

Platelet Number Enumeration Technique and Pitfalls

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Content

Platelet enumeration techniques

Spurious (false) platelet counts



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Spurious (false) platelet counts



Methods for platelet enumeration

**Manual direct
count**

**Estimation from
blood smear**

**Automated
analyzer**

**Immunological
count**

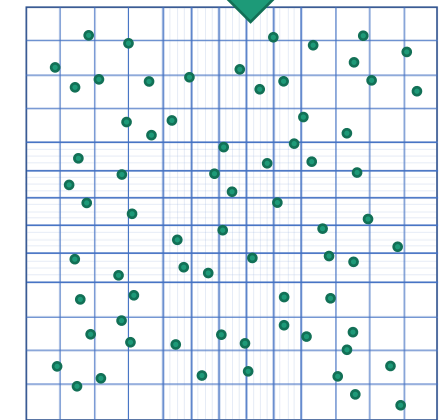
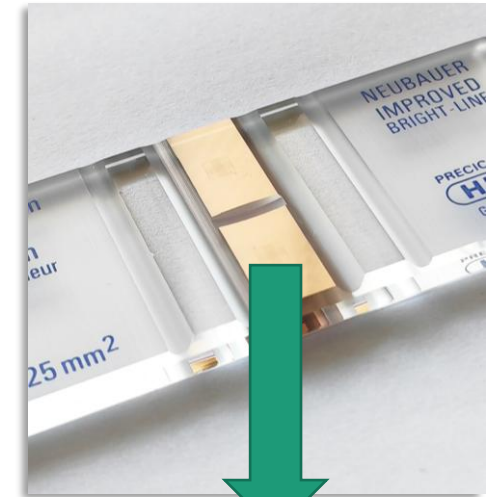
Methods for platelet enumeration

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1 mm 1 mm 1 mm

Methods for platelet enumeration

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Pros

- **Low cost**
- **Abnormal platelet morphology**

Cons

- **Imprecision**
- **Labor-intensive procedure**

Methods for platelet enumeration

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1

2

Label



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Representative area

Examine at 1000x (200 RBC/oil field)

At least 10 oil fields

“Adequate” platelet 5-25 /OF

“Decreased” platelet < 5 /OF

“Increased” platelet > 25 /OF

เกณฑ์มาตรฐานการจัดระดับความผิดปกติของเม็ดเลือดแดง. สภาเทคนิคการแพทย์.

Methods for platelet enumeration

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Multiplication factors

Numbers of platelet per OF from smear

x

9.4×10^3 to 20×10^3

Multiplication factor

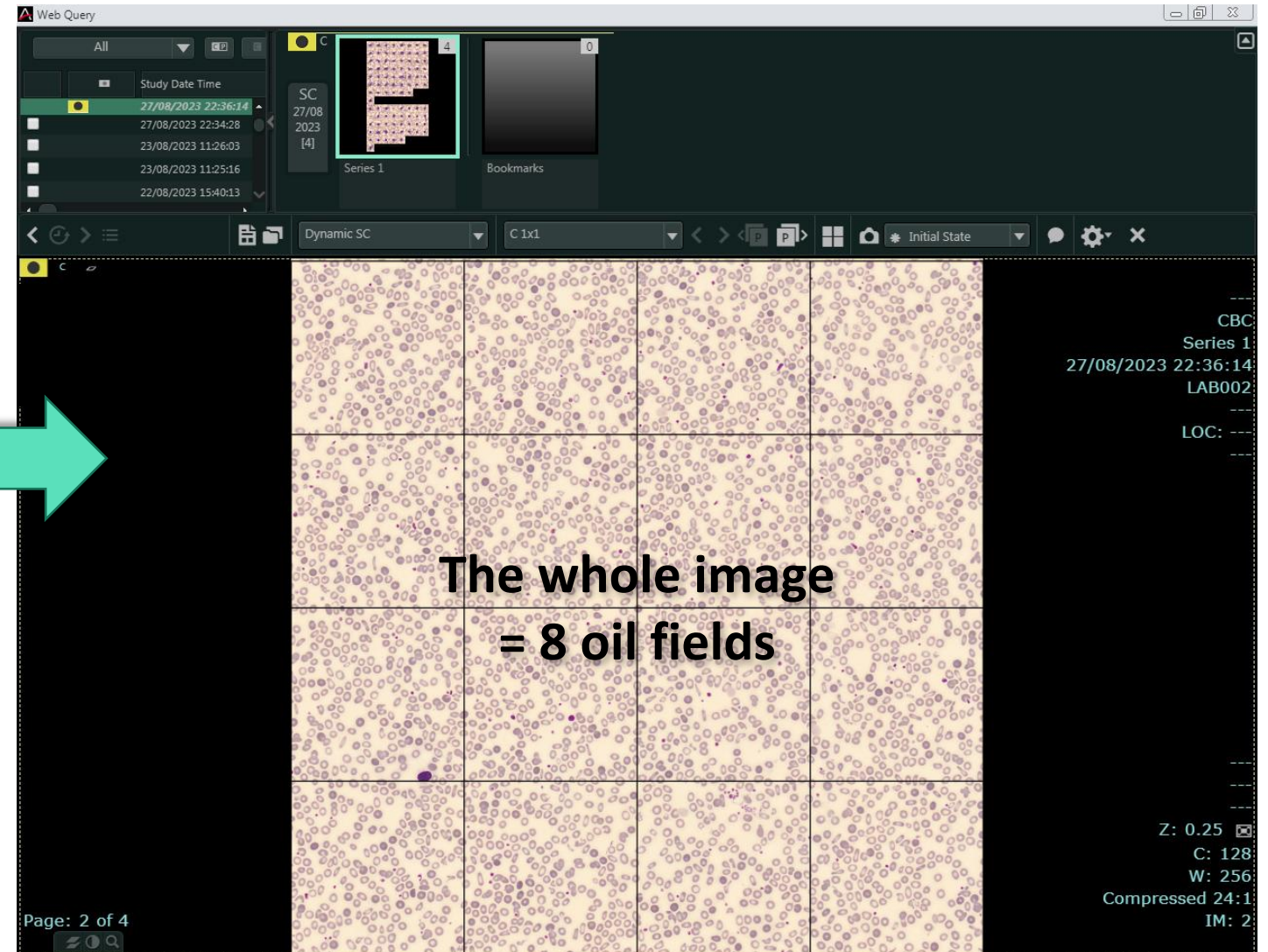
=

Estimated platelet count

Conventional blood smear examination



Online Blood Smear



Methods for platelet enumeration

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Pros

- Low cost and simple
- PLT clumping and other cells

Cons

- Indirect measurement
- Imprecision

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Impedance method

Optical method

Methods for platelet enumeration

Manual direct
count

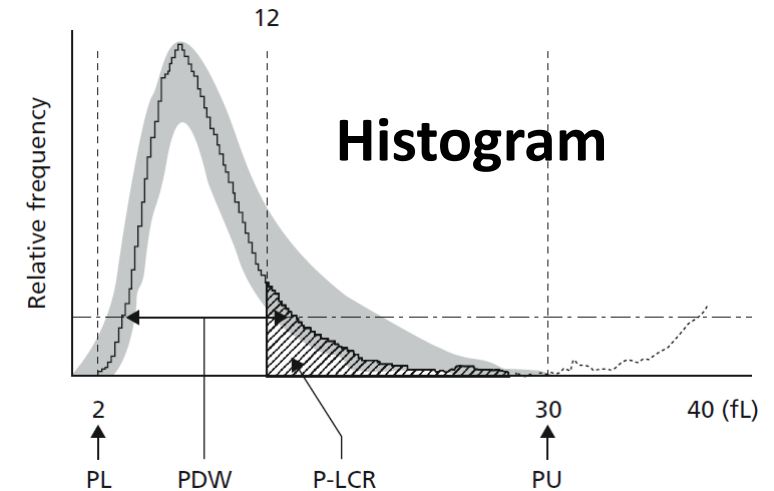
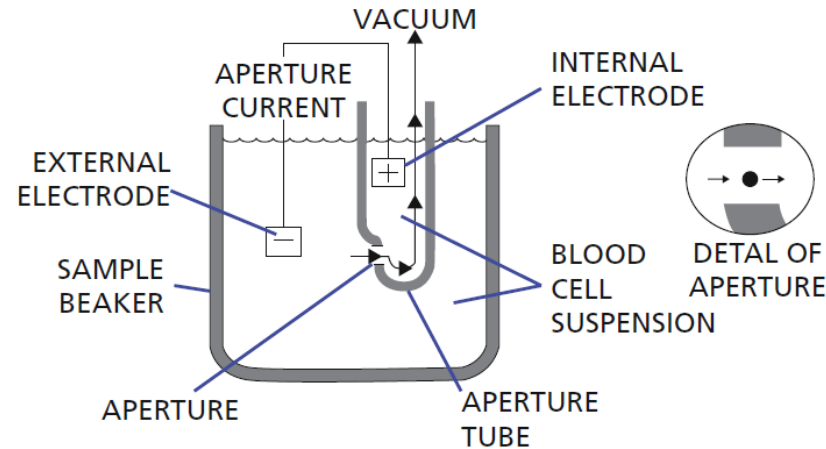
Estimation from
blood smear

**Automated
analyzer**

Immunological
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Impedance method

Optical method



Briggs C, et al. ISLH Laboratory Hematology Practice, 2012.

