BSAC
Belgian Society for Advancement of Cytometry

Annual meeting of the Belgian Society for Advancement of Cytometry

Platelet counting: current issues and solutions

Prof. François Mullier, Prof. Bernard Chatelain
Université catholique de Louvain, CHU UCL Namur
Laboratory of clinical biology

UCL Université catholique de Louvain

NTHC Namur Thrombosis & Hemostasis Center

CHU UCL NAMUR

Outline

- Importance of the preanalytical step
- Methods available to count platelets: manual phase-contrast microscopy, impedance, optical, fluorescence, image analysis and flow cytometry
- Interferences on the measurement of platelet counts on haematology analyzers (impedance and optical)
- Added value of PLT-F and flow cytometry
- Particular case: platelet concentrates: an unsolved issue…
- Other platelet parameters: MPV, IPF, (P-LCR, MFPV, PCT)
- Conclusions

Preanalytical step: blood collection and anticoagulant

- Blood collection: - Careful
  - Processing within 30min
  - Light tourniquet or not at all
  - 21-G (or larger bore) needle
  - Smooth draw (good flow)
  - Discard the first 2 ml of blood drawn

- Anticoagulant

Table 6.10.2 Anticoagulants Used in the Study of Platelets

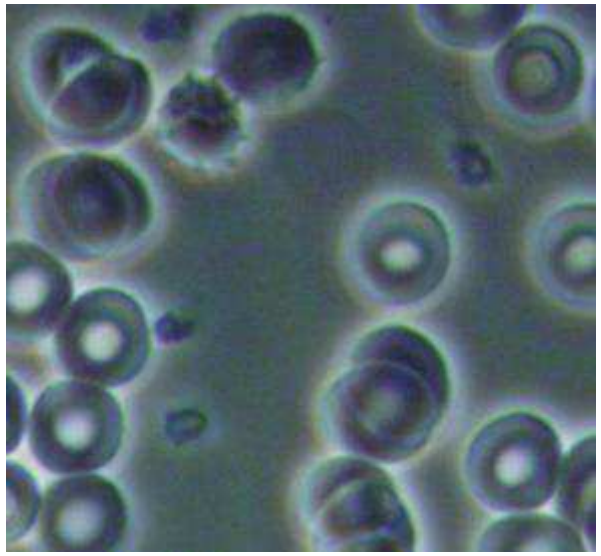
Anticoagulant	Mechanism of action
Acid citrate dextrose (ACD)	Weak Ca^{2+} chelator
Citrate theophylline adenosine dipyridimole (CTAD)	Chelates Ca^{2+} and increases intracellular cAMP, keeping platelets "quiet"
Corn trypsin inhibitor	Activated coagulation factor XII inhibitor
EDTA[a]	Strong Ca^{2+} chelator, dissociates GPIIb-IIIa complex
Heparin[a]	Combines with anti-thrombin III to inhibit thrombin activity
Hirudin	Direct thrombin inhibitor
D-Phenylalanyl-L-prolyl-L-arginine chloromethyl ketone (P-PACK)	Direct thrombin inhibitor
Sodium citrate	Weak Ca^{2+} chelator

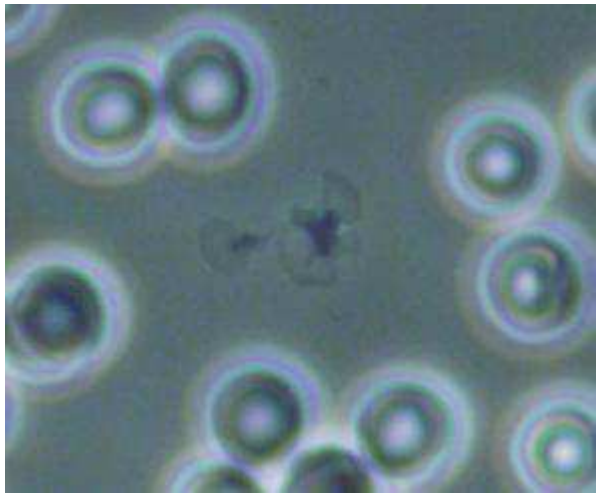
[a]These anticoagulants should be avoided for evaluation of platelet function studies by flow cytometry (see Strategic Planning).

LA Krueger et al. In: Current Protocols in Cytometry (2002) Unit 6.10

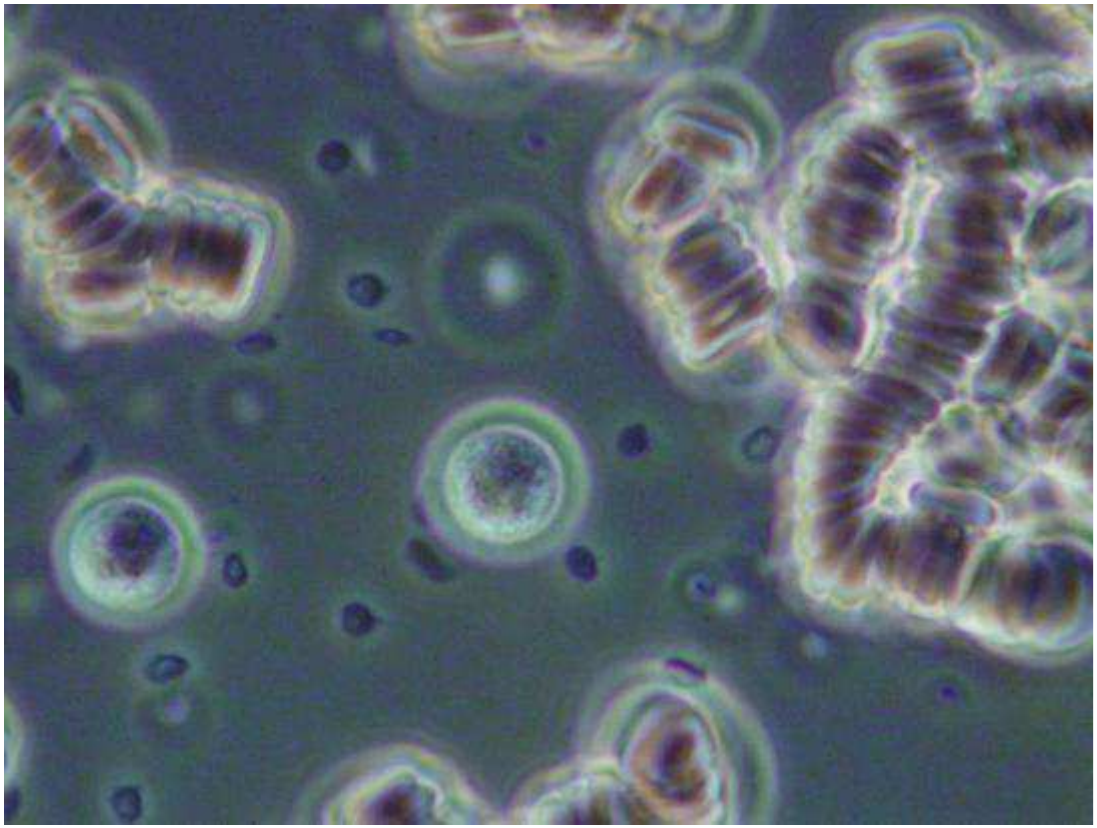
Anticoagulant

EDTA K2





CTAD



EDTA bad anticoagulant because it modifies the platelet volume

Preanalytical step: Sample handling, whole blood

- Sample handling: - Minimize time between drawing and preparation to reduce spontaneous platelet activation
  - Properly mix antocoagulant
  - Avoid unnecessary agitation prior testing
  - Container: nonwettable surfaces: siliconized glass or polypropylene → to avoid initiation of clotting resulting from contact activation
  - Transport: No pneumatic (platelet activaton)
    No extreme temperatures

- Whole blood

Methods available to count platelets

- Reference method: Manual Phase-contrast microscopy (Burker)
- Impedance: f(volume)
- Optical: f(surface and refractive index)
- Fluorescence: Sysmex XN
- Flow cytometry
- Image-analysis (DM-96)

1956: Coulter impedance measurement

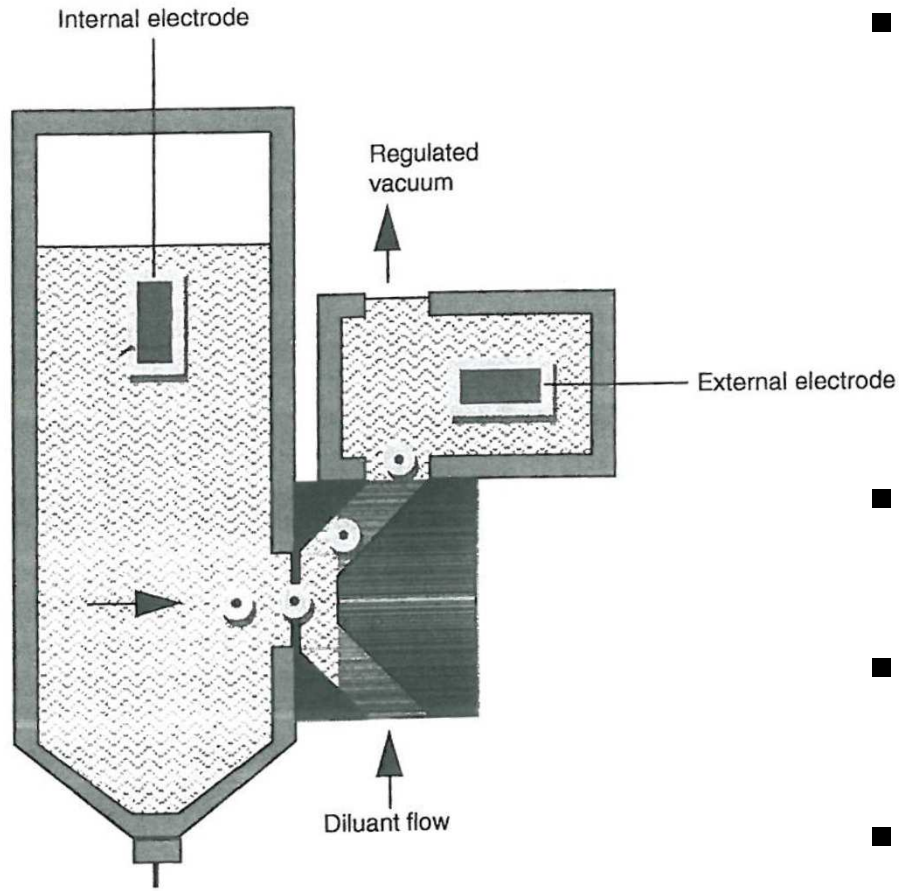


Fig. 1 Measuring chamber (coulter principle)

Salignac S. et al. Methods Mol Biol. 2013

- Based on the increase in electrical impedance when a blood cell in a conducting solution passes trough a small aperture encompassed by two electrodes
- Blood cells are very poor conductors of electricity
- The impedance increase is proportional to the cell volume.
- Individual cells counting and volume determination

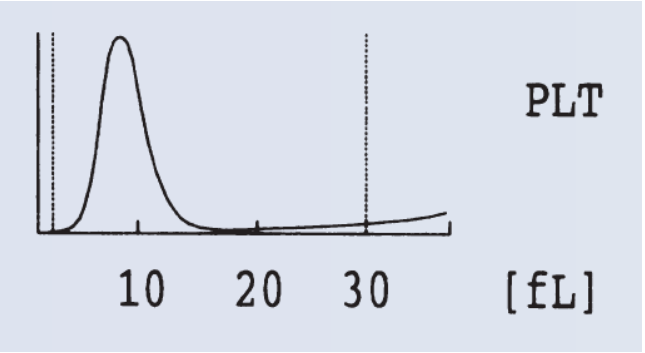
1970: Coulter impedance measurement

- Cells should pass one by one through the aperture to avoid coincidence, which would lead to inaccurate platelet count
- To avoid or at least to reduce this phenomenon, coincidence correction and hydrodynamic focusing (directing cells to the center of the aperture) have been developed on some analyzers during the 1970s

→ improvement of the cell counting apertures

⇨ More accurate cell sizing

⇨ Reliable and accurate platelet counts.



