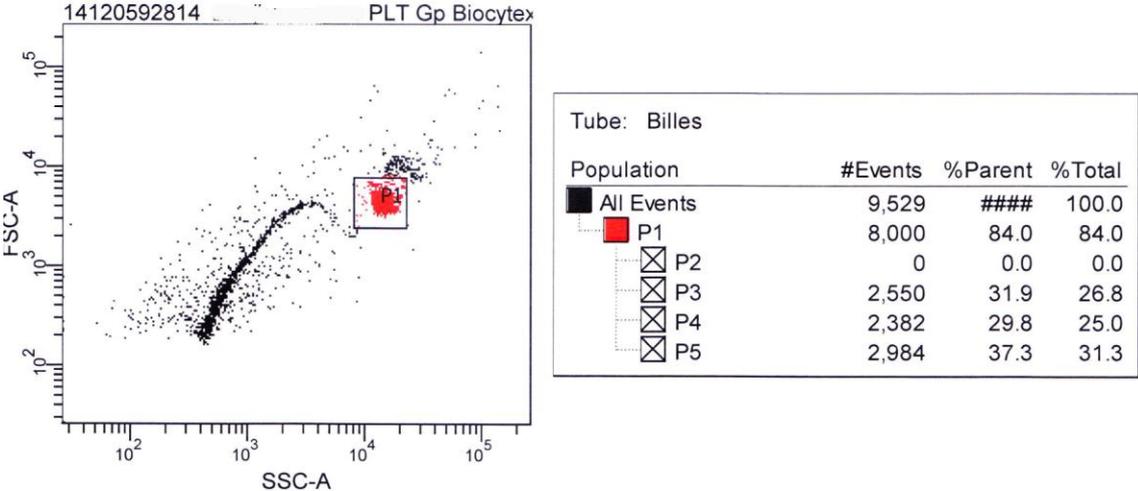
NEUTY: 69.93
 MNY: 15.7

COL: 02.02.16 08:25
 AGE: J:16 M:203 W:812 T:

Quantification des Glycoprotéines (Cytoquant Biocytex)



Quantification des Glycoprotéines (Cytoquant Biocytex)



Quantification des Glycoprotéines (Cytoquant Biocytex)

Opérateur :

Lot N° : 151102
Date Exp. : 04-2017

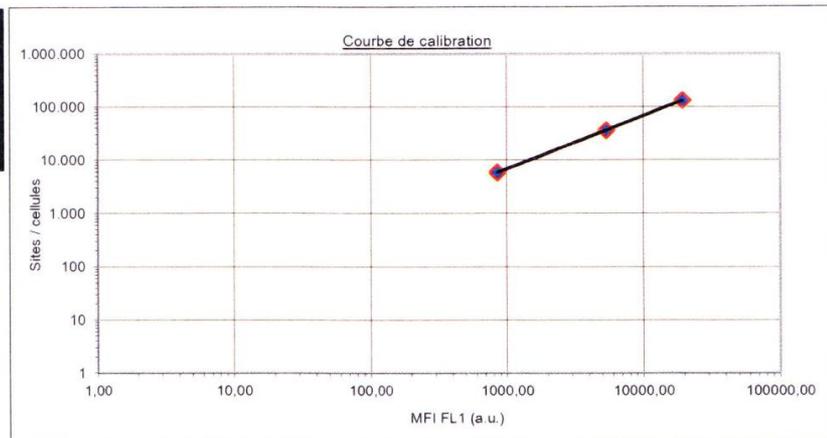
Echantillon :

Identification	Nom
14120592814	

MPV
19

Courbe de calibration

Billes	ABC	MFI
A	5.900	851,00
B	36.000	5327,00
C	133.000	19471,00



Données de la droite (y=ax+b)

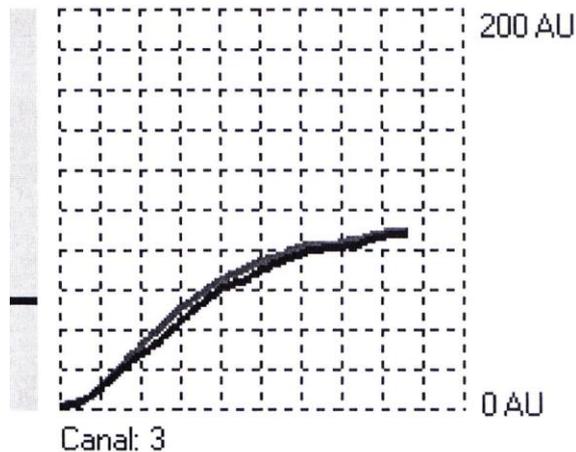
Pente (a)	0,995
Origine (b)	0,8546
r	0,99998
r ²	0,99996

Résultats Anticorps :

Anticorps	Spécificité	MFI	ABC	sABC	Valeurs attendues	Ratio
R2a	Mouse IgG	57	399			
R2b	CD62P	60	420	21	< 1 000	1,23
R2c	CD41	14.058	95.568	95.169	51 000 +/- 14 000	5598,20
R2d	CD42b	8.349	56.917	56.517	38 000 +/- 11 000	3324,56

Agrégométrie impédance (Multiplate)

Multiplate® analyse des fonctions plaquettaires - V2.03.13



ID du patient :

Nom du test : ASPItest (sang citraté), V1

Début du test : 02. Fév. 2016, 14:38:03 (Durée de mesure

Aire sous la courbe : 50 U

Agrégation : RUO: 88.9 AU

Vitesse : RUO: 11.8 AU/min.