Methods for platelet enumeration

Manual direct
count

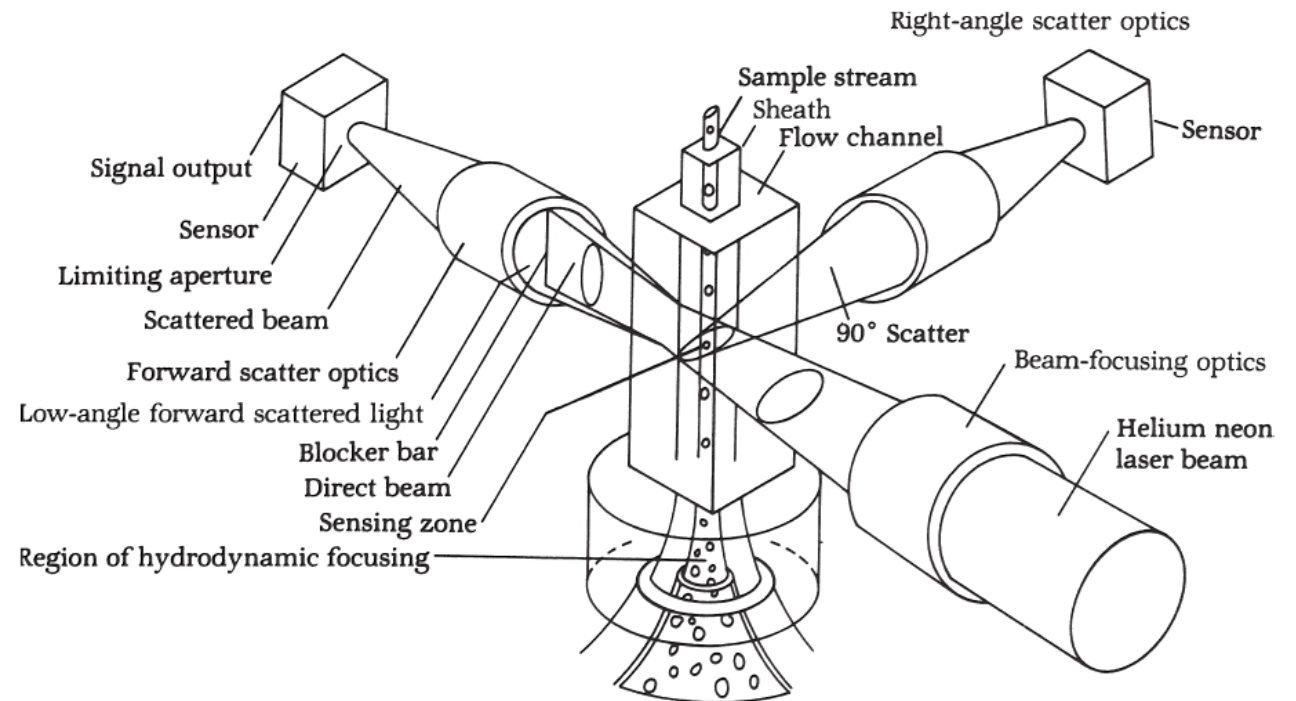
Estimation from
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**Automated
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Immunological
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Impedance method

Optical method



Clinical Hematology Theory and Procedures, 2018.

Methods for platelet enumeration

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Impedance method

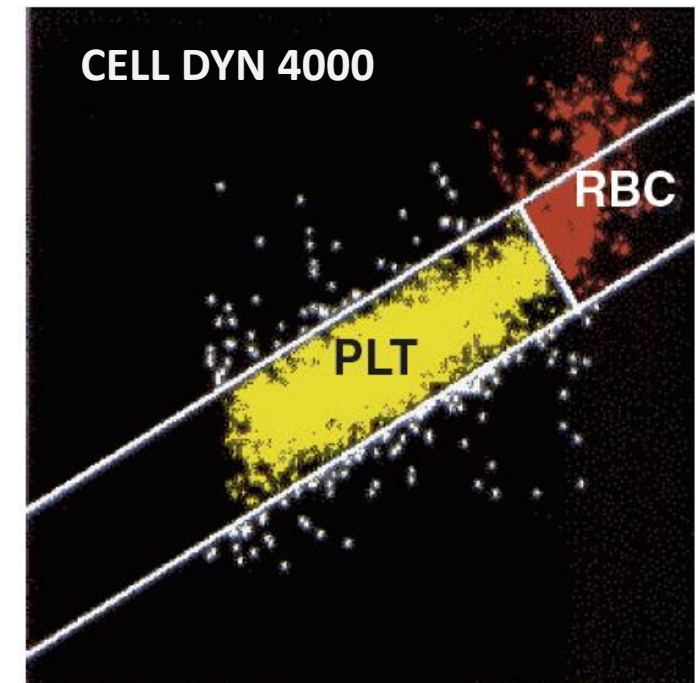
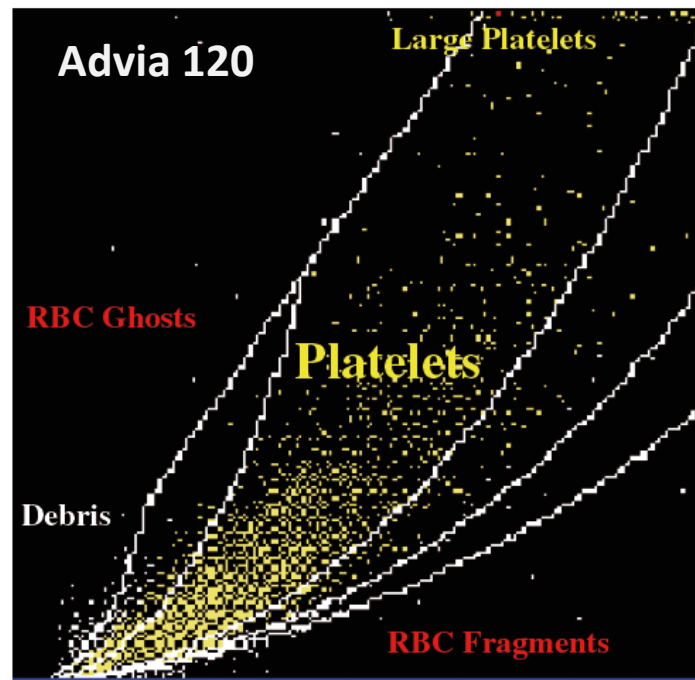
Optical method

Estimation from
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**Automated
analyzer**

Immunological
count

Scattergram



Methods for platelet enumeration

Manual direct
count

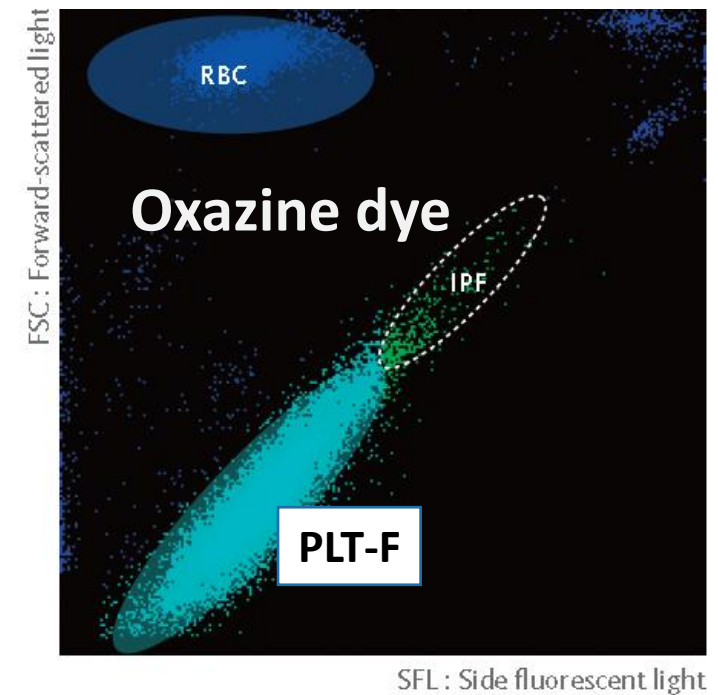
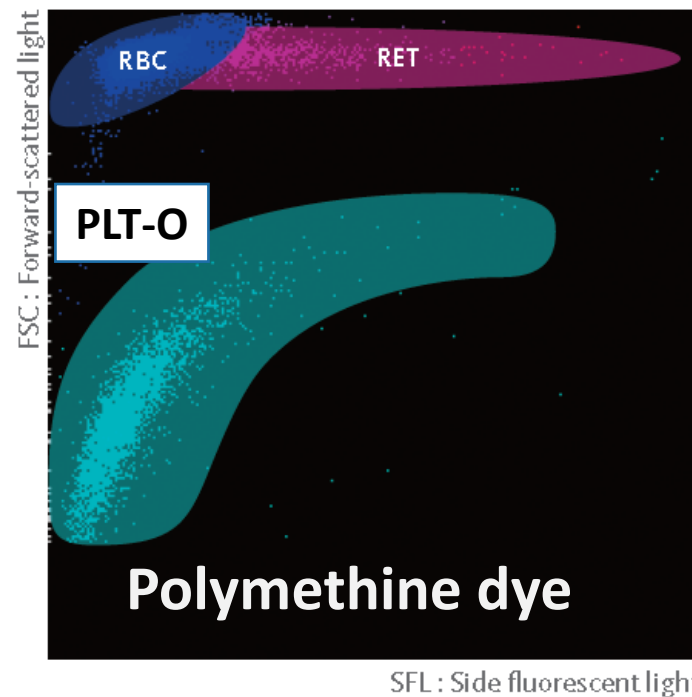
Impedance method

Optical method

Estimation from
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Methods for platelet enumeration