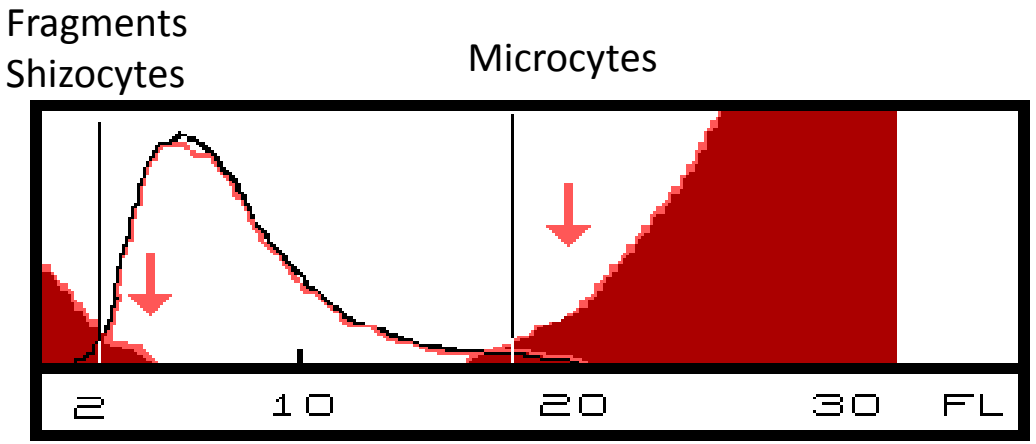
Salignac S. et al. Methods Mol Biol. 2013

Limitations of the impedance method

- Major disadvantages:

→impossibility to distinguish large platelets from extremely small red cells or fragment of red cells (Coulter instruments: flag “interference zone”)

→ Risk of underestimation of platelet count when large platelets are present



Salignac S. et al. Methods Mol Biol. 2013

Optical methods

- Based on light diffraction
- Cells pass trough a laser beam and permit a cell by cell analysis of volume and structure (in two-dimensional method)
- Platelets are sphered without modification of their volume analysis (SDS and glutaraldehyde).
- The measure is based on Mie theory of light scattering for homogenous spheres

Salignac S. et al. Methods Mol Biol. 2013

Optical Fluorescence Platelet counting methods: XE-2100 (Sysmex)

- Optical fluorescence count performed in the reticulocyte channel in addition to the impedance count
- Use of a fluorescent dye to stain nucleic acids of reticulocytes and platelets
- As optical count is not always more accurate thant the impedance count, a switching algorithm is available to report the most accurate platelet count

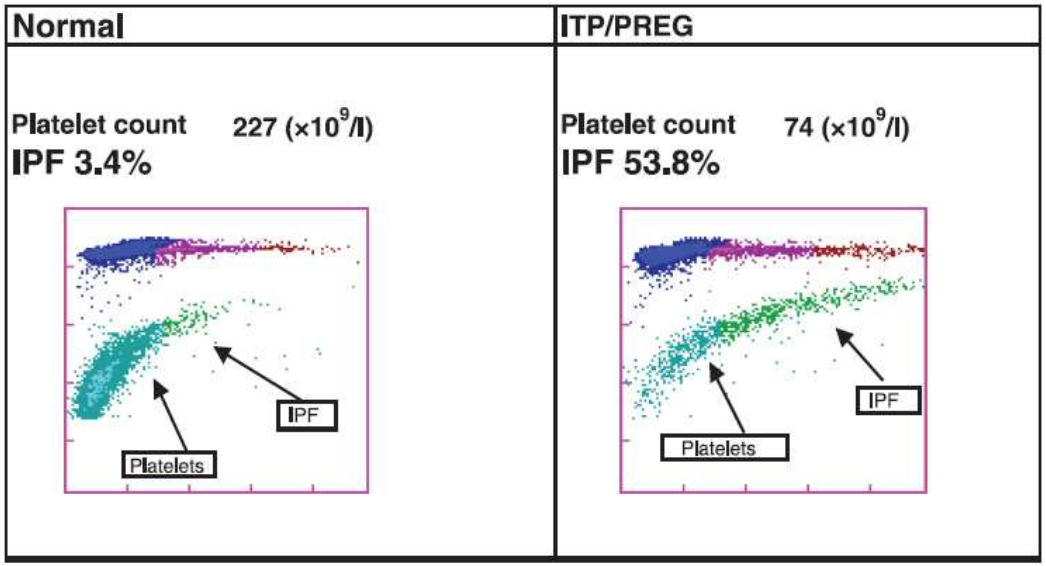
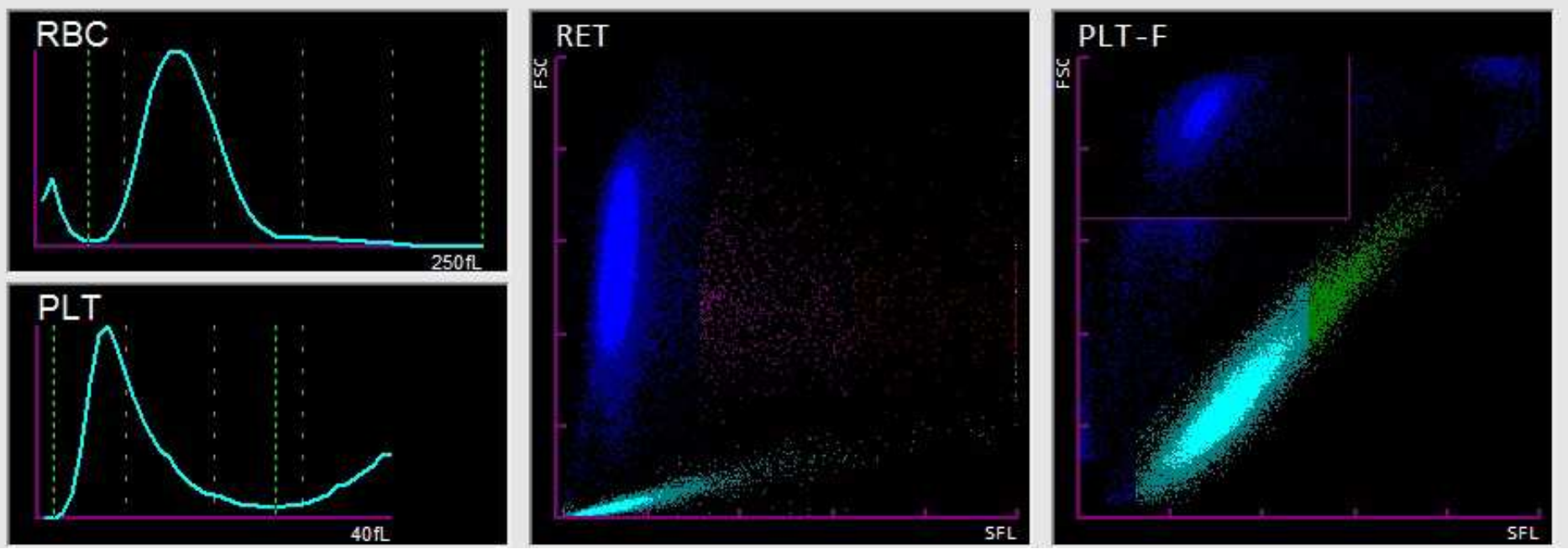


Figure 3. Sysmex XE optical platelet scattergrams from a healthy individual with a normal immature platelet fraction (IPF) and a patient with a high IPF. Mature platelets appear as blue dots, green dots represent the IPF with increased cell volume and higher fluorescence intensity compared with mature platelets.

Salignac S. et al. Methods Mol Biol. 2013

Briggs C et al Int Jnl Lab Hem 2009

Platelet counting by fluorescence: XN-PLT-F (Sysmex)



Good separation between red blood cells, microspherocytes and platelets

X: Fluorescence

Y: Size after spherization

PLT-F should be requested by the user

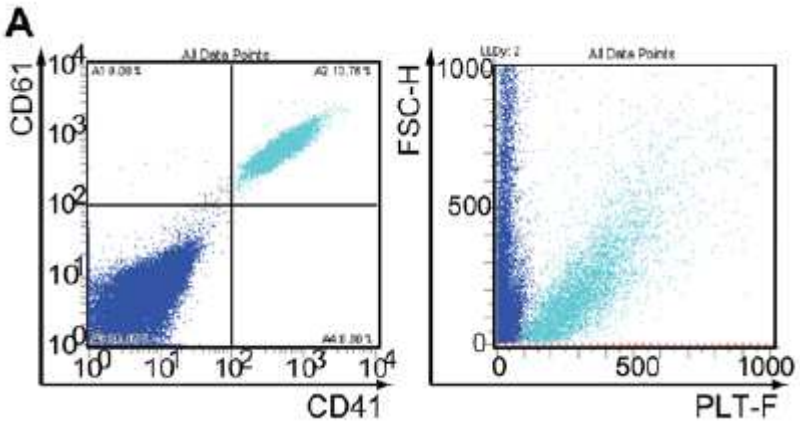
Algorithm to report the most accurate platelet count

Platelet counting by fluorescence: XN-PLT-F (Sysmex)

PLT-F reagent strongly stained some intraplatelet organelles labeled with anti-Grp75, but only faintly stained the plasma membrane of both platelets and erythrocytes.

→platelets and fragmented erythrocytes are clearly distinguished

→contributes to the platelet-counting accuracy of the PLT-F system.



Wada A et al. Plos One 2014

Platelet counting by fluorescence: XN-PLT-F (Sysmex)

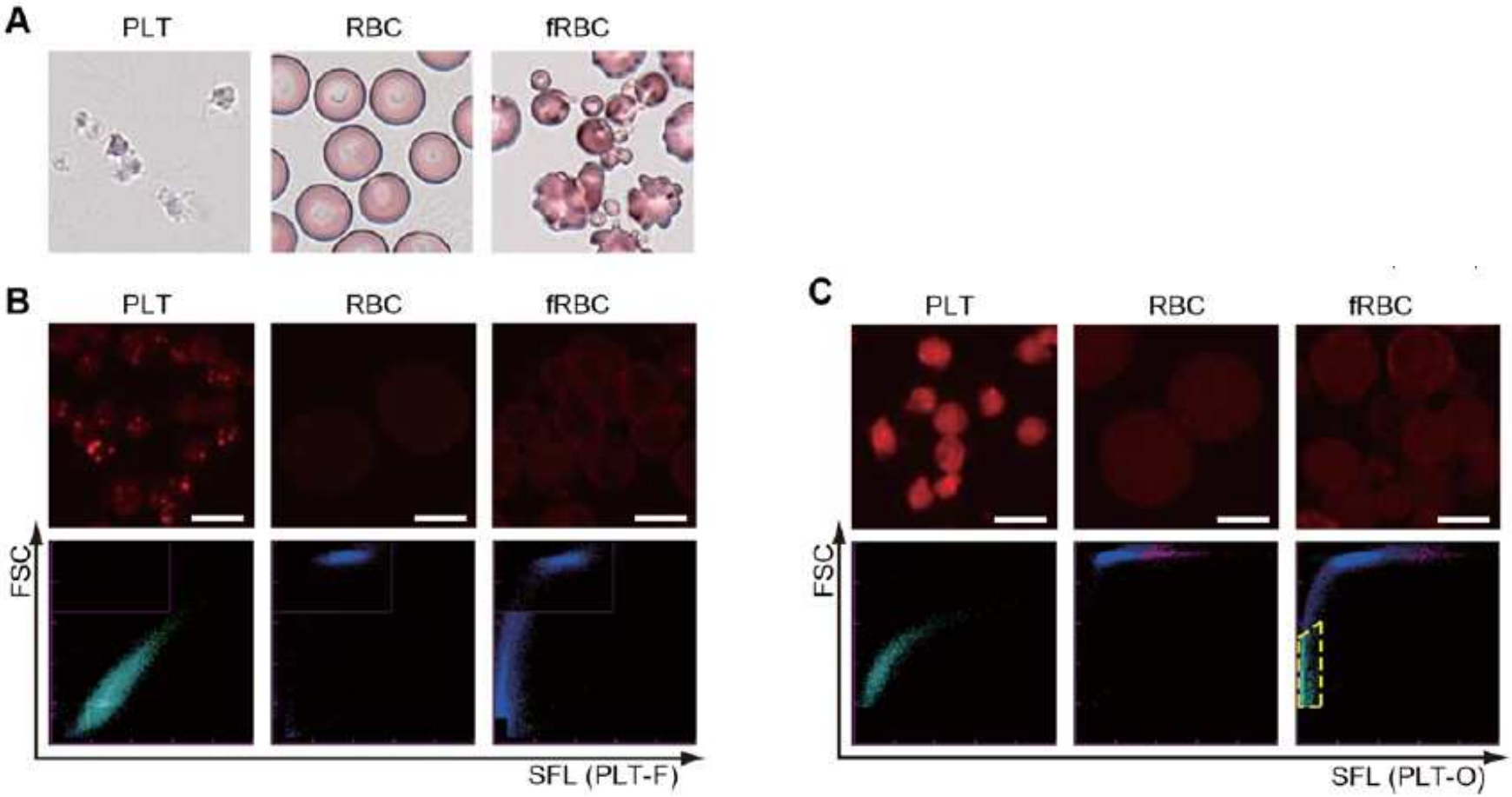


Fig 2. Platelets and fragmented erythrocytes were distinguished with PLT-F reagents. In each row, the left, middle, and right columns show platelets (PLT), erythrocytes (RBC), and fragmented erythrocytes (fRBC), respectively. (A) May-Giemsa-stained cells. (B) (Upper) PLT-F-stained cells. (Lower) PLT-F scattergrams. (C) (Upper) PLT-O-stained cells. (Lower) PLT-O scattergrams. Pale blue dots surrounded by yellow dashed line indicate fRBC misidentified as platelets. Bars: 5 μm.

Wada A et al. Plos One 2014

Platelet counting by fluorescence: XN-PLT-F (Sysmex)

Extended mode: counting on a higher volume → improved reproducibility

Table 1
Results for Reproducibility of PLT-I, PLT-O, and PLT-F Counts on the Sysmex XN2000 Analyzer at 4 Different Levels

		Reproducibility (CV%)		
Sample	PLT count, $\times 10^3/\mu L$ ($10^9/L$)	PLT-I	PLT-O	PLT-F
1	20 (20)	9.3	8.5	3.0
2	32 (32)	9.6	12.4	4.3
3	51 (51)	7.0	3.4	1.5
4	79 (79)	2.8	2.4	1.1

CV, coefficient of variation; PLT, platelet; PLT-F, platelet counting with fluorescence; PLT-I, impedance method of platelet counting; PLT-O, optical method of platelet counting.