Ecart-type : 2.994 %

Coefficient de corrélation : 0.998

Conclusions

A l'examen microscopique au MGG, mise en évidence d'inclusions cytoplasmiques bleutées (pseudo corps de Döhle) dans les polynucléaires neutrophiles ainsi que de plaquettes de grande taille, parfois vacuolées et avec un contenu granulaire important. Ceci évoque un syndrome de MYH9.

La numération plaquettaire par Immunocomptage (technique de référence) est de 53.900 plt/mm³. Notons que la technique d'impédance sous-estime fortement la numération plaquettaire. Le volume plaquettaire et l'augmentation de l'IPF sont liés à la macrothrombocytose.

L'analyse des glycoprotéines plaquettaires montre une augmentation des GpIb et IIbIIIa par plaquette, mais la densité de ces glycoprotéines est normale ainsi que le rapport entre elles. L'agrégation plaquettaire par impédance a été réalisée en dépit de la thrombopénie (technique validée lorsque la thrombocytose est > 100.000 plt /mm³), elle montre une agrégation quasi normale pour les différents inducteurs testés, comme rapporté dans la littérature pour les thrombopathies MYH9.

Anomalie de May-Hegglin

- Maladie héréditaire AD rare
- Triade
 - Thrombocytopénie
 - Plaquettes géantes
 - Inclusions leucocytaires
- Tableau clinique variable
 - Degré variable de purpura
 - Tendance au saignement
- Découverte souvent fortuite
 - → incidence probablement sous-estimée



Anomalie de May-Hegglin

- Frottis sanguin (MGG)
 - Grandes inclusions fusiformes (jusqu'à 5 μm de longueur) bleu pâle dans les granulocytes (neutrophiles, éosinophiles et basophiles) et les monocytes
 - Ressemblent à des corps de Döhle

Anomalie de May-Hegglin

- Thrombopénie généralement modérée
 - $40-80 \times 10^3/\mu\text{L}$
 - Diamètre jusqu'à $15 \mu\text{m}$ (MPV jusqu'à $30 \mu\text{m}^3$)
- Pas de corrélation claire entre la gravité de la tendance au saignement et le degré de la thrombopénie

Anomalie de May-Hegglin

- Le + souvent
 - Fonction plaquettaire normale
 - Expression des glycoprotéines plaquettaires normale
 - Durée de vie des plaquettes normale
- L'anomalie plaquettaire serait liée à un défaut de fragmentation des mégacaryocytes

Anomalie de May-Hegglin

- Traitement
 - Généralement pas nécessaire parce que tendance au saignement modérée
 - Corticostéroïdes ou splénectomie : aucun effet
- Importance de poser un diagnostic correct afin d'éviter une thérapie inefficace et d'exclure des syndromes apparentés, qui ont des conséquences graves

Affections apparentées

- Macrothrombocytopenies héréditaires
 - Constituent un groupe d'affections avec une transmission AD ou AR
 - A l'origine, ont été classées arbitrairement selon la présence d'inclusions ou non dans les leucocytes
 - Sans inclusions
 - Syndrome de Bernard-Soulier (transmission AR, et touche la glycoprotéine « GPIb-IX-V »)
 - Syndrome des plaquettes grises (transmission AD)
 - Macrothrombocytopenie + myélofibrose + splénomégalie

Affections apparentées

- Avec inclusion « Döhle-like » dans les leucocytes
 - L'anomalie de May-Hegglin est le prototype
 - Syndrome de Fechtner
 - Triade de l'AMH + surdité + cataracte + néphrite
 - Au microscope, inclusions + petites + arrondies que dans l'AMH
 - Variant du syndrome d'Alport
 - Syndrome de Sebastian
 - Macrothrombocytopénie + inclusions leucocytaires
 - Syndrome d'Epstein
 - Macrothrombocytopénie + surdité + néphropathie + inclusions

Affections apparentées

- Toutes les affections avec inclusions leucocytaires représentent des variants alléliques du même gène MYH9 (situé sur le chromosome 22q11.2)
- Ces affections sont classifiées comme « MYH9-related macrothrombocytopenias »
- Ce gène code pour la chaîne lourde de la myosine non musculaire de type IIA
- Les mutations sur la position R702 donnent
 - Une macrothrombocytopénie
 - Une surdité
 - Une néphrite



Comité d'organisation :

Pr Christian Chatelain, Pr. Bernard Chatelain, Dr Béangère Devalet, Dr Anne-Sophie Dincq, Pr Jean-Michel Dogné
Dr Jonathan Douxfils, Pr Maximilien Gourdin, Dr Geoffrey Horlait, Dr Sarah Lessire, Dr Valérie Mathieux,
Pr François Mullier, Dr Jean-Baptiste Nicolas, Pr Anne Spinewine, Pr Jean-Baptiste Watelet



Le Namur Thrombosis and Hemostasis Center a le grand plaisir de vous inviter à son

6^{ème} symposium annuel le jeudi 28 avril 2016

au Château de la Poste, Domaine de Ronchinne 25, 5330 Maillen