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Pros

- Widely available in most labs
- Precise and accurate

Cons

- Inaccurate in many conditions
- High cost

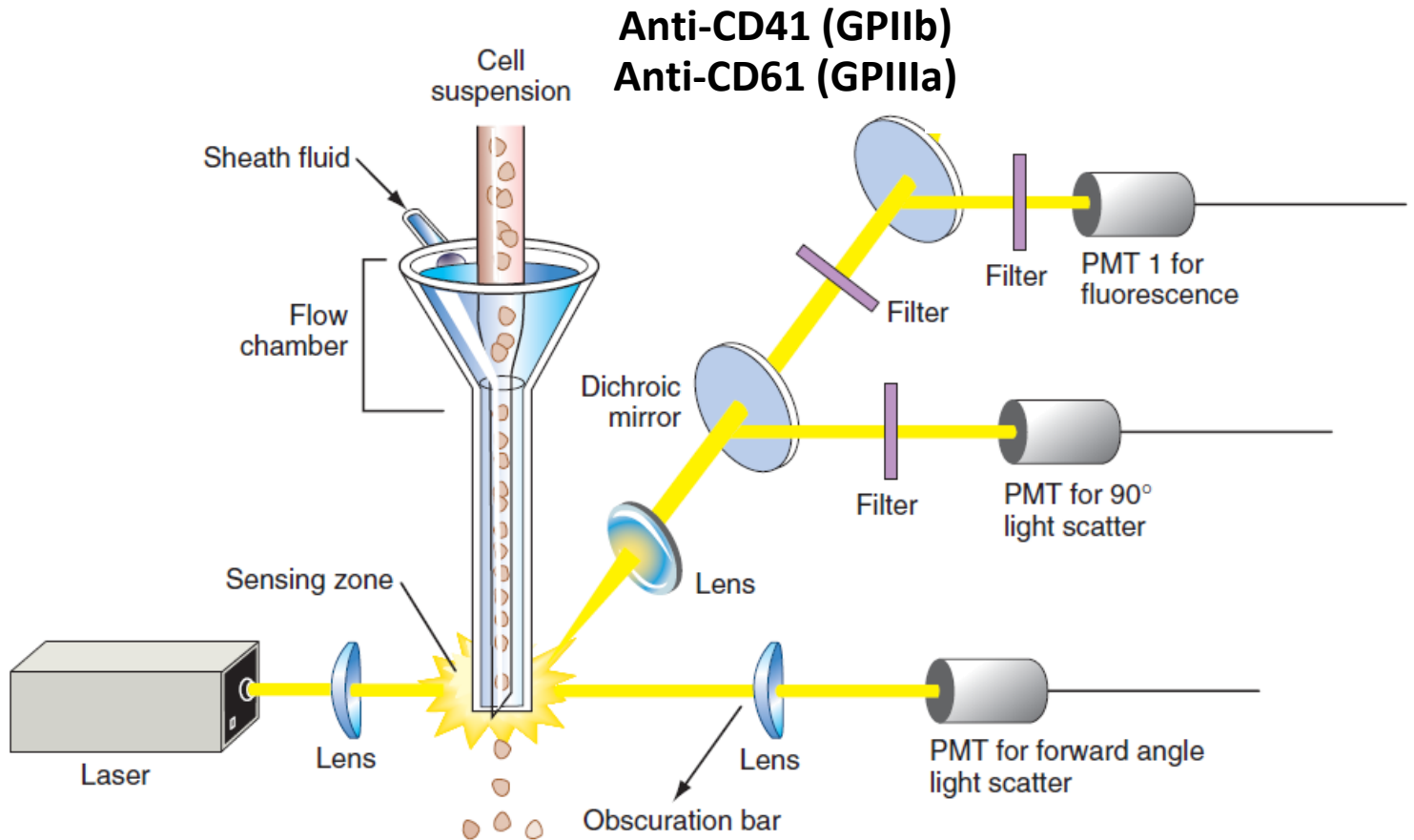
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Henry's clinical diagnosis and management by laboratory methods, 2017.

Methods for platelet enumeration

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Coagulation and Transfusion Medicine / REFERENCE METHOD FOR PLATELET COUNTING

Platelet Counting by the RBC/Platelet Ratio Method

A Reference Method

International Council for Standardization in Haematology Expert Panel on Cytometry and
International Society of Laboratory Hematology Task Force on Platelet Counting†*

Key Words: Flow cytometry; Platelets; Platelet count; Reference method; Value assignment; Whole blood calibration

Am J Clin Pathol. 2001;115:460–4.

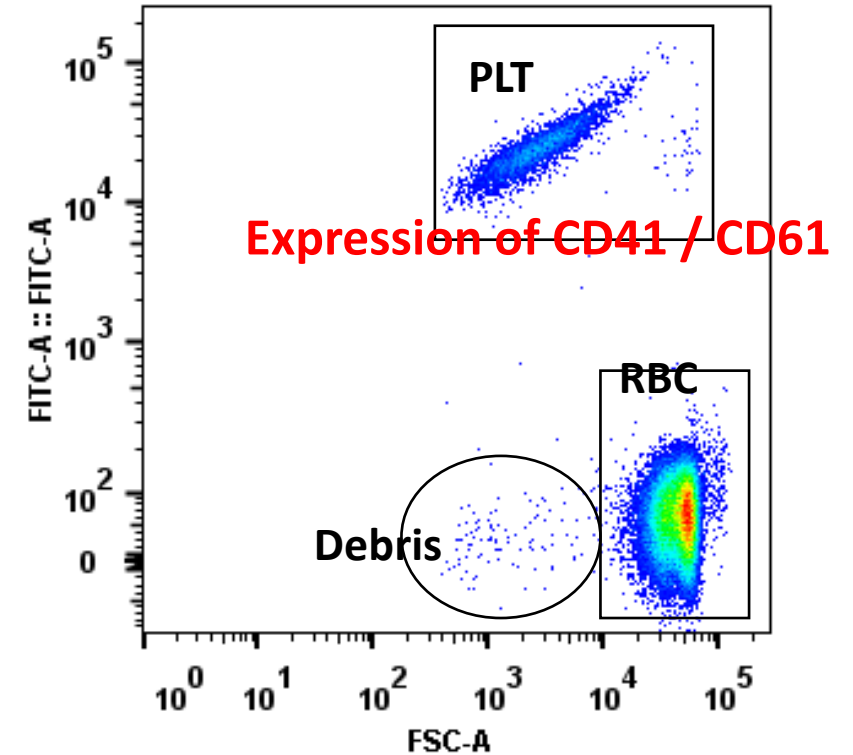
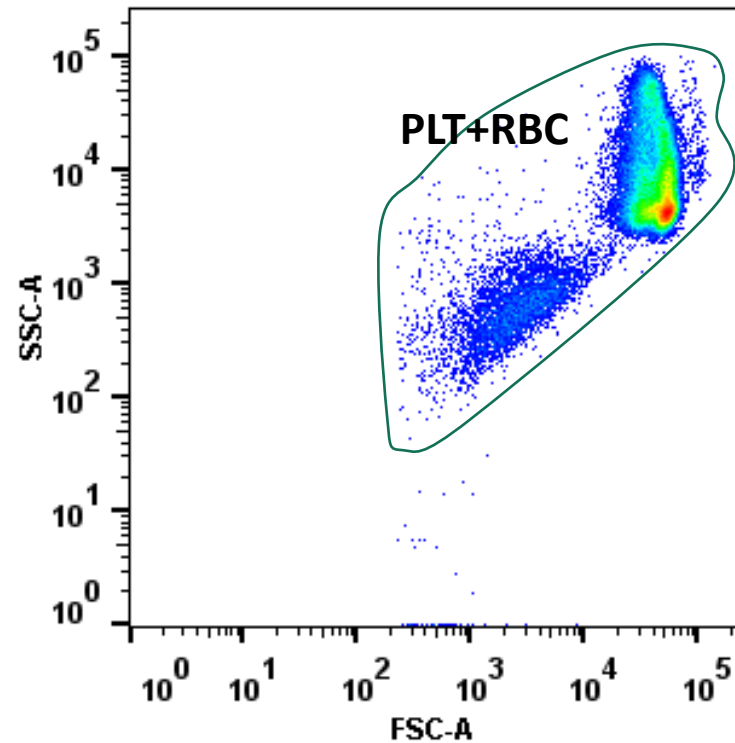
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Methods for platelet enumeration

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Pros

- New reference method
- Very precise and accurate

Cons

- Sophisticated technology
- High cost / turnaround time

Content

Platelet enumeration techniques

Spurious (false) platelet counts

(Focus on automated method)



Spurious (false) platelet counts

Falsely low

Falsely high

Spurious (false) platelet counts

Falsely low

Falsely high

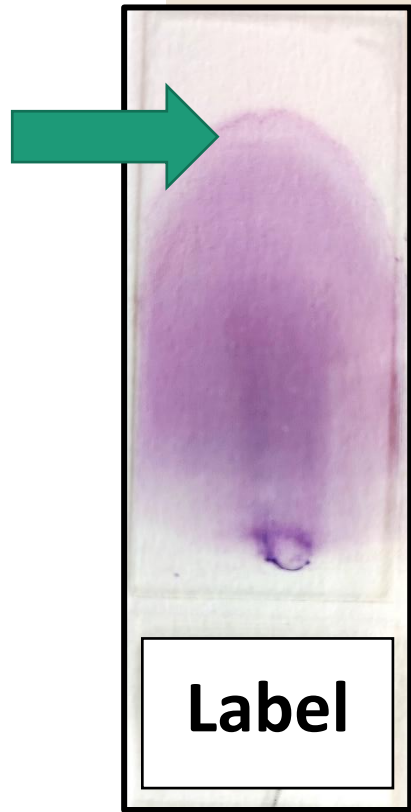
Case scenario 1

A 45-year-old male came for annual health check up.

He has no abnormal symptom.