Schoorl M et al. Am J Clin Pathol 2013

Excellent performance of PLT-F: at 5000/µl: CV lower than 10% (personal data)

Thrombocytopenia in a young child

CBC

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	%

DIFF

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	%

RET

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

PLT-F

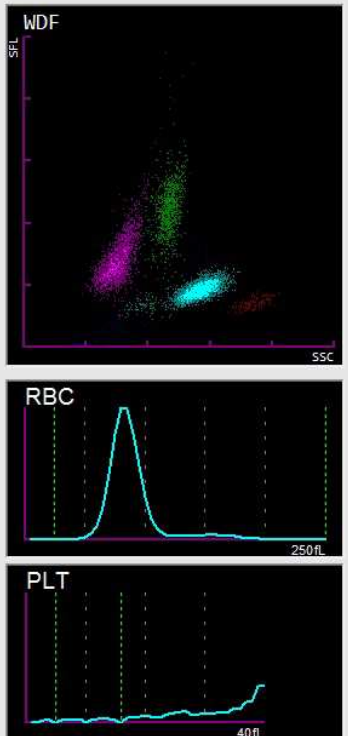
Item	Data	Unit
IPF	20.2 *	%

WBC Flag(s)

RBC Flag(s)

PLT Flag(s)

PLT Abn Distribution
Thrombocytopenia



Inaccuracy of impedance/optical methods in platelet counting when platelet count is below 20G/L→ responsible for different decision regarding transfusion of platelets

Salignac S. et al. Methods Mol Biol. 2013

Segal HC et al. Br J Haematol. 2005 Overestimation by most analysers in severe thrombocytopenia

Inaccuracy of impedance/optical methods at low platelet counts

- De la Salle BJ et al. Am J Clin Pathol 2012
- CV ranging from 15 to 43% for several different analyser at platelet levels between 5000 and 10000/µl
- Lozano et al. Vox Sanguinis 2014
- 4 samples/82 labs/nine countries/6 manufacturers

Table 3 Summary of the results of platelet counts for samples counted by the IRM and haematology analysers

	IRM ($N = 4$)		Haematology analysers ($N = 82$)	
	Platelet count ($\times 10^9$/l) Mean (range)	CV (%) Mean (range)	Platelet count ($\times 10^9$/l) Mean (range)	CV (%) Mean (range)
LP1	6·3 (4·6–8·0)	1·8 (0·0–3·8)	9·0 (2·3–31·1)	17·5 (1·9–65·6)
LP2	13·3 (11·1–15·1)	2·9 (0·8–6·6)	16·2 (10·8–47·8)	9·8 (1·9–41·0)
LP3	21·6 (14·8–27·3)	3·3 (0·6–4·6)	23·0 (10·1–60·3)	8·3 (0·6–44·7)
LP4	53·0 (43·4–58·3)	2·2 (0·8–4·3)	57·6 (44·5–90·1)	4·5 (0·8–11·0)

CV, coefficient of variation.

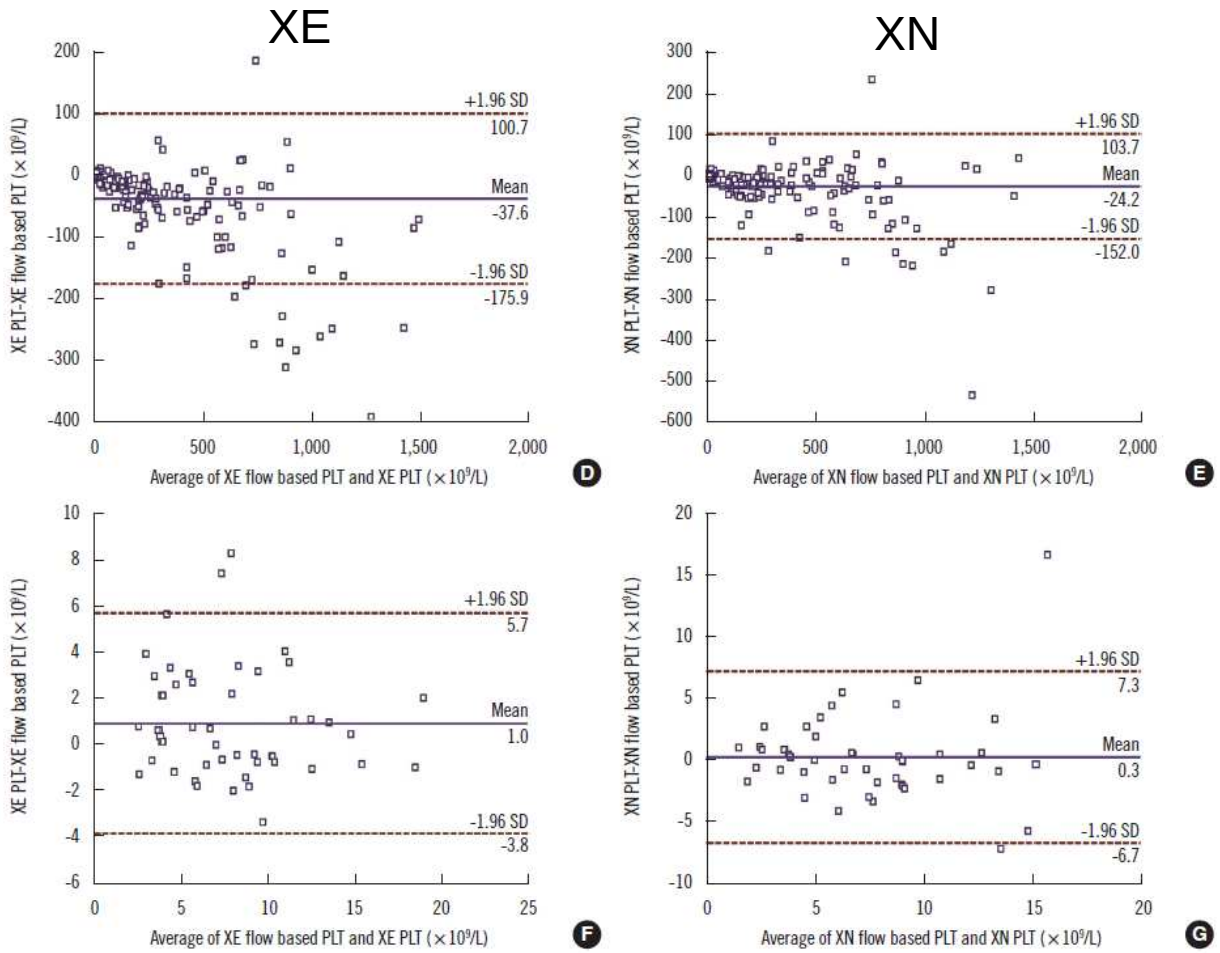
Lozano et al Vox Sanguinis 2014

Inaccuracy of impedance/optical methods at low platelet counts

Transfusion threshold <20 × 10^9/l			
% samples below trigger	% samples above trigger	% under-Tx[a]	% over-Tx[b]
92·8	7·2	7·2	0
83·8	16·2	16·2	0
23·1	76·9	0	23·1
0	100	0	0

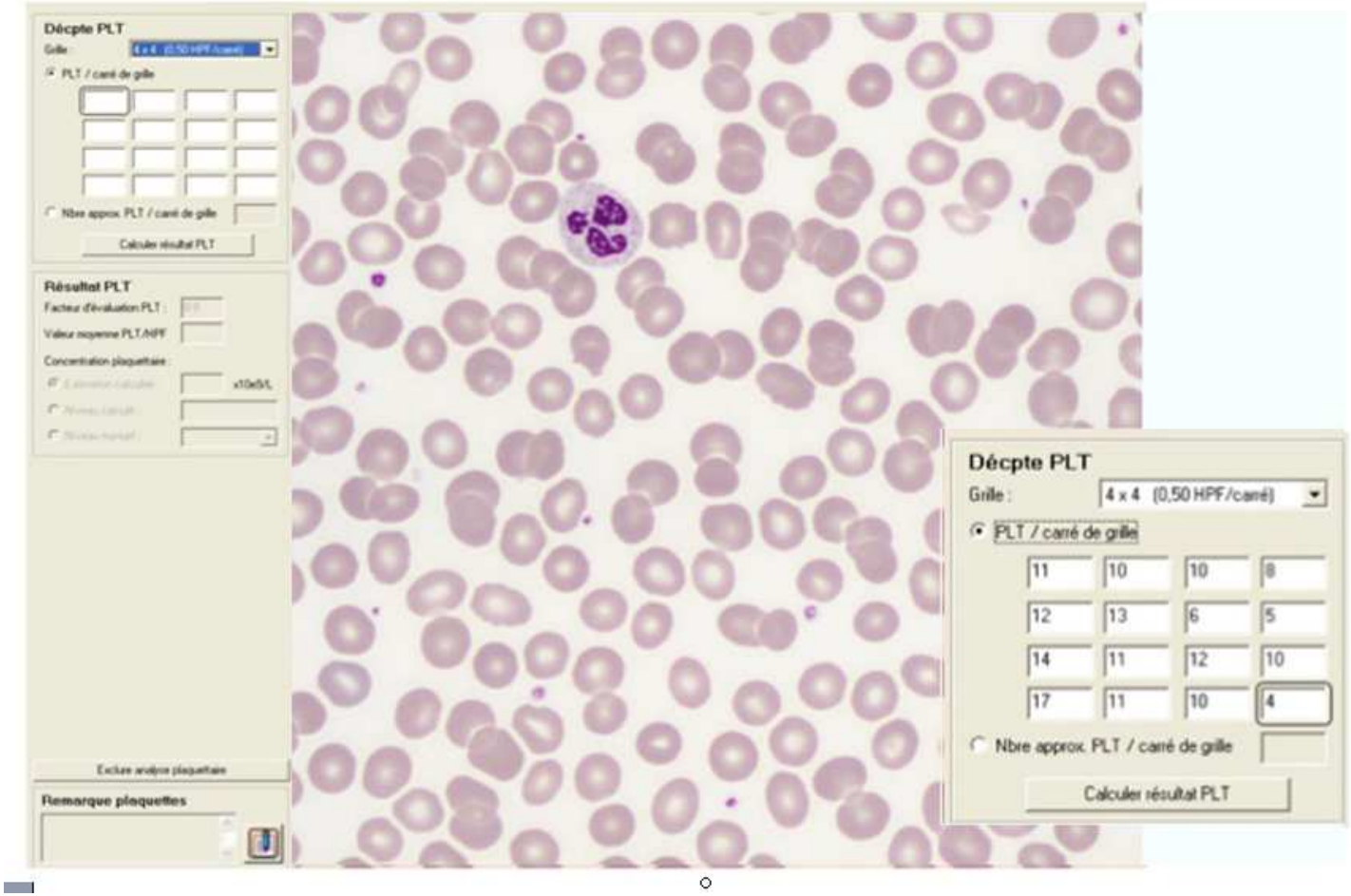
Lozano et al Vox Sanguinis 2014

Accuracy of PLT-F

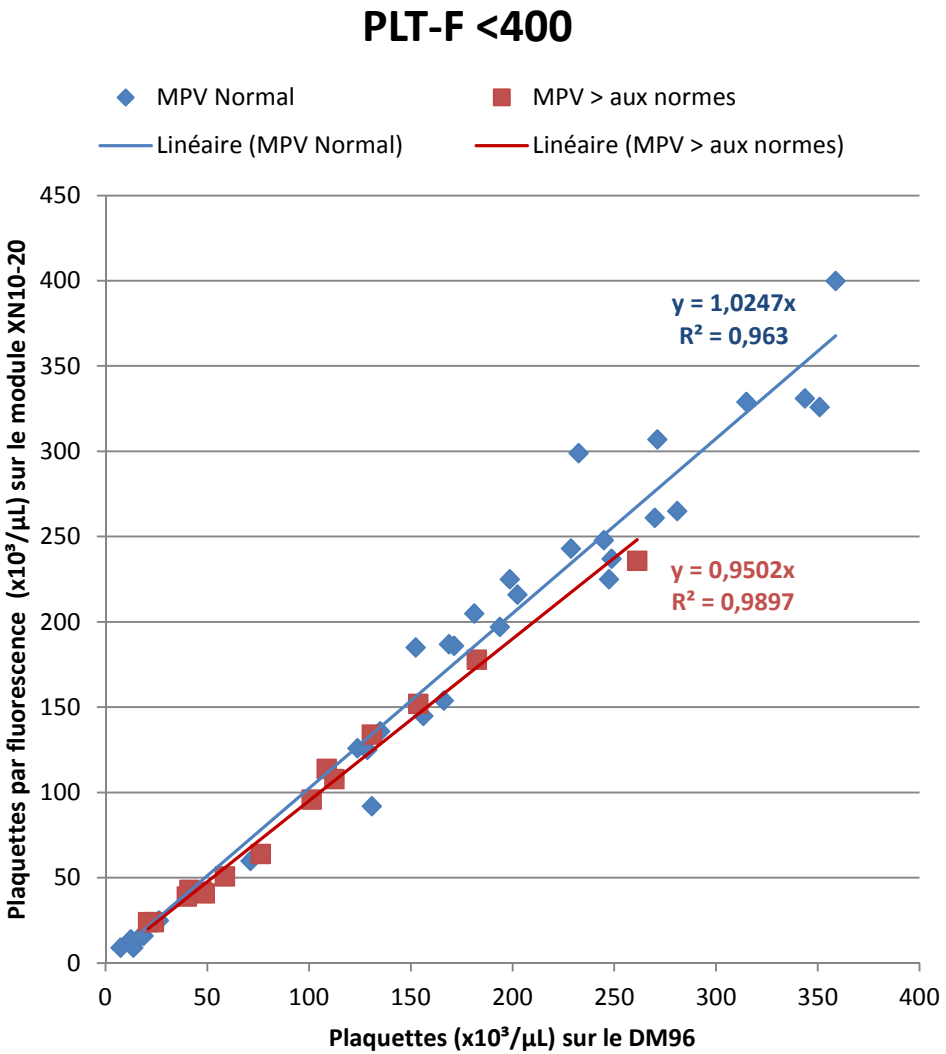


Park et al. Ann Lab Med 2014

Platelet counting on DM96



PLT-F and DM96



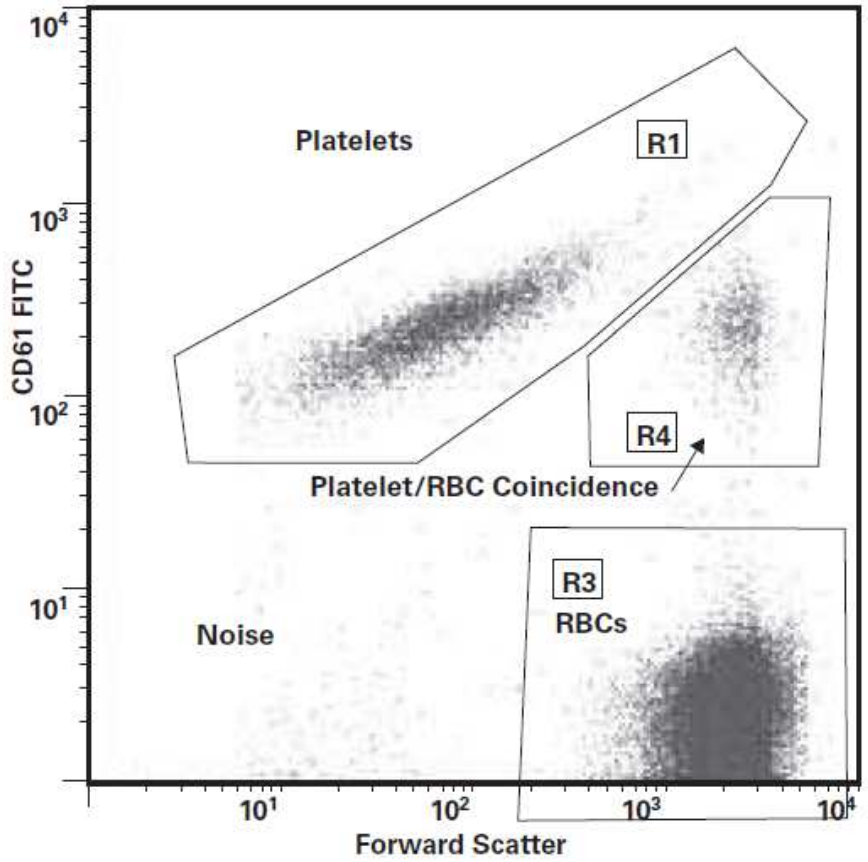
DM96: excellent performance even in case of thrombocytopenia

PLT-F is not influenced by the presence of large platelets (contrary to optical/impedance)

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

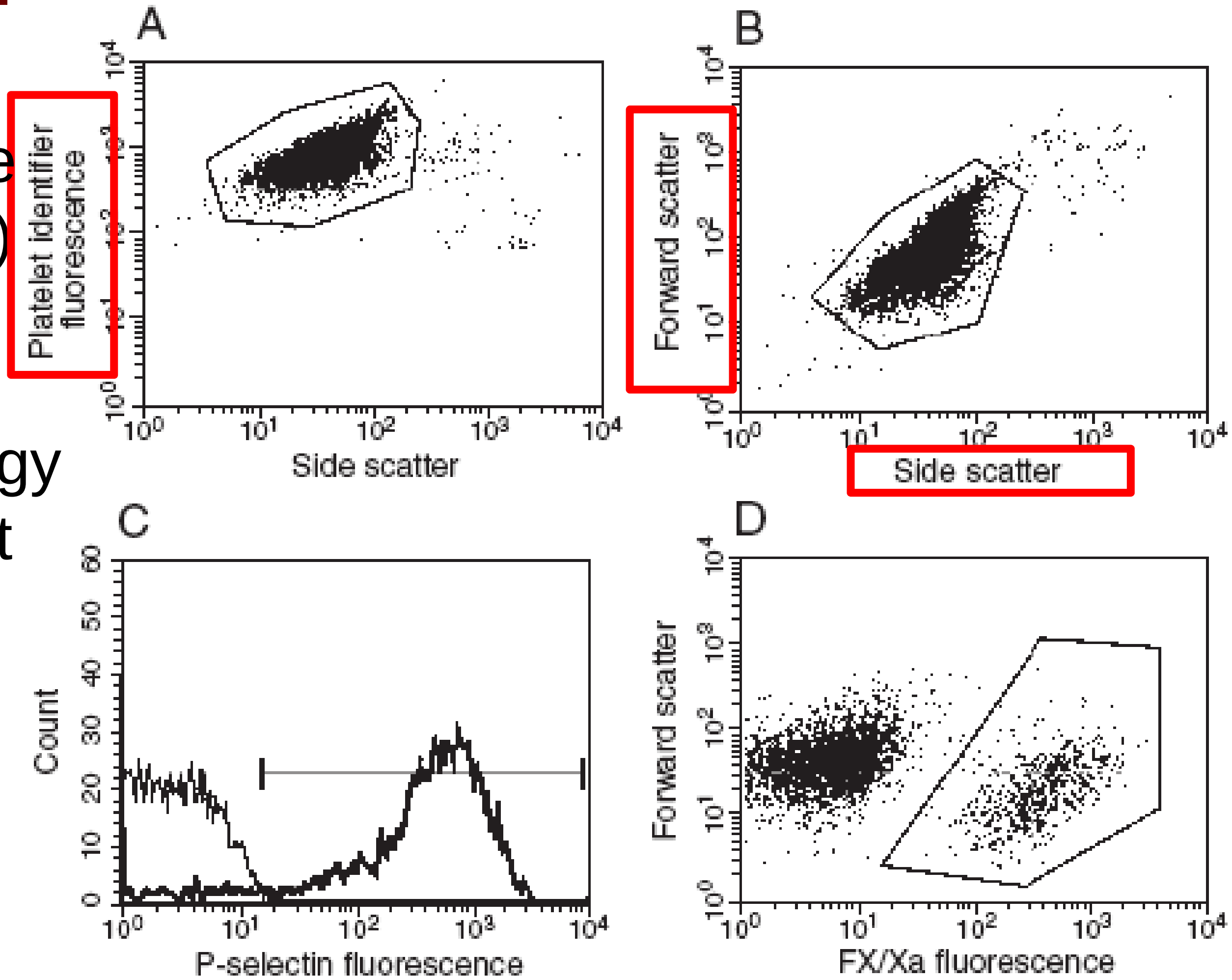
Received 7 October 1999; accepted for publication 11 October 1999



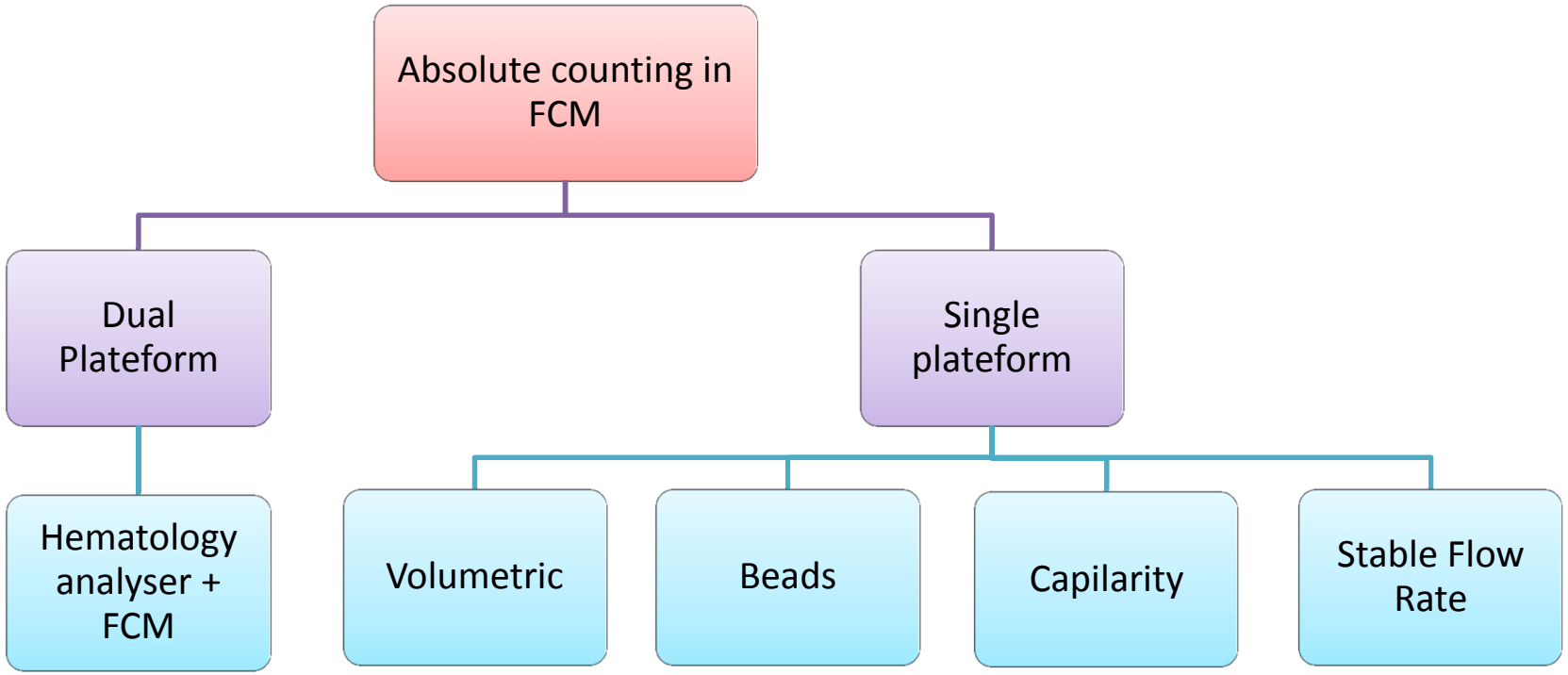
+ : SPECIFICITE ↑↑↑

Platelet counting by flow cytometry

To minimize platelet coincidence



Platelet counting by flow cytometry

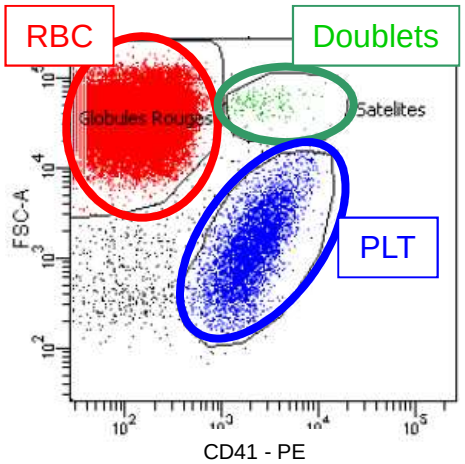


Platelet counting by flow cytometry

- Dual platform system
  - [RBC]→ Hematimetry (ex: XE-2100®)

Item	Data		Unit
WBC	33.11	+	10^3/uL
RBC	3.84		10^6/uL
HGB	11.8		g/dL
HCT	34.7		%
MCV	90.4		fL
MCH	30.7		pg
MCHC	34.0		g/dL
PLT	167		10^3/uL

  - FCM



$$\frac{\text{PLT events}_{FCM} + \text{Doublets events}_{FCM}}{\text{RBC events}_{FCM} + \text{Doublets events}_{FCM}} \times \text{RBC}_{CBC} = \text{PLT / µL in whole blood}$$

Platelet counting by flow cytometry

- Dual platform system

- Needs the RBC concentration → non usable for platelet concentrates and PRP
- Needs to define specifically the population of interest.
- Take care to the limits of linearity of the hematimeter.
- Needs to check reproducibility, accuracy, controls of the 2 instruments
- Errors of both instruments are multiplied by themselves

Platelet counting by flow cytometry: dual platform system: Importance of BSA

- Dilute your platelet sample in a buffer with proteins (no mention in the « Harrison paper » of 1999, only mentioned in van der Meer Transfusion 2012)
  - Ex: PBS + BSA 1%

- Why?
  - Platelets/Beads tend to stick to the tube wall
  - Induce by weak electrostatic charge
  - Proteins from BSA could avoid/minimize this phenomenom (the phenomenom is amplified by the vortex)

Cytometry. 2001 Feb 1;43(2):154-60.

The "vanishing counting bead" phenomenon: effect on absolute CD34+ cell counting in phosphate-buffered saline-diluted leukapheresis samples.

Brando B[1], Göhde W Jr, Scarpati B, D'Avanzo G; European Working Group on Clinical Cell Analysis.