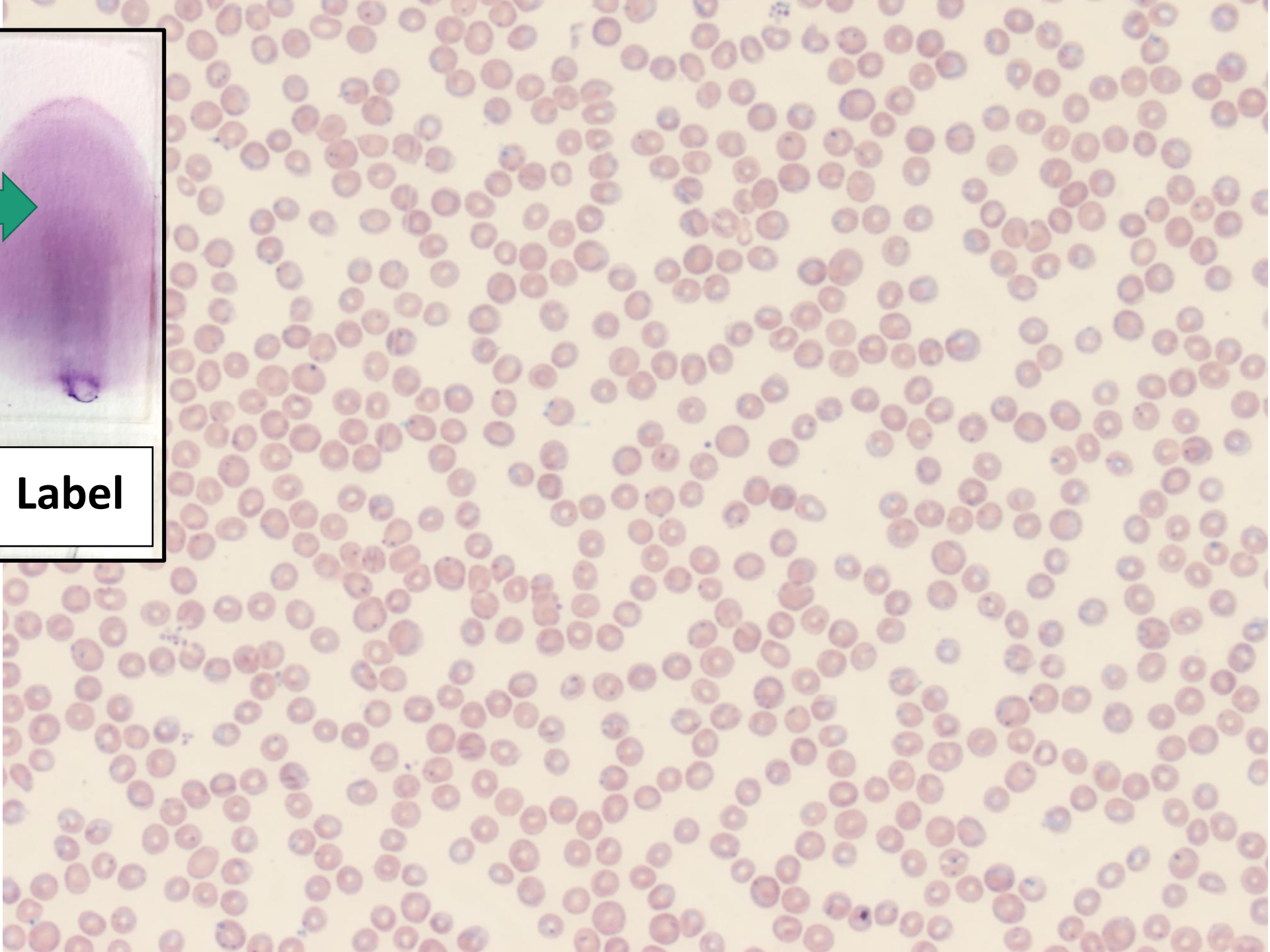
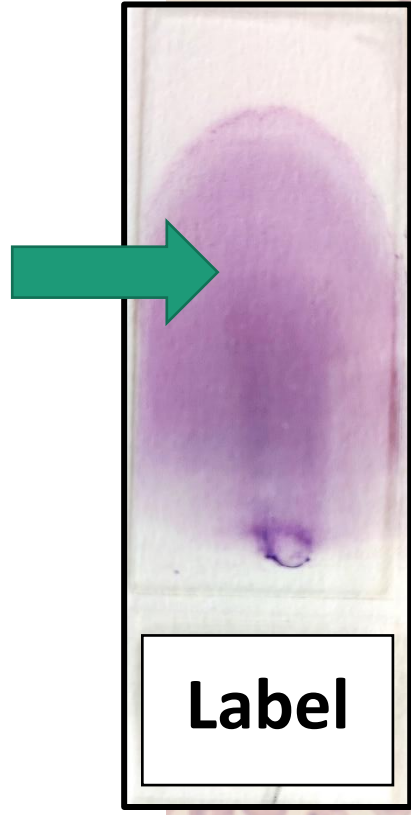
CBC: Hb 11.9 g/dL, Hct 35.4%, WBC 3,180/ μ L, N 31.8%,
L 57.2%, M 6.9%, E 3.8%, Platelet 16,000/ μ L Review smear

The patient has been referred to a hematologist.



Platelet clumping

Pseudothrombocytopenia

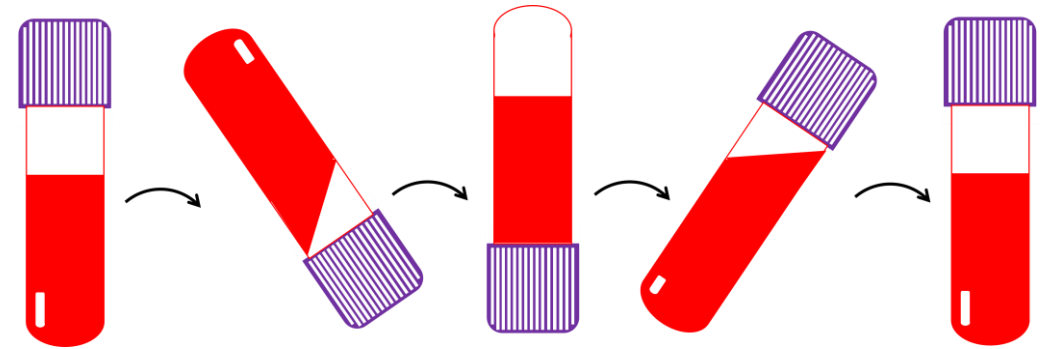


Causes of platelet clumping

**Preamanalytical
problem**

Traumatic venipuncture

***In vitro* blood clot**

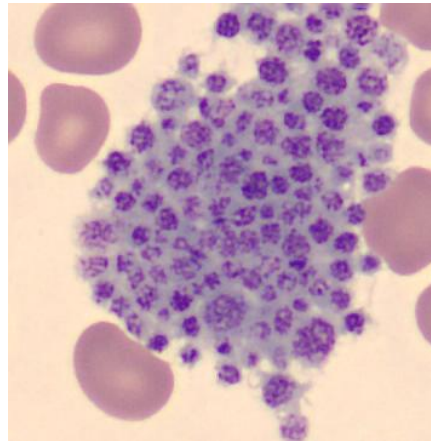


1 inversion mix

< 8

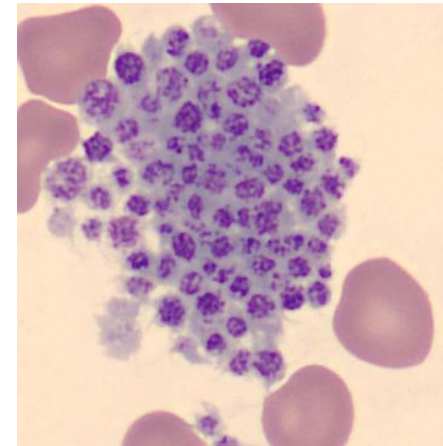
CBC result of a 73-year-old male with cancer

21-03-14 06:26	
Hemoglobin	7.4
Hematocrit	23.6
Rbc count	2.54
MCV	92.9
MCH	29.1
MCHC	31.4
Red cell distribution RDW	16.4
Wbc count	17.17
NRC/ 100 WBC	0.0
Platelet count	24
Absolute neutrophils	13.39
% Neutrophils	78.0
% Lymphocytes	10.5
% Monocytes	6.8
% Eosinophils	4.5
% Basophils	0.2



CBC result of a 73-year-old male with cancer

	21-03-14 06:26	21-03-14 09:07	21-03-14 11:07
Hemoglobin	7.4	7.5	7.7
Hematocrit	23.6	23.3	23.6
Rbc count	2.54	2.53	2.56
MCV	92.9	92.1	92.2
MCH	29.1	29.6	30.1
MCHC	31.4	32.2	32.6
Red cell distribution RDW	16.4	16.6	16.6
Wbc count	17.17	17.77	19.87
NRC/ 100 WBC	0.0	0.0	0.0
Platelet count	24	40	14
Absolute neutrophils	13.39	15.99	15.02
% Neutrophils	78.0	90.0	75.6
% Lymphocytes	10.5	2.0	14.2
% Monocytes	6.8	4.0	6.3
% Eosinophils	4.5	4.0	3.7
% Basophils	0.2	-	0.2



Causes of platelet clumping

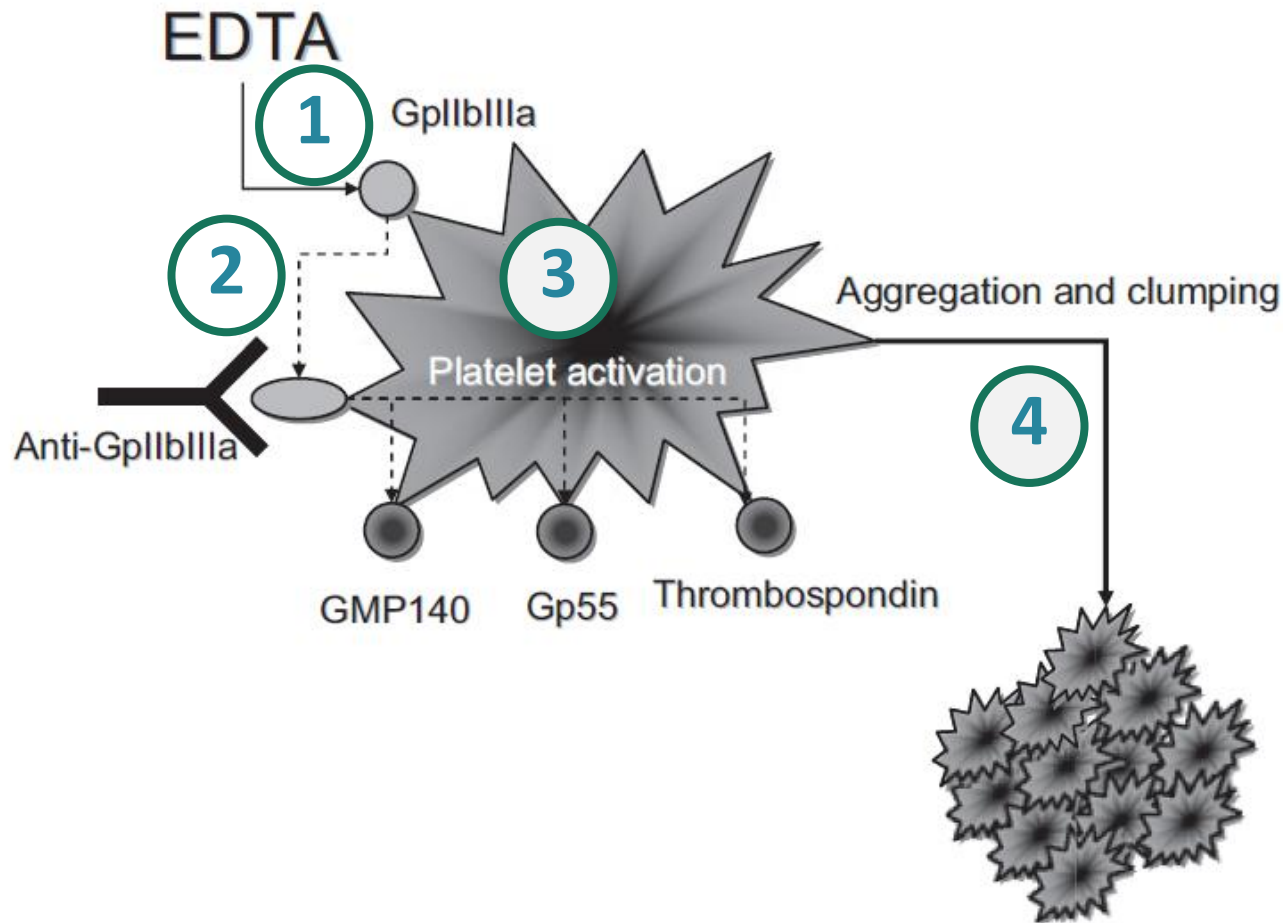
**Preamanalytical
problem**

Traumatic venipuncture

***In vitro* blood clot**

**EDTA-dependent
pseudothrombocytopenia
(EDTA-PTCP)**

EDTA-PTCP



(Exclude pre-analytical error)