⊕ **Author information**

Platelet counting by flow cytometry: Single platform system

- Mainly, two kinds of beads
  - Becton Dickinson TruCount®
  - Beckman Coulter FlowCount®
  - … … … … … …
- Global principle:

A known amount of beads is added to a known volume of blood

Platelet counting by flow cytometry: Single platform system: Counting beads

- Simultaneous acquisition of beads and PLTs
- "big" beads: quick sedimentation limits analysis time.
  (Thorough mixing between microbeads and sample must be ensured in order to reduce the risks of beads sedimentation or floating (bubble or foam formation from energic agitation should be avoided)).
- Ratio Bead/MV: no more than 10/1-1/10
- Acquisition of at least 1000 bead events → statistical robustness
- Control of bead quantity or concentration by the manufacturer

Platelet counting by flow cytometry: Single platform system: Counting beads: Size matters

- Precision in the pipetting steps!
- Beads aggregates may influence the number of bead events to be used for counting.
- Overlapping of fluorescence signals from PLTs and beads
- Drop in microbead concentrations (the “vanishing bead phenomenon”). (Depend on the plasma protein concentration)
- Expired fast enough (for some)

Platelet counting by flow cytometry: Single platform system: Volumetric systems

- Disadvantages:
  - Requires a FCMr with a constant flow
  - A disruption of the FCMr requires a re-calibration
  - Depends on hemolytic solution
  - Depends on pressure
  - Acquisition speed dependent

- Advantages:
  - Calibration is easy and fast (less than 60min)
  - QC easy and fast
  - User friendly & cheap
  - Available on every FCMrs*

Platelet counting by flow cytometry: Single platform system: Volumetric systems

- Principle:
  - If the flow rate of the cytometer is steady (FCMr stability needs to be checked)
  - Then sample volume acquired during a T-Time is constant

  - If we could define the volume acquired.
  - Then we could quantify the acquired events in a V-Volume during a T-Time

Platelet counting by flow cytometry: Single platform system: Volumetric systems

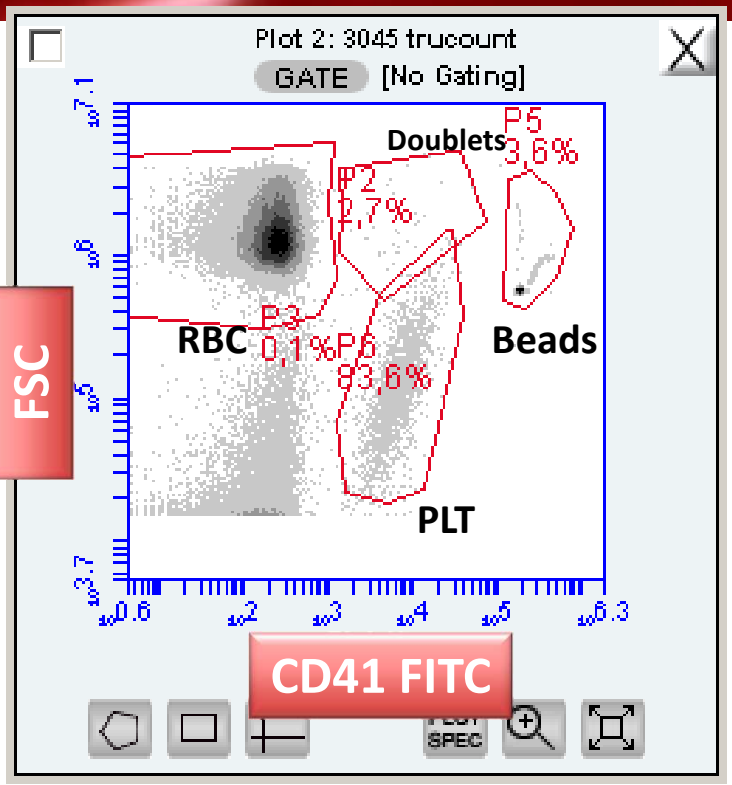
- Use the Poiseuille law!

- Flow Rate (*Q*) depends on some parameters:
  - Flow radius (*r*)
  - the length of the flow cell(*l*)
  - the pressure difference across the flow cell(*p*)
  - viscosity of the sample (η)

$$Q = \frac{\pi r^4}{8 \eta l} \times p$$

Platelet counting by flow cytometry: Accuri C6: Simple and dual platform

On BD Accuri C6:



- **Accuri SP (v/s beads)**
- **Accuri DP (v/s Sysmex XE2100)**
- **Accuri SP (volumetric)**

Cytometer able to give directly an absolute count

$$\frac{\text{(number of platelet events) x dilution factor}}{\text{(volume of aspiration)}}$$

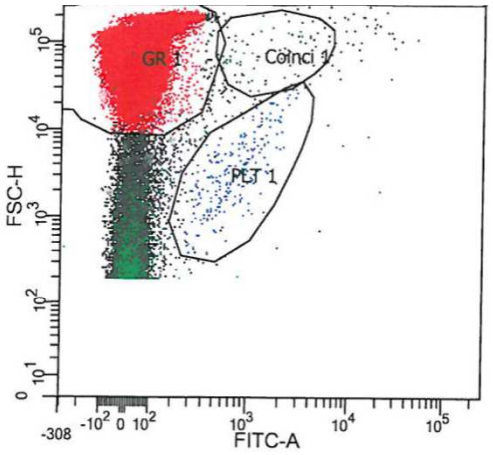
Altered platelet counts on haematological analysers (impedance/optical)

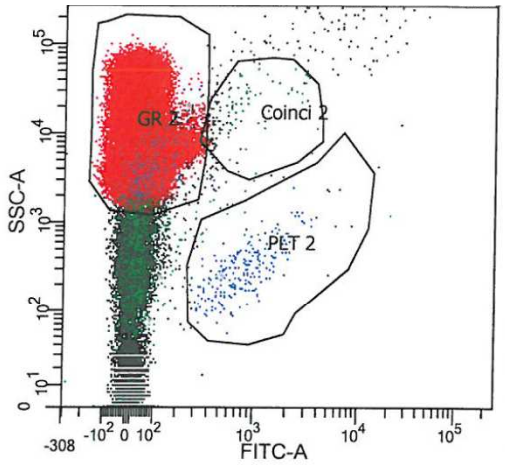
Table 1. Situations leading to altered platelet counts on haematology analysers

	Alteration of other parameters
Spurious decrease	
PLT agglutination (EDTA, but other anticoagulants may be concerned)	PLT aggregates enumerated as WBC
PLT satellitism (mainly related to EDTA)	
Around polymorphs	
Around other WBC (normal; pathological)	
PLT-neutrophil agglutination (mainly related to EDTA)	WBC count spuriously low
Large PLT (outside the normal range)	Enumerated together with WBC
Coagulation within the sample	Abnormal CBC
Overfilling the sample (inadequate mixing)	Abnormal CBC
Spurious increase	
Fragmented RBC (schistocytes, severe iron deficiency anaemia, burns)	RBC count spuriously low (anecdotal)
Cytoplasmic fragments of nucleated cells (leukaemia, lymphoma cells)	
Cryoglobulins, cryofibrinogen	WBC count spuriously increased
Bacteria	
Fungi (*Candida*)	
Lipids (samples taken after a meal, lipid drips)	WBC and haemoglobin spuriously high

Zandecki M. et al. Int Jnl Lab.Hem. 2007

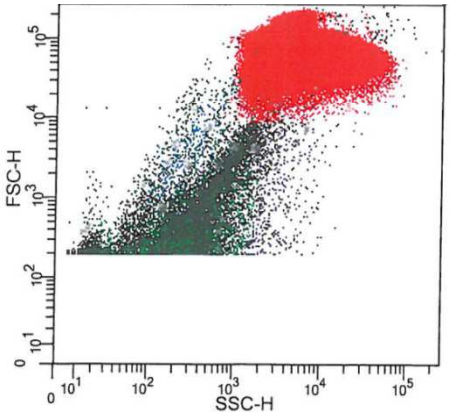
Interference of blasts on platelet count

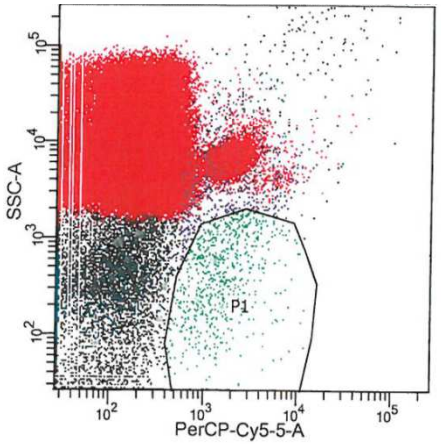




Tube: Tube_003

Population	#Events	%Parent	%Total
All Events	174,876	####	100.0
Time	174,876	100.0	100.0
PLT 1	268	0.2	0.2
Coinci 1	99	0.1	0.1
GR 1	157,498	90.1	90.1
PLT 2	237	0.1	0.1
Coinci 2	169	0.1	0.1
GR 2	159,229	91.1	91.1
Plaquettes	212	0.1	0.1
Coincidence	50	0.0	0.0
Globules rouges	157,256	89.9	89.9
P2	1,016	0.6	0.6
P1	663	0.4	0.4





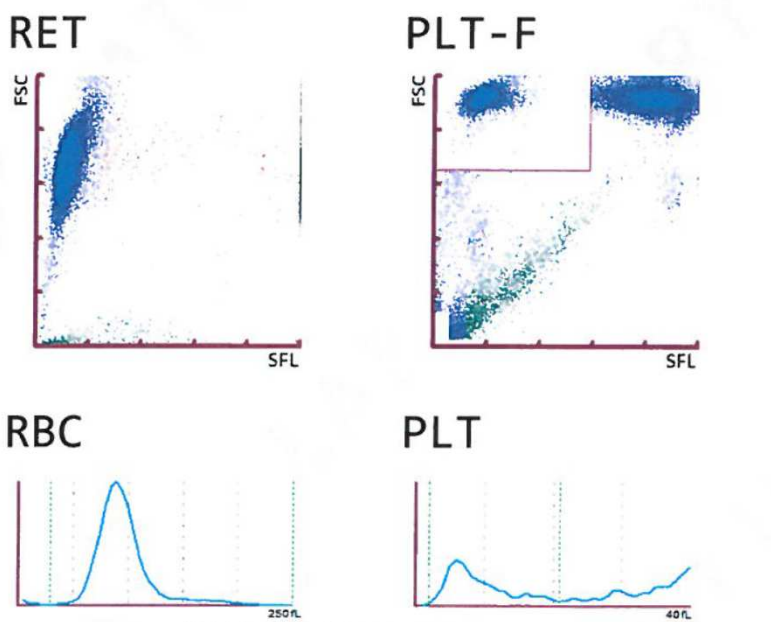
PLT: (20+188/126144)* 4060000=
6694 PLT/microl

Confirms the results of Frotscher et al. Int Jnl Lab Hem 2015

Interference of blasts on platelet count: PLT-F

08/09/2016 15:02:57

PLT &F	15 *	[10^3/uL]
RDW-SD	52.0	[fL]
RDW-CV	16.0	[%]
PDW	10.8 *	[fL]
MPV	9.4 *	[fL]
P-LCR	24.9 *	[%]
PCT	0.02 *	[%]



PLT IP Message
Thrombocytopenia
PLT Clumps?

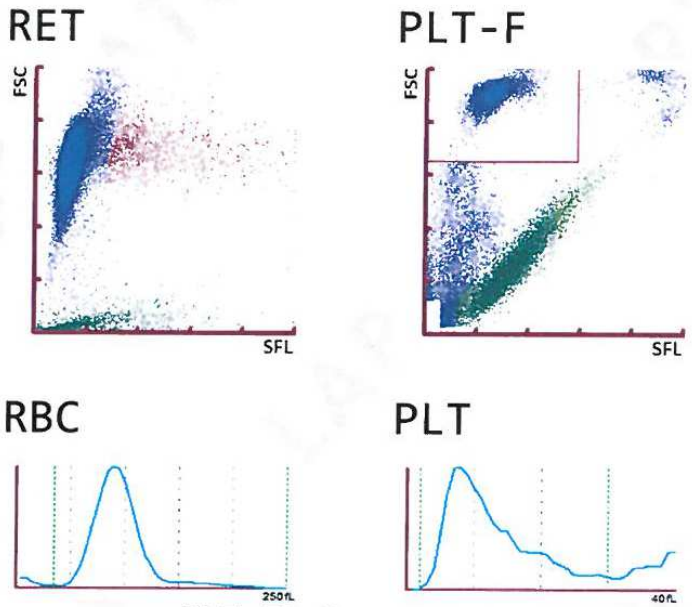
Interference of blasts: PLT-F or flow cytometry

Transfusional efficiency post acute leukemia : PLT-F or flow cytometry

PLT-I	21 * [10^3/uL]
PLT-O	19 * [10^3/uL]
PLT-F	15 * [10^3/uL]

Interference of hemolysis on platelet count

PLT &F	77	[10^3/uL]
RDW-SD	59.0 +	[fL]
RDW-CV	17.6 +	[%]
PDW	19.0 +	[fL]
MPV	12.7	[fL]
P-LCR	44.2 +	[%]
PCT	0.16 -	[%]



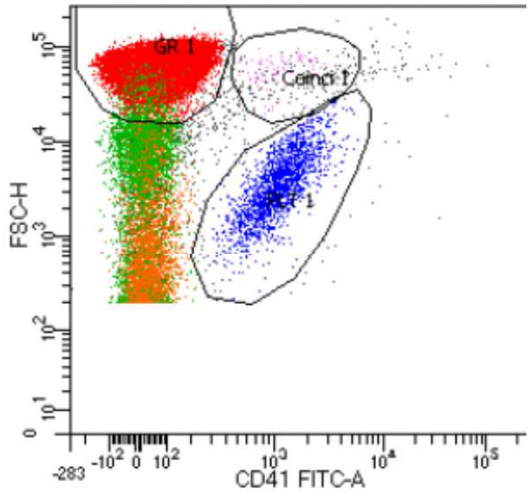
PLT IP Message
Thrombocytopenia

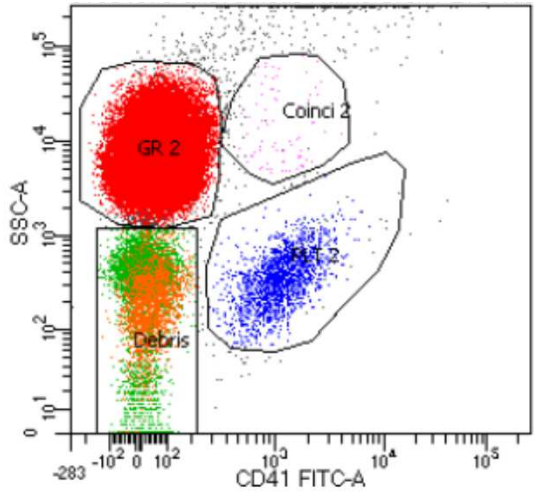
No CI to bone marrow aspiration

CI to bone marrow aspiration

PLT-I	129	[10^3/uL]
PLT-O	97	[10^3/uL]
PLT-F	77	[10^3/uL]

Interference of hemolysis on platelet count

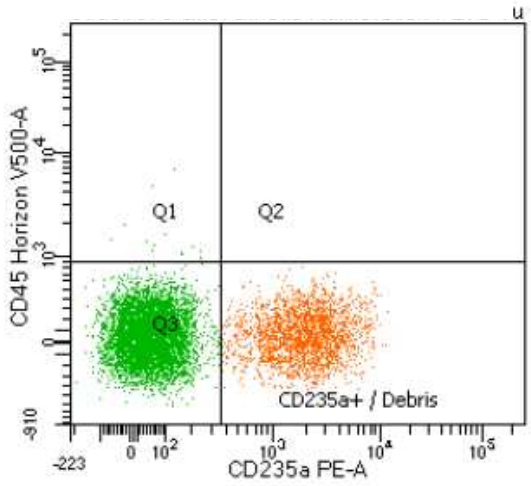


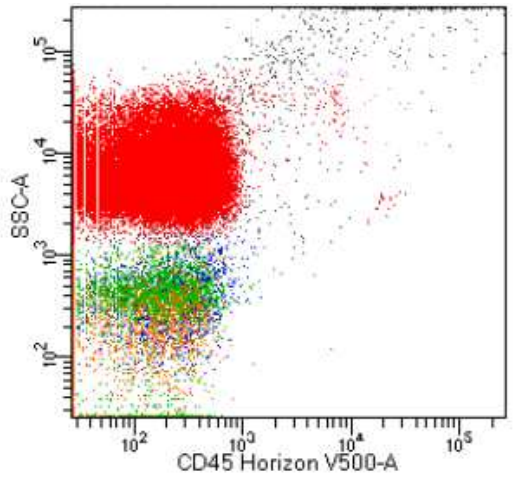


Tube: Tube_001

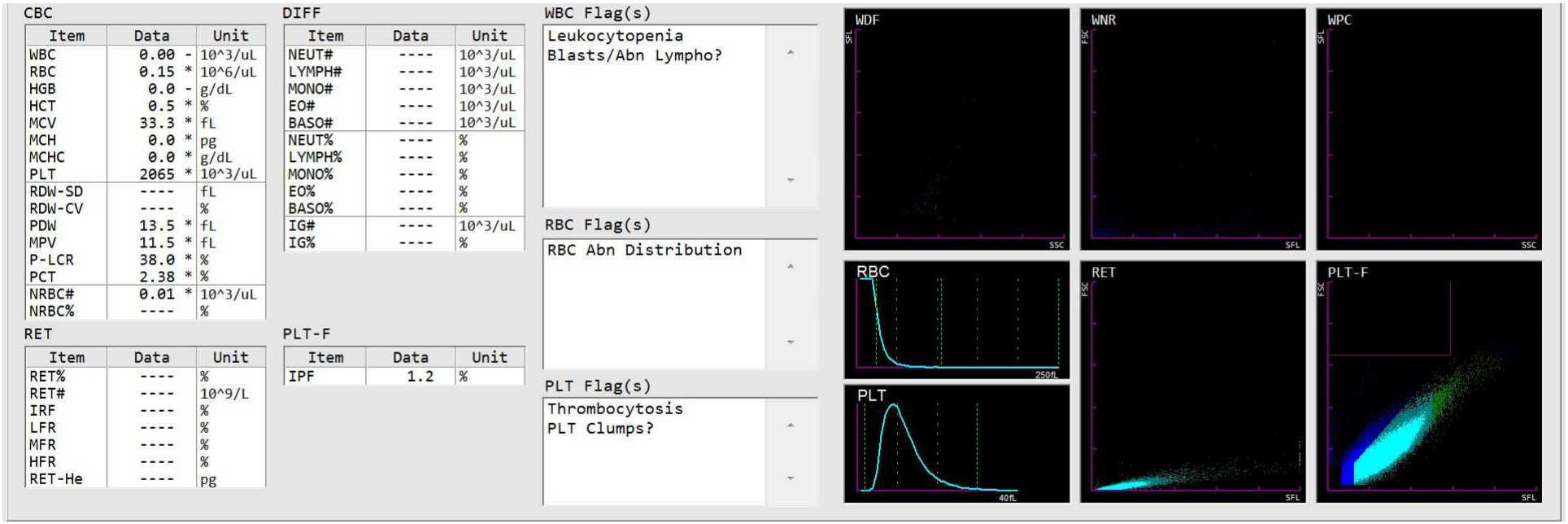
Population	#Events	%Parent	%Total
All Events	118,325	####	100.0
Time	118,325	100.0	100.0
PLT 1	1,877	1.6	1.6
Coinci 1	259	0.2	0.2
GR 1	107,529	90.9	90.9
PLT 2	1,882	1.6	1.6
Coinci 2	113	0.1	0.1
GR 2	107,826	91.1	91.1
Plaquettes	1,837	1.6	1.6
Coincidence	81	0.1	0.1
Globules rouges	106,857	90.3	90.3
Debris	8,082	6.8	6.8
Q1	13	0.2	0.0
Q2	0	0.0	0.0
Q3	5,879	72.7	5.0
CD235a+ / Debris	2,190	27.1	1.9

Flow cytometry:
81069 PLT/microl
95989 CD235a+ RBC fragments/microl





Platelet concentrates: example on XN (optical/impedance)



CBC

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%	----		%

DIFF

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%	----	%

RET

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

PLT-F

Item	Data	Unit
IPF	1.2	%

WBC Flag(s)

Leukocytopenia
Blasts/Abn Lympho?

RBC Flag(s)

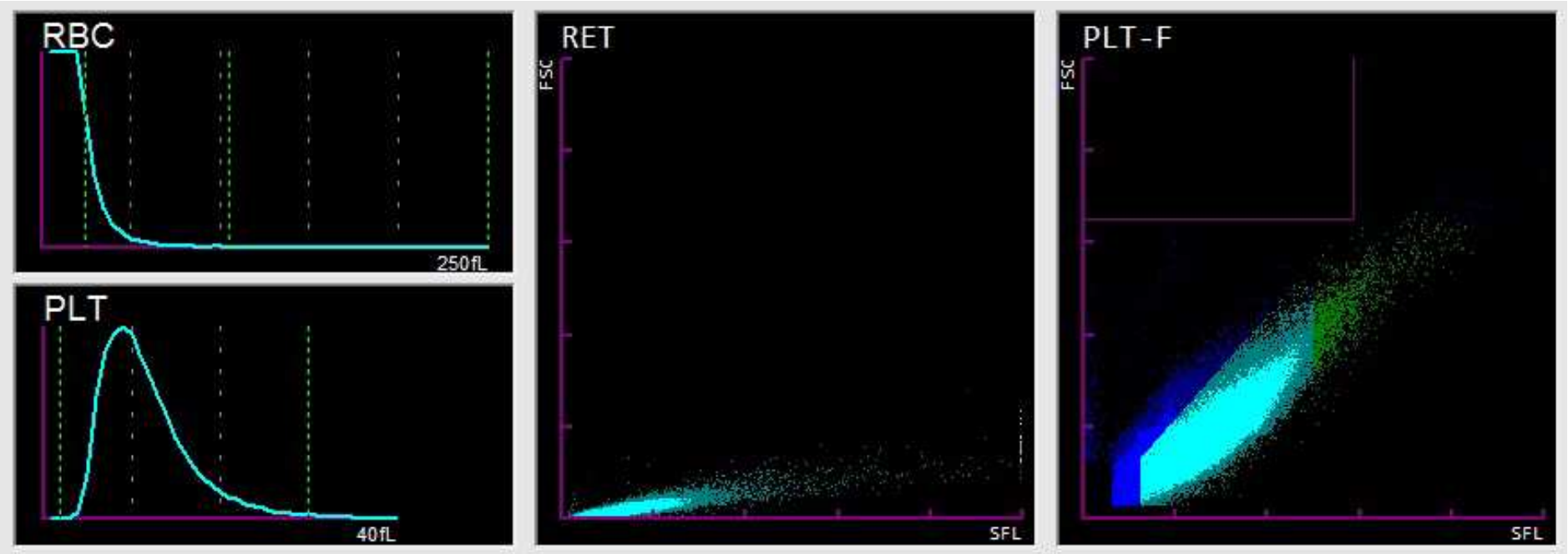
RBC Abn Distribution

PLT Flag(s)

Thrombocytosis
PLT Clumps?

High intermethod variation for optical and impedance methods

Platelet concentrates: example on XN (fluorescence)



Mobile threshold: The absence of red blood cells in platelet concentrates make the threshold vary

The platelets in dark blue not counted → platelet underestimation

Platelet concentrates

- 5 samples/8 centers/ 6 FCM and 15 haematological analysers

TABLE 1. Overview of differences in flow cytometric PLT counting methods as developed in different laboratories

Variable	Variables	Critical?
Flow cytometer	All from BD Biosciences, but different types	No, final comparison showed no difference among different types.
Antibody	CD41a, CD61, CD42a	No, all are PLT-specific antibodies.
Label	FITC, PE, PerCP-Cy5	No, provided gating is performed in correct channel.
Diluent	Without and with EDTA	No, experiments showed no effect on PLT count. However, large effect on RBC count in case of ICSH method.
	Without and with protein	Yes, to prevent beads from sticking; source of protein had no effect.
IgG control	Either used or not used	No, not needed when counting PLT concentrates.
Counting beads	All used TruCount tubes	No, since all used the same method.
Dilution factor	Ranging from 1:100 to 1:5000	No, provided the event rate during measurement remains below 2000/sec.
Staining	10 to 30 min	No.
Poststaining dilution	Ranging from no dilution to 1:20	No.
Acquisition	Acquisition was stopped based on the number of beads counted, PLTs counted, or on time elapsed	No, provided sufficient "neat" volume is counted to have accurate PLT and bead counts.
Identification of PLT events	All used events that had fluorescence of the corresponding anti-PLT monoclonal antibody	No, since all used the same method.
Calculation	As provided by the manufacturer of the TruCount tubes	No, since all used the same method.

Van der Meer PF et al. Transfusion 2012

Platelet concentrates

- 5 samples/8 centers/ 6 FCM and 15 haematological analysers

TABLE 2. Comparison of in-house–developed flow cytometric PLT counting methods (n = 6) with hematology analyzers (n = 15), shown as mean ± SD of four PLT samples counted in triplicate, in $\times 10^9$ PLTs/L

	Flow cytometers		Hematology analyzers	
Sample	Mean ± SD	CV (%)	Mean ± SD	CV (%)
1	1030 ± 171	16.6	930 ± 88	9.5
2	1752 ± 188	10.7	1597 ± 138	8.6
3	2111 ± 249	11.8	1991 ± 156	7.8
4	1124 ± 285	25.4	1106 ± 101	9.2

TABLE 3. Comparison of a uniform flow cytometric PLT counting method used at seven centers and various hematology analyzers (n = 15), shown as mean ± SD of five PLT samples counted in triplicate, in $\times 10^9$ PLTs/L

	Flow cytometers		Hematology analyzers	
Sample	Mean ± SD	CV (%)	Mean ± SD	CV (%)
1	1558 ± 108	6.9	1446 ± 109	7.5
2	1430 ± 88	6.1	1343 ± 98	7.3
3	1278 ± 81	6.3	1202 ± 93	7.8
4	1598 ± 97	6.1	1485 ± 111	7.5
5	1165 ± 68	5.9	1075 ± 83	7.7

« The BEST flow cytometric method has a smaller intercenter CV and a smaller center-to-center deviation from the group mean compared to hematology analyzers. Conversely, individual hematology analyzers are more precise than the flow cytometric method.

Thus, the flow cytometric method provides a calibration tool to allow comparisons between centers, but there is no need to replace routine counting with hematology analyzers ».

Van der Meer PF et al. Transfusion 2012

Issues with platelet concentrates (1/2)

- Haematological analysers: high interinstrument variation (sometimes higher than 50%!!!)