Low platelet count < 100,000/ μ L

EDTA-anticoagulated specimens

Evidence of platelet aggregates

No sign of platelet disorders

Platelet count falling over time

Recommendations for EDTA-PTCP

Warming the samples

Use **alternate anticoagulants** e.g. Na citrate, Li heparin, ammonium oxalate, β -hydroxyethyltheophylline, Na fluoride, pyridoxal 5'-phosphate and Tris (CPT)

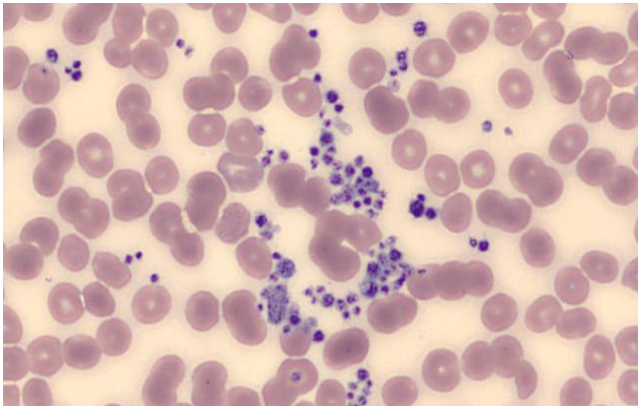
Use **antiplatelet agents** e.g. ASA, PGE1, apyrase, monoclonal Abs

Use **antibiotics** e.g. aminoglycosides

CBC result of a 73-year-old male with cancer

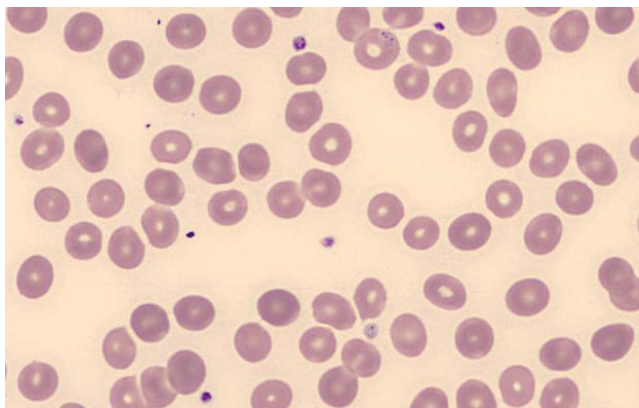
	21-03-14 06:26	21-03-14 09:07	21-03-14 11:07
Hemoglobin	7.4	7.5	7.7
Hematocrit	23.6	23.3	23.6
Rbc count	2.54	2.53	2.56
MCV	92.9	92.1	92.2
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Red cell distribution RDW	16.4	16.6	16.6
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EDTA blood



Platelet count 14,000/ μ L

Na citrate blood



Platelet count 183,000/ μ L

In case of citrated blood analysis, **x 1.1** to
RBC count, Hb, Hct, WBC count, absolute WBC count

Kanamycin supplement



1.5 mg K₂EDTA:15 mg of kanamycin

87.5%: platelet recovery to over 100,000/ μ L

93.8%: no serious abnormal PLT histogram

Vortex to disaggregate platelet clumps

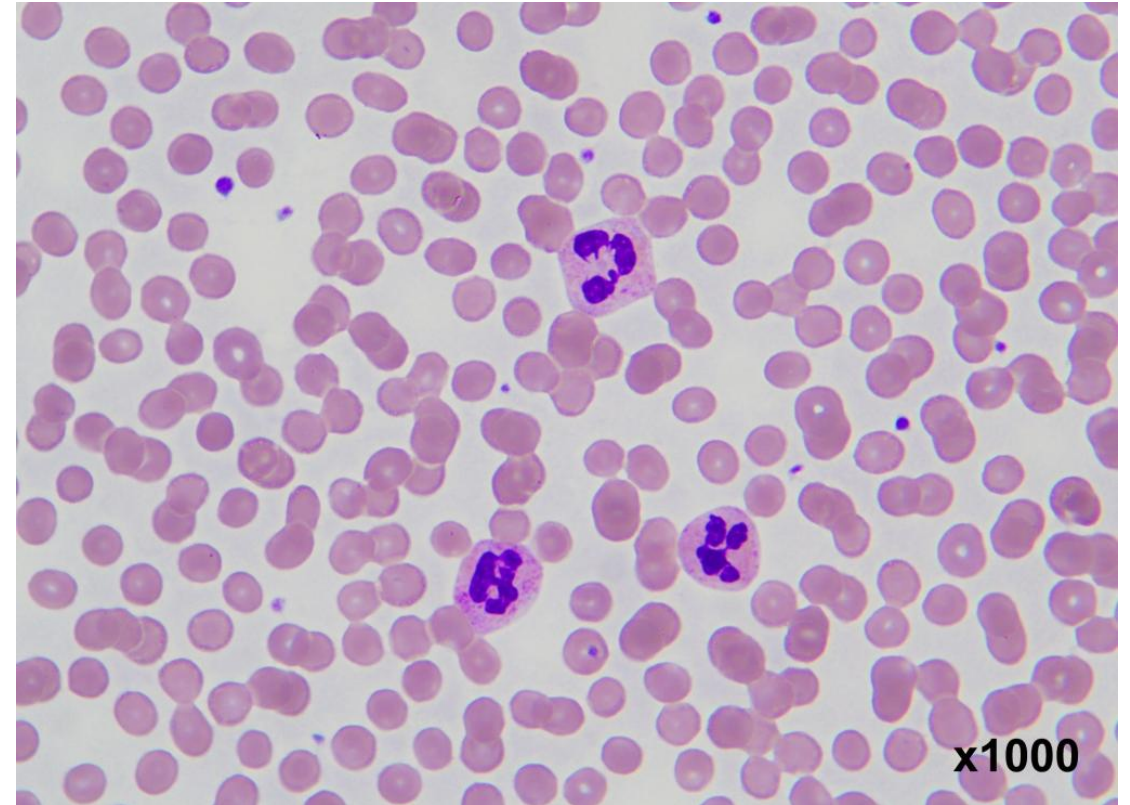
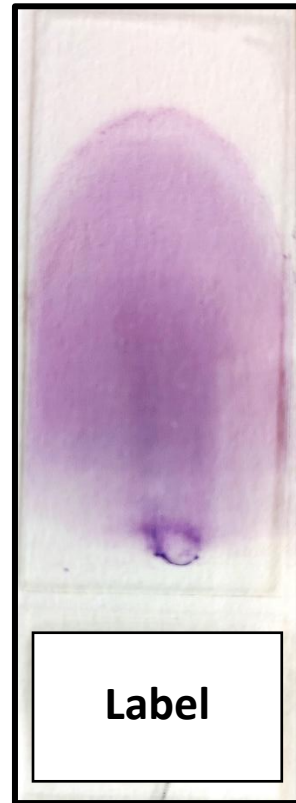


Specimens from 48 patients with **EDTA-PTCP**

At 1,500 RPM for 2 minutes

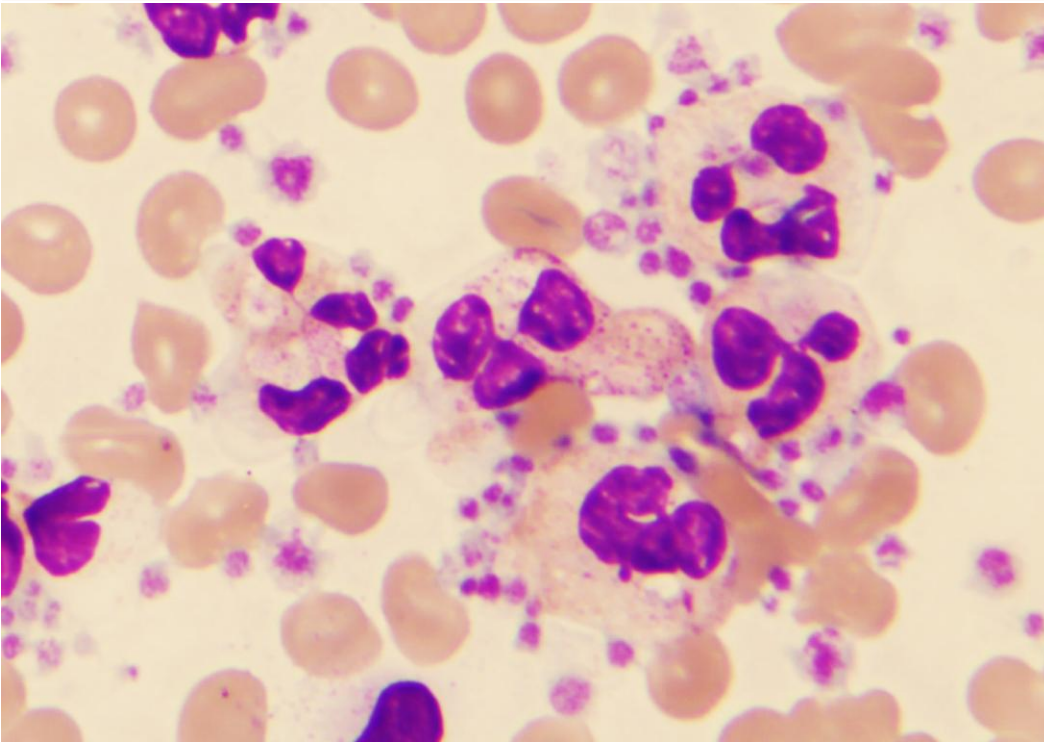
39.6%: platelet recovery to over 100,000/ μ L