- High platelet concentration may be a problem for some analysers (absence of linearity at concentration higher than 1500000/µl)

→PLT-PLT coincidence: multiple PLT are counted as one platelet → underestimation of the actual number of PLTs

→ Diluted samples may provide higher values than undiluted samples

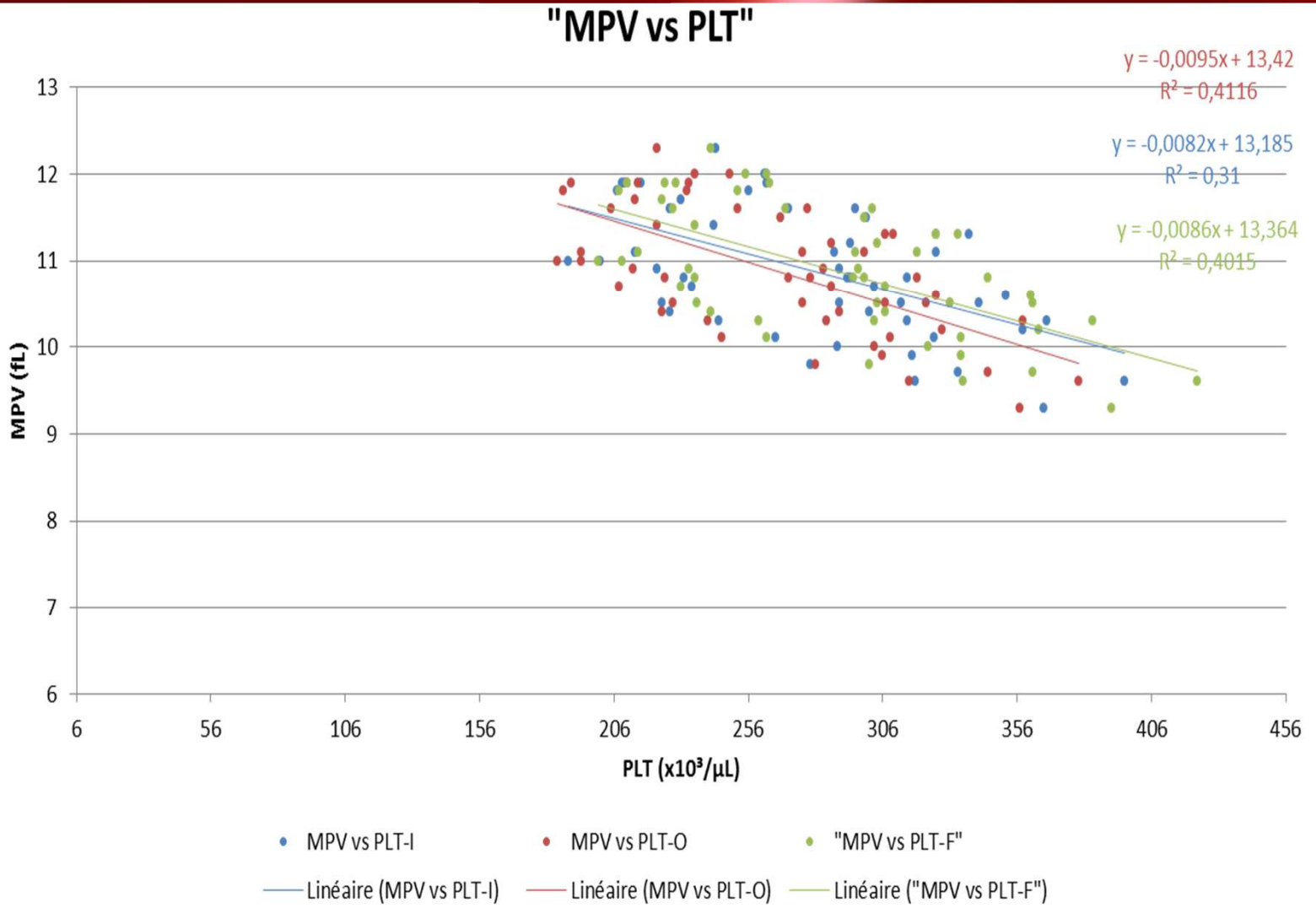
→ Van der Veer Transfusion 2009: diluted samples sometimes lower values (unexplained, hypothesis: dilution in Isoton → PLT swelling)

- Other anticoagulant (Citrate): modification of refractive index
  - Always put in K2 EDTA

Issues with platelet concentrates (2/2)

- Absence of red blood cells
  - Difference of viscosity
  - When counting whole blood, a correction factor is needed to compensate for PLT-RBC coincidence. In PLT concentrates, this erroneously leads to an overcorrection of the actual number of platelets (van der Veer Transfusion 2009)
  - Other additional correction factors or software algorithms of the analyser may introduce inaccuracy when counting PLT concentrates
  - Difference of position of mobile threshold for impedance counting (Sysmex) → inaccurate results (Hervig Vox Sang 2004, van der Veer Transfusion 2009)
- No reference technique for calibration
- Absence of adapted controls (contain red blood cells)
- Presence of aggregates (van der Meer Vox Sanguinis 2015)
- Presence of microparticles (Maurer-Spurej E Transfus Apher Sci. 2016, Rank A et al. Vox Sang.2011)

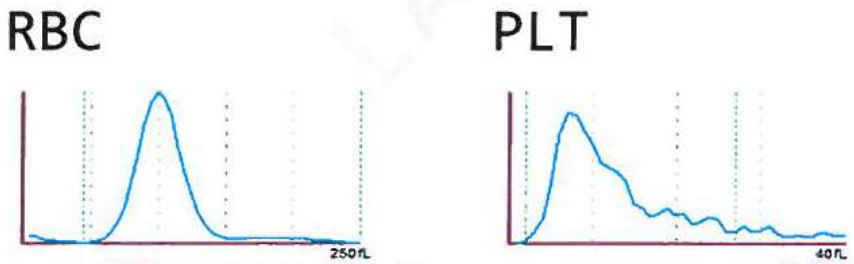
Relation MPV- Platelet count



MPV is unreliable!

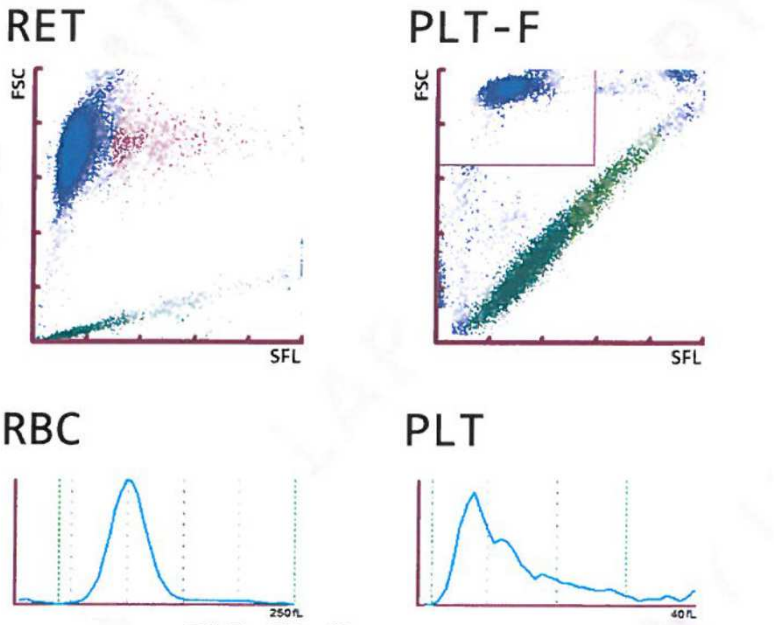
18/10/2016 13:43:29

PLT	65 -	[10^3/uL]
RDW-SD	53.1	[fL]
RDW-CV	14.5	[%]
PDW	12.9	[fL]
MPV	11.7	[fL]
P-LCR	38.7	[%]
PCT	0.08 -	[%]



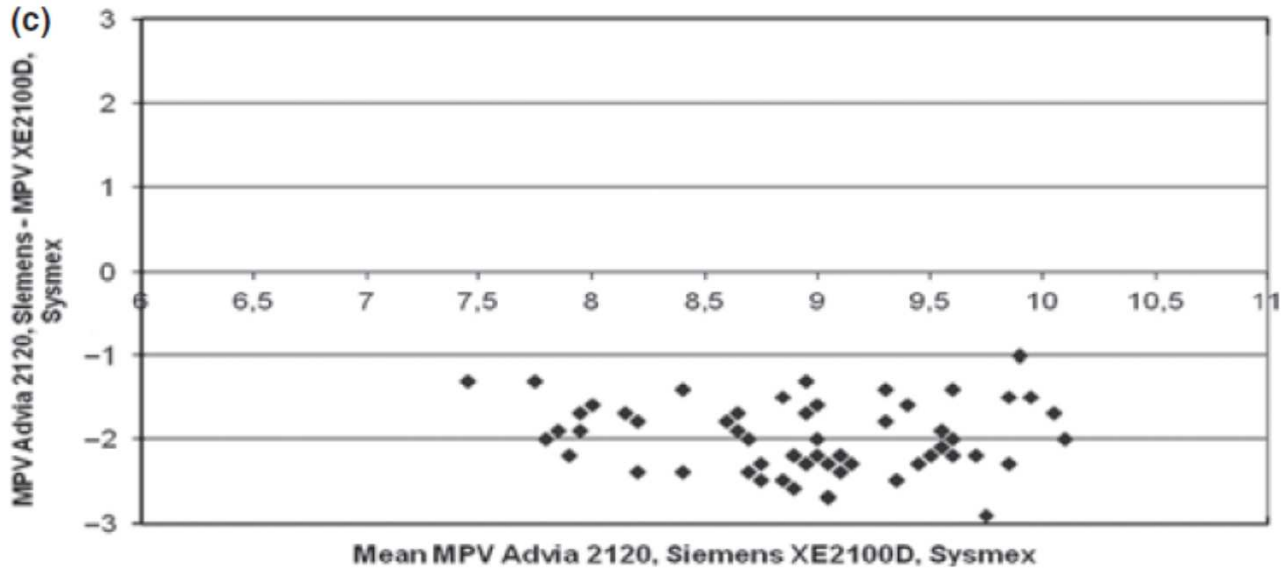
18/10/2016 16:00:12

PLT &F	76	[10^3/uL]
RDW-SD	53.0	[fL]
RDW-CV	14.4	[%]
PDW	16.4	[fL]
MPV	12.7	[fL]
P-LCR	44.5 +	[%]
PCT	0.08 -	[%]



Progressive increase of MPV

- Important of platelet swelling for MPV measurement
- Plateau between 60 min (Bath PM et al. Thromb Haemost 1993) and 120 min (Lancé MD et al. Lab Hematol 2010)
- Lack of standardization (Noris et al. Platelets 2016, Latger-Cannard et al. Int Jnl.Lab.Hematol.2012)



Minimal requirements for publications on MPV

- "Firstly, as with any clinical investigation, the study should be adequately powered with evidence of how the power calculations have been determined.

- Comparisons between groups should ideally be matched for age and gender and where this is not possible the data should be analysed for the effects of these demographic variables and adjusted appropriately.

- All blood samples should be collected, handled and processed in the same way so that the effect of pre-analytical variables between the groups is minimised".

Harrison P. and Goodal AH. Platelets 2016

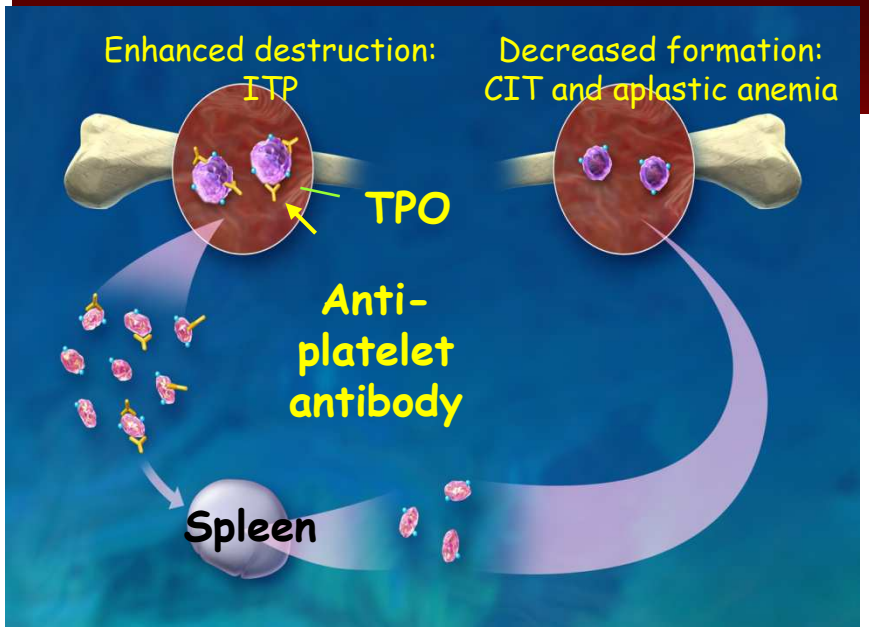
Minimal requirements for publications on MPV

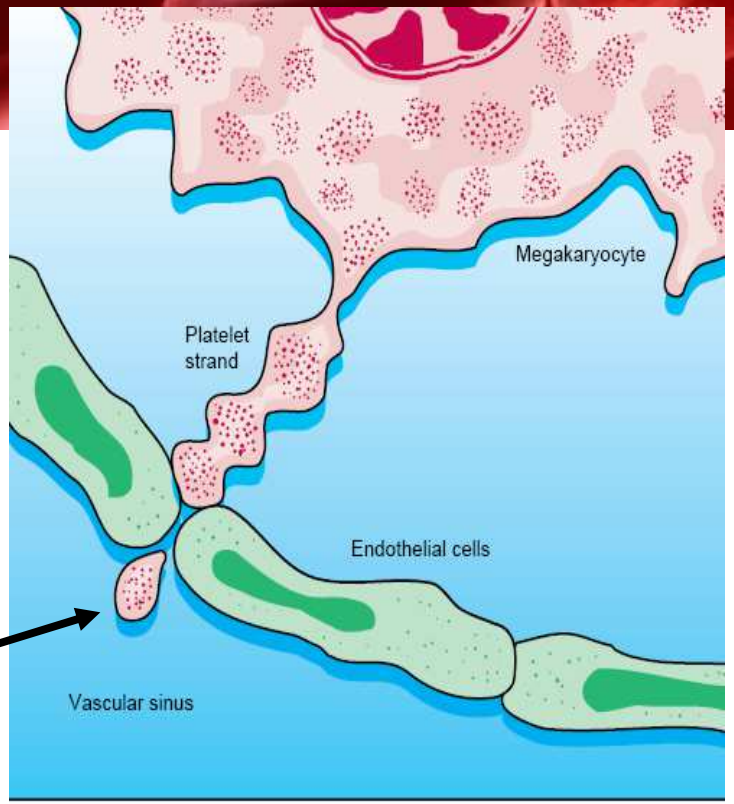
- "Pre-analytical variables including anticoagulation, accuracy of tube filling and mixing, temperature and time delays between sampling and analysis must also be clearly stated. Ideally the time delay should be standardized but if not the range of times should be limited and stated in the manuscript.

- A full description of the technology used (manufacturer, instrument model, method principle etc), Internal and External Quality control procedures employed and ideally the coefficient of variation of MPV measurement (within and between assays) should be included".

Harrison P. and Goodal AH. Platelets 2016

Immature platelet fraction





Young, reticulated platelet

Why?

- Bone marrow megakaryocyte activity and platelet kinetics
- Differentiate low platelet production from enhanced consumption
- May decrease the invasive BM aspiration need and eliminates superfluous platelet transfusions

Immature platelet fraction

Flow cytometry

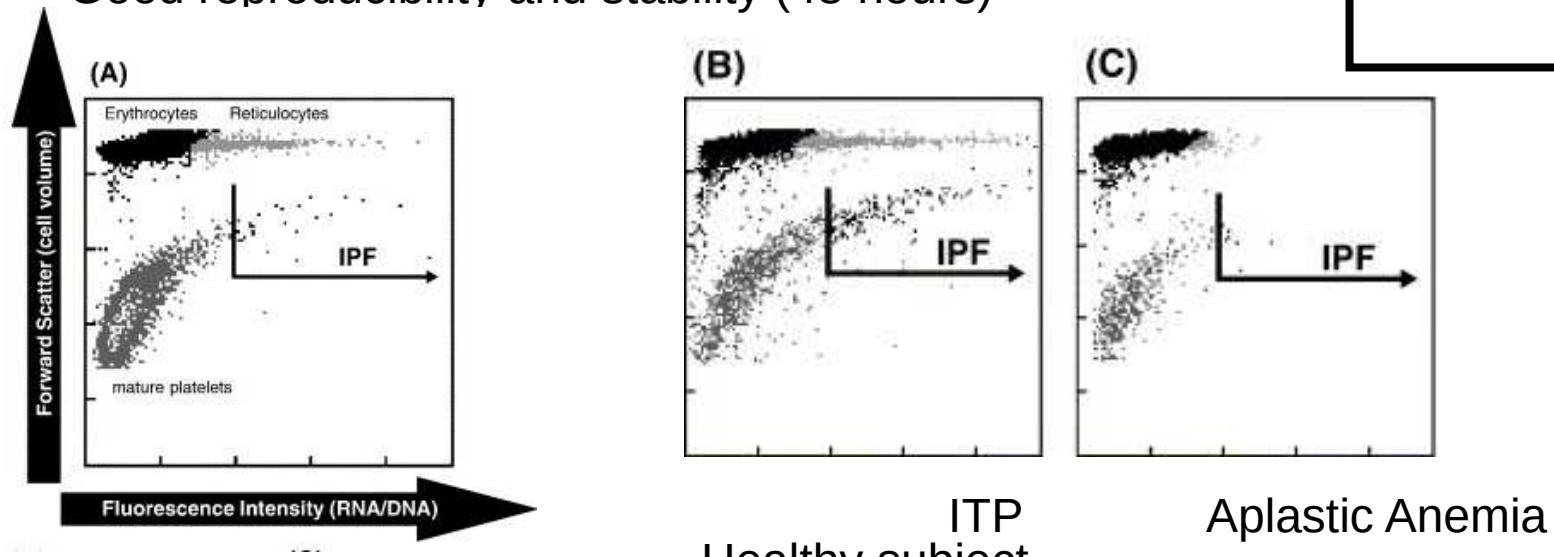
Lack of standardisation
Flow cytometer and a cytometrist required
Not widespread as a daily routine test

Reference range:
1.1-6.1%

Autoimmune thrombocytopenia:
IPF=17-22%

Hematimetry (ex: XE 2100)

Precise, automated, relatively inexpensive, non-ivasive
Good reproducibility and stability (48 hours)



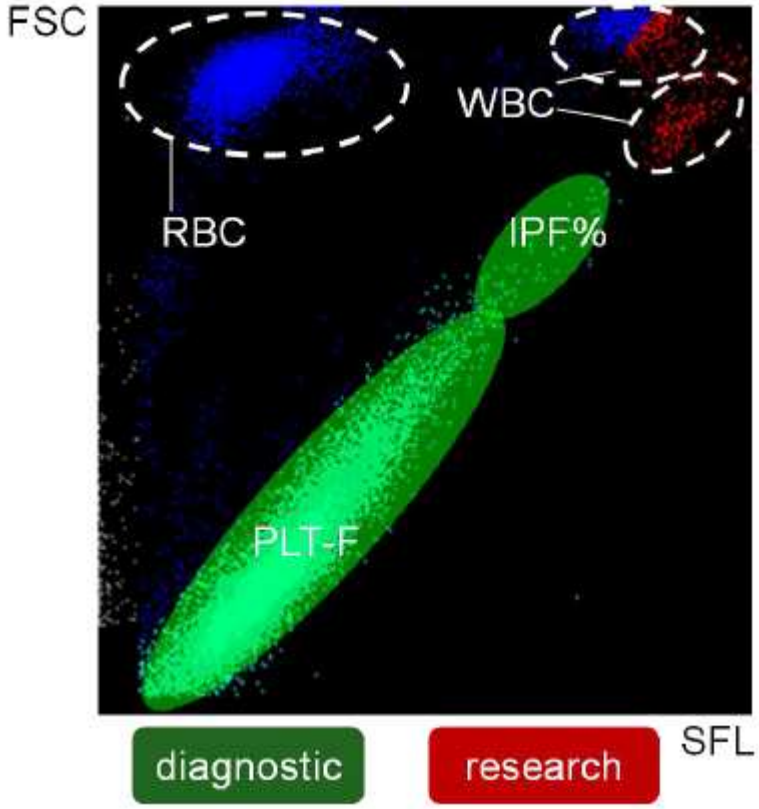
ITP
Aplastic Anemia
Healthy subject

Immature platelet fraction

- IPF comes from platelet fluorescence channel
  - FSC: Axial Diffusion
  - SFL: Fluorescence after Oxazine

- On FSC vs SFL scale , IPF= at the upper right of platelet population

- Fixed threshold on fluorescence (SFL) → IPF is dependent on the platelet volume



IPF Comparison between XE-2100 and XN

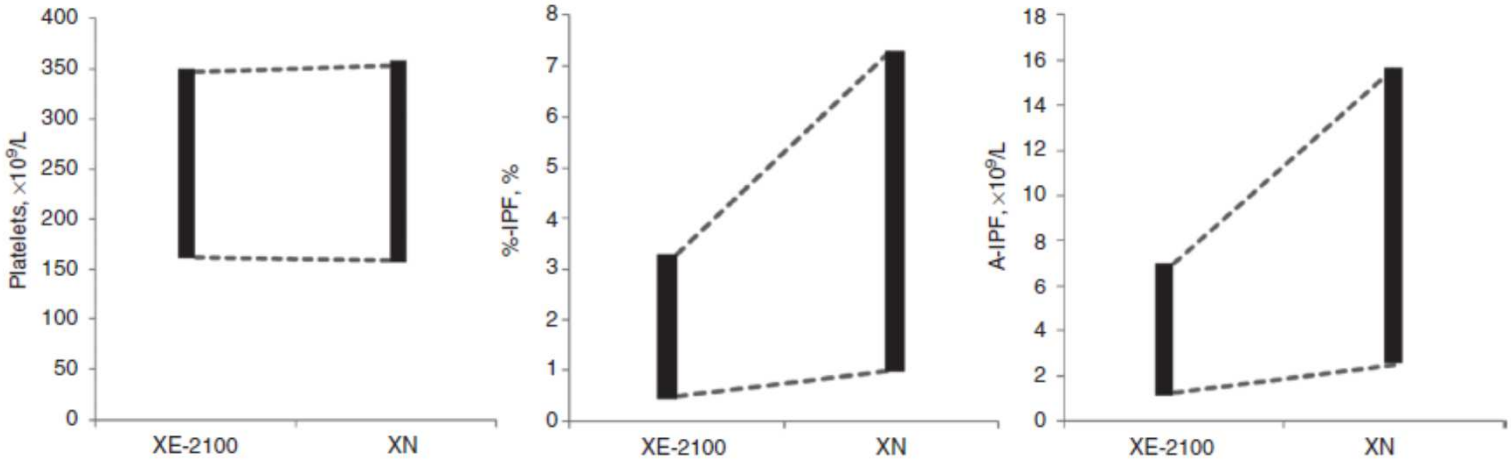


Figure 3 Comparison of reference intervals for platelet counts and immature platelet fraction (IPF) between Sysmex XN and XE-2100 in healthy individuals.
The present data on Sysmex XN (n=2104) were compared with the previously reported data on Sysmex XE-2100 (n=2152) by Ko et al. [13].

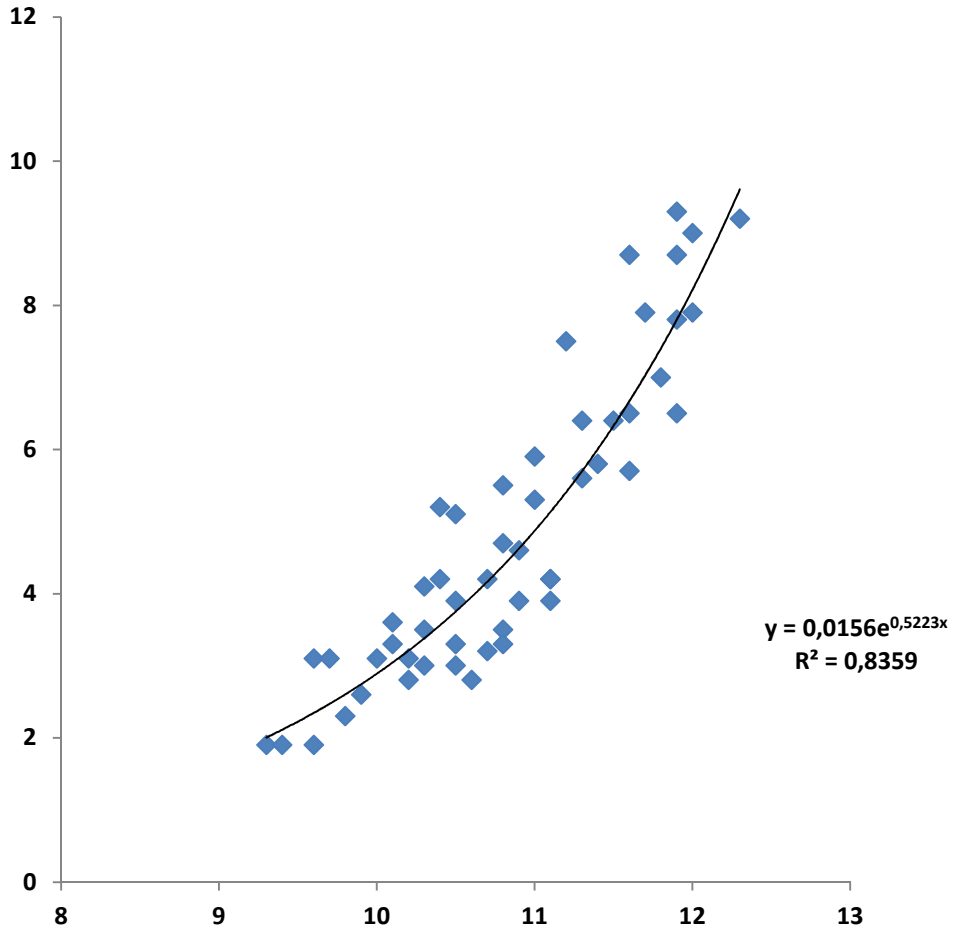
Different reference intervals for IPF between XE-2100 and XN

Ko et al. Clin Chem Lab Med 2015

IPF vs MPV

IPF (%)

IPF is a good indicator of the platelet volume (instead of RNA content)



MPV (fL)

Confirms previsous publication of Miyazaki Hematology 2015

Conclusions about platelet counting

- Inaccuracy of impedance/optical in many clinical situations: low platelet counts, fragmented red blood cells, leukemia/lymphoma cells, cryoglobulins, bacteria, lipids, haemolyis,..
- PLT-F is more accurate than PLT-I and PLT-O in low platelet counts, fragmented red blood cells, leukemia cells and haemolysis (but less than Flow Cytometry)
- Flow cytometry: gold standard for whole blood but there is still place for improvement for platelet concentrates, importance of BSA
- Platelet concentrates: Many unsolved technical issues
- Platelet-derived parameters: lack of standardization, low clinical interest nowadays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THANK YOU FOR YOUR
ATTENTION

Acknowledgments

- **Prof. Bernard Chatelain**
- **Ph. Hugues Jacqmin**
- **Dr Amandine Magnette**
- **Mr Nicolas Bailly**
- **Mr Sebastien Walbrecq**
- **Mr Adrien Collard**
- **Mr Javier Hernandez**
- **Mme Jeanine Dumont**

Announcement

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

7ème symposium annuel le jeudi 30 mars 2017

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