Use finger-prick blood



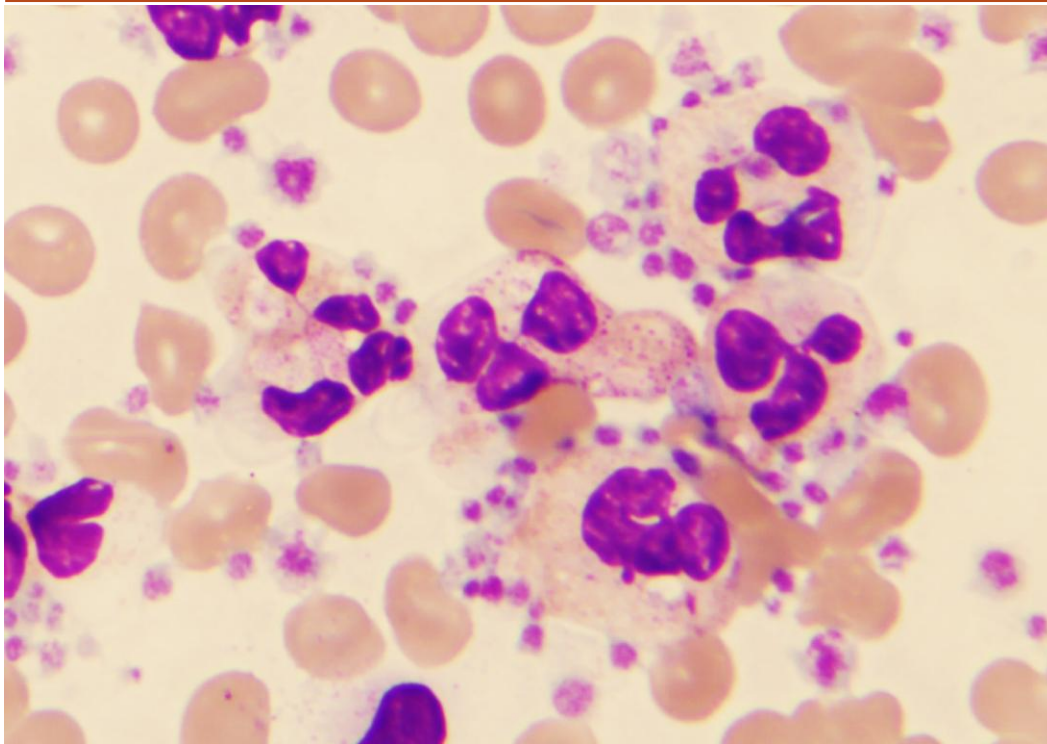
Other causes of falsely low automated platelet count

**Platelet satellitism
(satellitosis)**

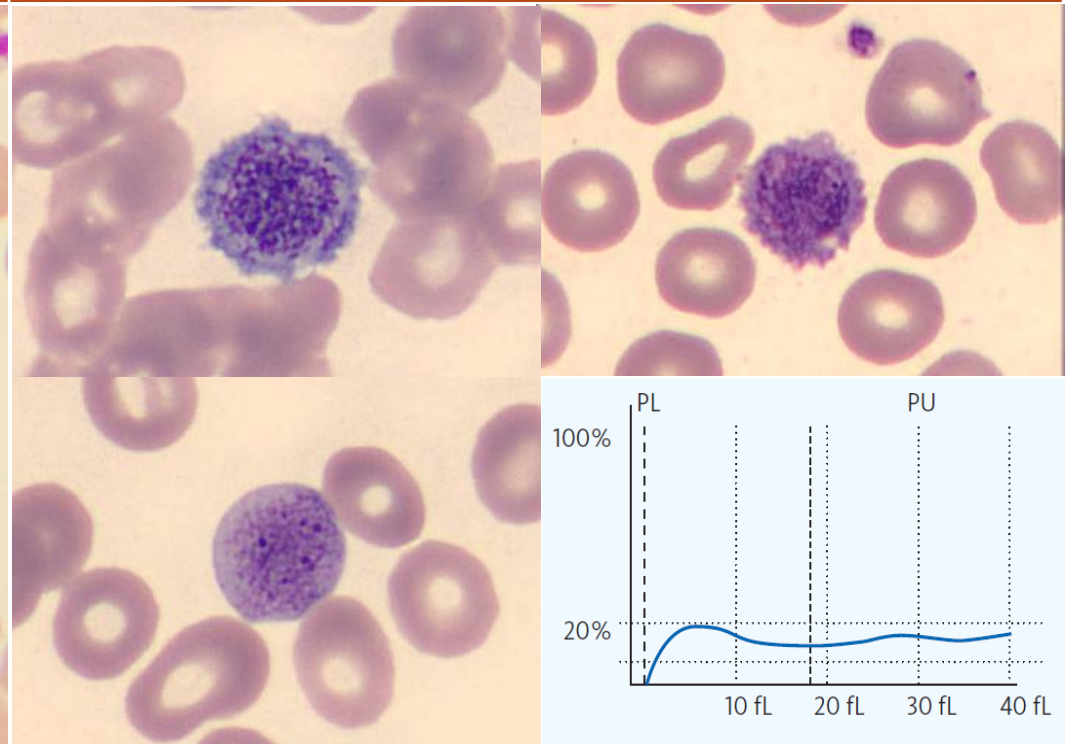


Other causes of falsely low automated platelet count

**Platelet satellitism
(satellitosis)**



Giant platelets



Sysmex Xtra Online | June 2011.

Spurious (false) platelet counts

Falsely low

Falsely high

Case scenario 2

A 1-year-old girl with biliary atresia and cirrhosis was admitted with high grade fever and diarrhea.

The diagnosis was infectious diarrhea with *Klebsiella pneumoniae* septicemia.

Case scenario 2

During the admission, the patient developed **petechiae** and **bleedings** at the puncture and catheter sites.

Case scenario 2

Measurement Parameters			
Item	Data		Unit
WBC	3.49		$10^3/\mu\text{L}$
RBC	3.42		$10^6/\mu\text{L}$
HGB	8.5		g/dL
HCT	25.7	-	%
MCV	75.1	-	fL
MCH	24.9	-	pg
MCHC	33.1		g/dL
PLT	217	*	$10^3/\mu\text{L}$
RDW-SD	56.7	+	fL
RDW-CV	21.6	+	%
PDW	----		fL
MPV	----		fL
P-LCR	----		%
PCT	----		%

Measurement Parameters			
Item	Data		Unit
NEUT#	2.25		$10^3/\mu\text{L}$
LYMPH#	0.81	-	$10^3/\mu\text{L}$
MONO#	0.28		$10^3/\mu\text{L}$
EO#	0.12		$10^3/\mu\text{L}$
BASO#	0.03		$10^3/\mu\text{L}$

Item	Data		Unit
NEUT%	64.5		%
LYMPH%	23.2		%
MONO%	8.0		%
EO%	3.4		%
BASO%	0.9		%

Flag(s)

	Ref Range	Units	08-03-14 07:17 N/A
PT	11 - 13	sec.	*16.9
APTT	23 - 32	sec.	◆85.5 
Fibrinogen	200.0 - 400.0	mg/dl	197.1
D-dimer	< 500	ug/L	7122.66

ISTH Scoring System for overt DIC

Does the patient have an underlying disorder associated with overt DIC?

If yes: proceed

If no: do not use this algorithm

Score the test results

1. Platelet count

(> 100x10⁹/L = 0, < 100x10⁹/L = 1, < 50x10⁹/L = 2)

2. Elevated fibrin marker (e.g. FDPs, D-dimer) 

(no increase = 0, moderate increase = 2, strong increase = 3)

3. Prolonged PT 

(< 3 s = 0, > 3 but < 6 s = 1, > 6 s = 2)

4. Fibrinogen level

(> 1 g/L = 0, < 1 g/L = 1)

Calculate score

≥ 5 compatible with overt DIC: repeat score daily

< 5 suggestive for non-overt DIC: repeat next 1–2 d

Case scenario 2

Measurement Parameters			
Item	Data		Unit
WBC	3.49		10 ³ /uL
RBC	3.42		10 ⁶ /uL
HGB	8.5		g/dL
HCT	25.7	-	%
MCV	75.1	-	fL
MCH	24.9	-	pg
MCHC	33.1		g/dL
PLT	217	*	10 ³ /uL
RDW-SD	56.7	+	fL
RDW-CV	21.6	+	%
PDW	----		fL
MPV	----		fL
P-LCR	----		%
PCT	----		%

Measurement Parameters			
Item	Data		Unit
NEUT#	2.25		10 ³ /uL
LYMPH#	0.81	-	10 ³ /uL
MONO#	0.28		10 ³ /uL
EO#	0.12		10 ³ /uL
BASO#	0.03		10 ³ /uL

Item	Data		Unit
NEUT%	64.5		%
LYMPH%	23.2		%
MONO%	8.0		%
EO%	3.4		%
BASO%	0.9		%

Flag(s)

	Ref Range	Units	08-03-14 07:17
			N/A
PT	11 - 13	sec.	*16.9
APTT	23 - 32	sec.	◆85.5
Fibrinogen	200.0 - 400.0	mg/dl	197.1
D-dimer	< 500	ug/L	7122.66

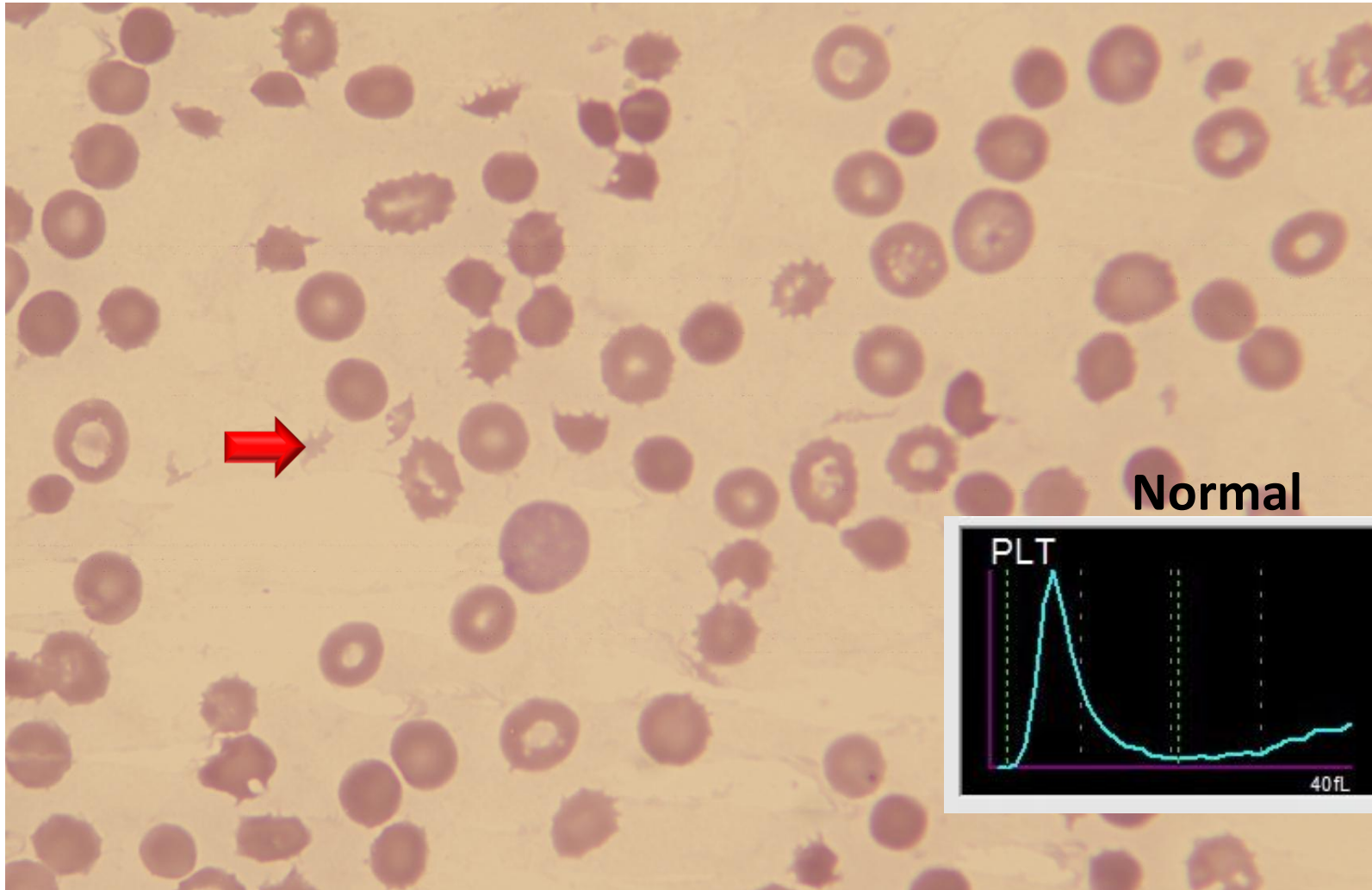
(ISTH DIC score = 4)

Case scenario 2

During the admission, the patient developed **petechiae** and **bleedings** at the puncture and catheter sites.

After the appropriate transfusion of fresh frozen plasma, the patient still had **mucocutaneous bleeding**.

Case scenario 2

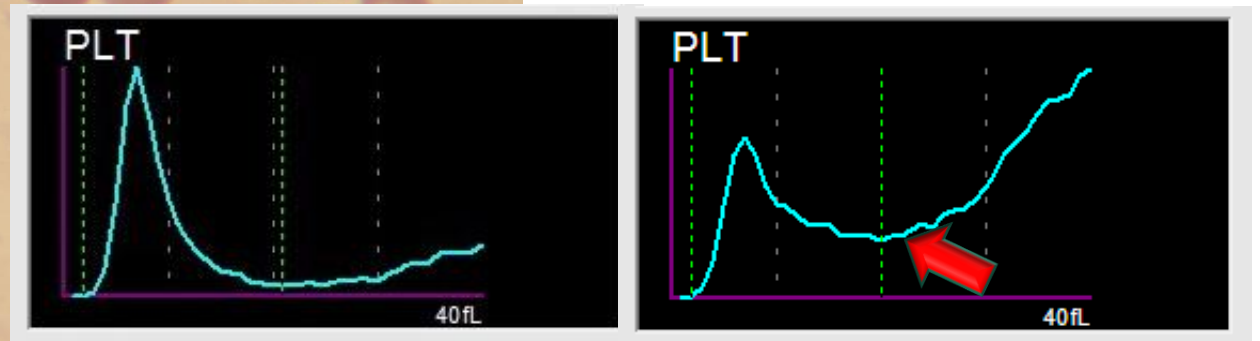


MAHA blood picture

Fragmented RBC

Normal

Patient



Case scenario 2

Types of analyzers	Principles of analyzers	Platelet counts (/μL)	Important flags
Analyzer A	Electrical impedance (PLT-I)	193,000	PLT Abn Distribution
	Optical fluorescent (PLT-O)	115,000	Microcytosis, Fragments? PLT Abn Distribution
	Optical fluorescent (PLT-F)	49,000	Fragments? PLT Abn Distribution Thrombocytopenia
Analyzer B	Electrical impedance	174,100	Cellular interference H&H Check failed
Analyzer C	Optical detection	255,000	RBC MORPH
Analyzer D	Optical detection	144,000	RBC FRAG +

Case scenario 2

A 1-year-old girl with biliary atresia and cirrhosis with *Klebsiella pneumoniae* septicemia.

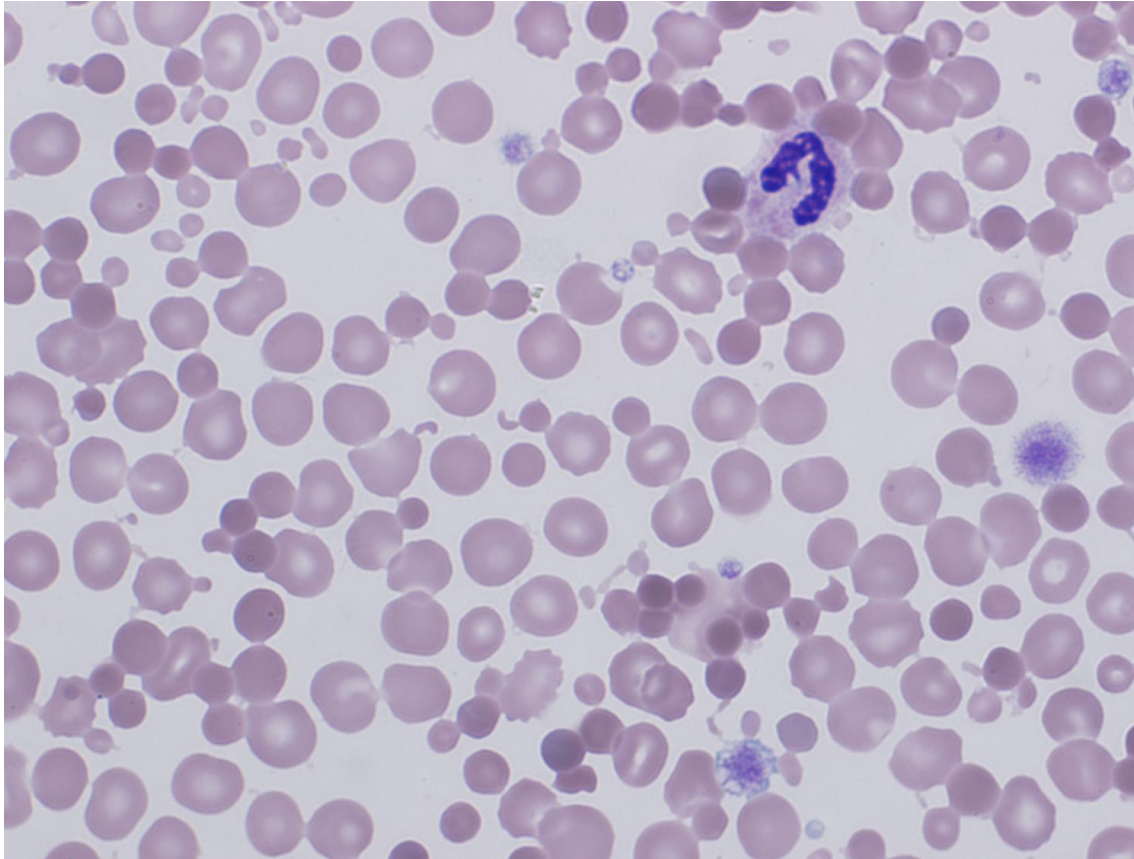
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PT	11 - 13	sec.	*16.9
APTT	23 - 32	sec.	◆85.5
Fibrinogen	200.0 - 400.0	mg/dl	197.1
D-dimer	< 500	ug/L	7122.66

Actual platelet count = 49,000/ μ L

ISTH DIC score = 6

The diagnosis is overt DIC

A 32-year-old male with severe burn



CBC

Item	Data	Unit
WBC	45.11 +	$10^3/\mu\text{L}$
RBC	6.24 *	$10^6/\mu\text{L}$
HGB	15.4	g/dL
HCT	45.0 *	%
MCV	72.1 *	fL
MCH	24.7 *	pg
MCHC	34.2 *	g/dL
PLT	1993 *	$10^3/\mu\text{L}$
RDW-SD	----	fL
RDW-CV	31.4 *	%
PDW	----	fL
MPV	----	fL
P-LCR	----	%
PCT	----	%
NRBC#	0.06	$10^3/\mu\text{L}$
NRBC%	0.1	%

RET

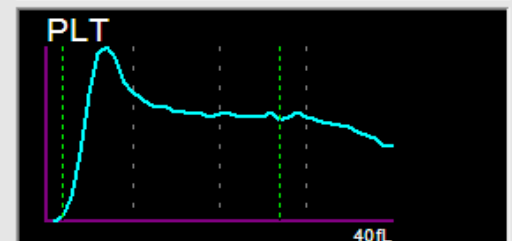
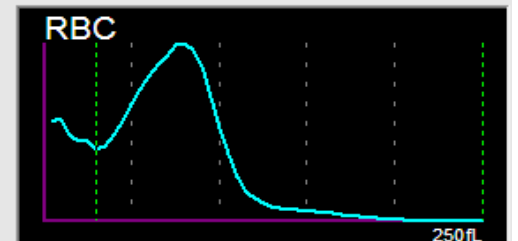
Item	Data	Unit
RET%		%
RET#		$10^6/\mu\text{L}$
IRF		%
LFR		%
MFR		%
HFR		%
RET-He		pg

RBC Flag(s)

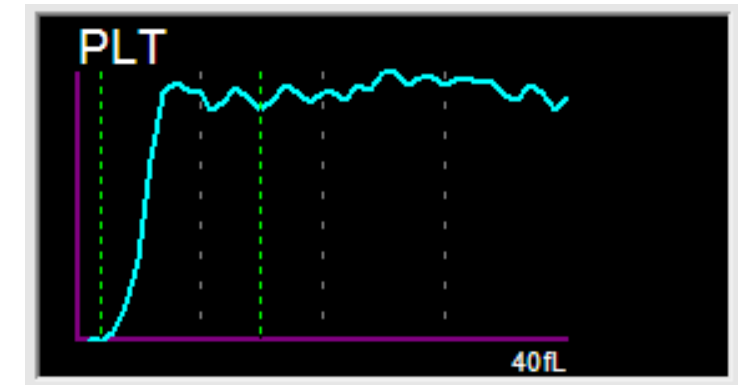
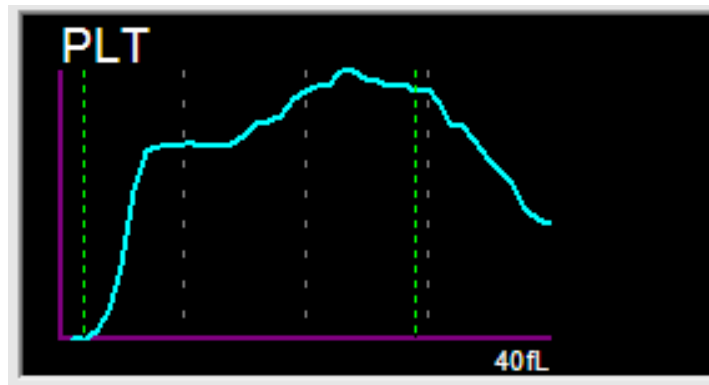
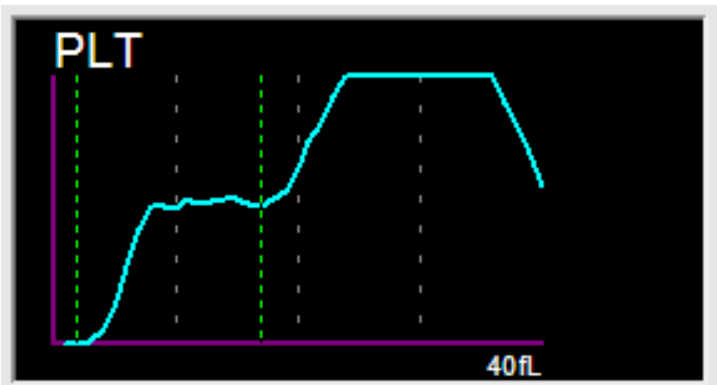
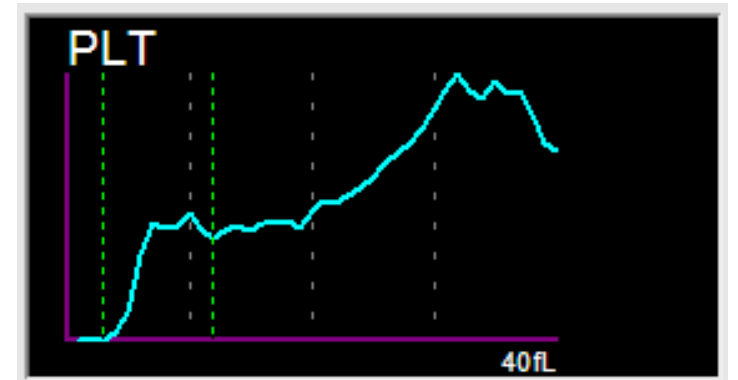
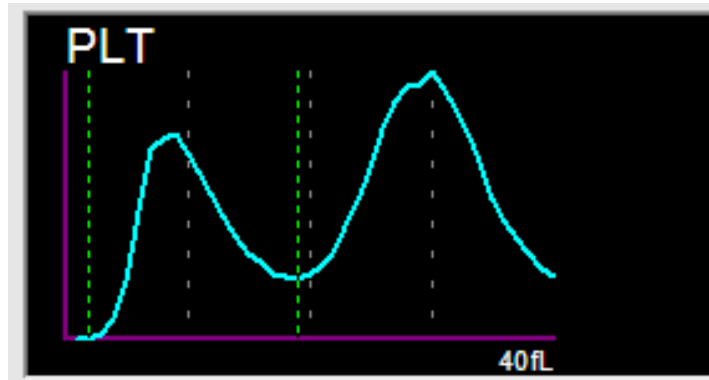
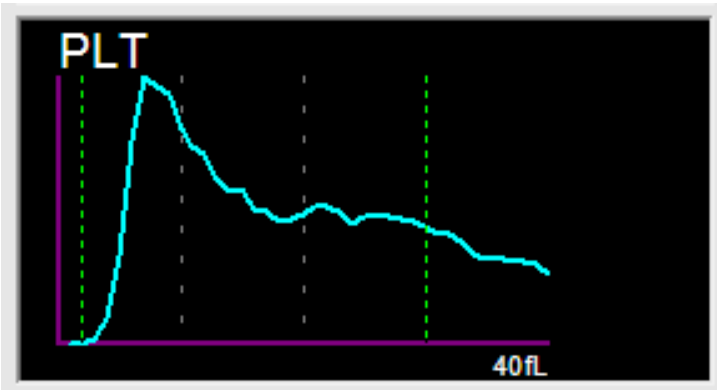
RBC Abn Distribution
Anisocytosis

PLT Flag(s)

PLT Abn Distribution
Thrombocytosis

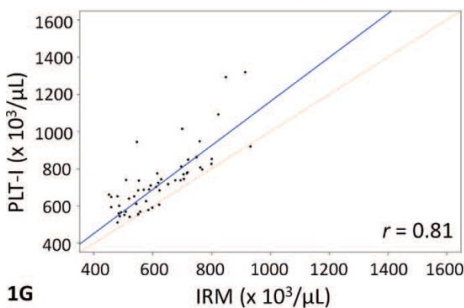
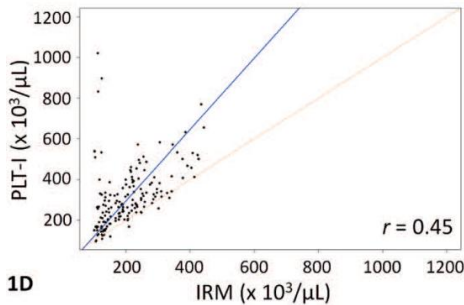
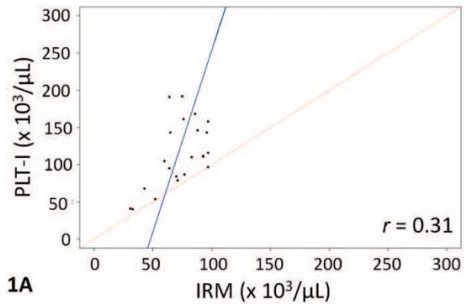


Examples of platelet histogram in thalassemia

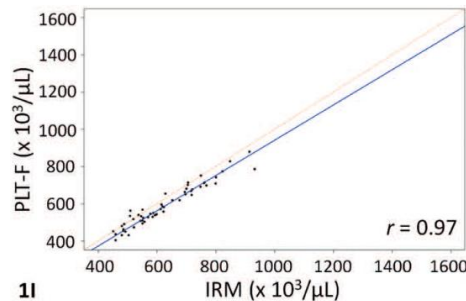
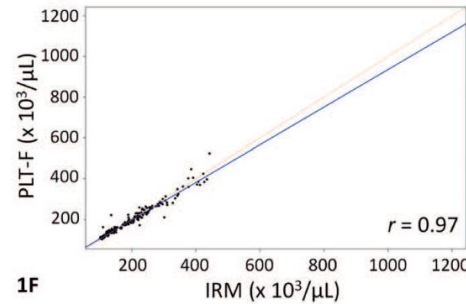
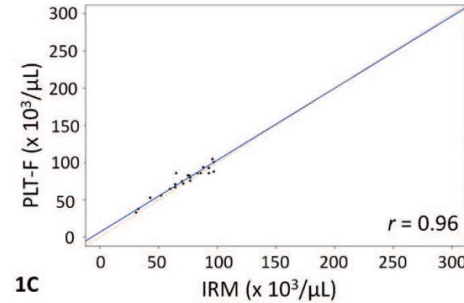


Spurious platelet count in thalassemia

Impedance



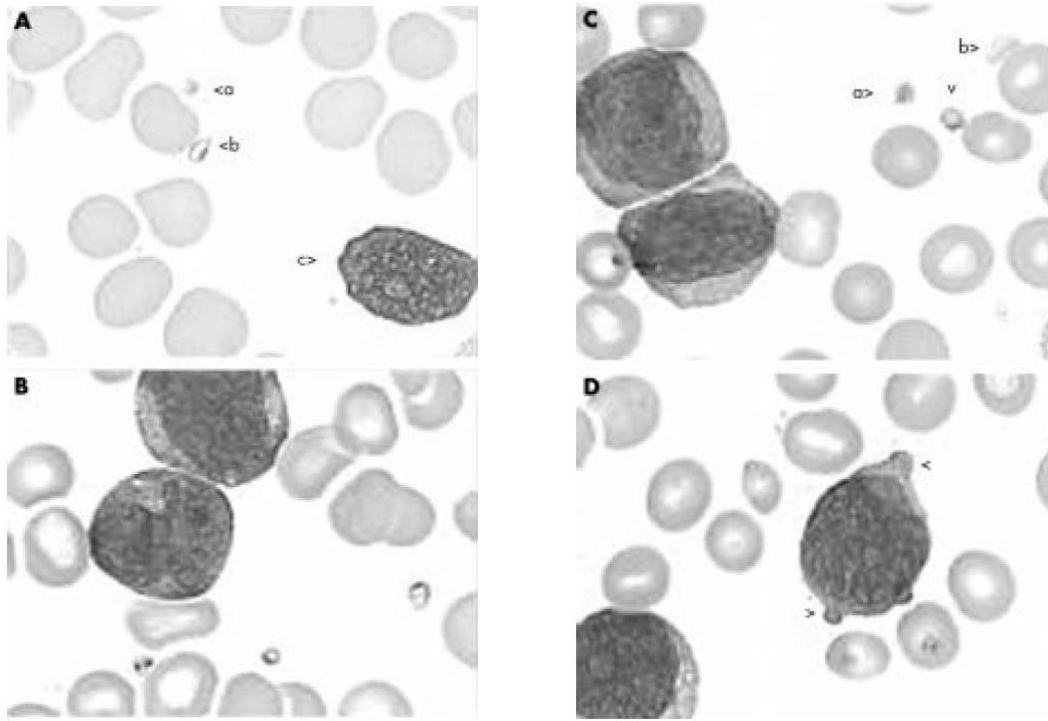
Optical fluorescence



High RDW-CV was an independent factor of inaccurate impedance platelet count

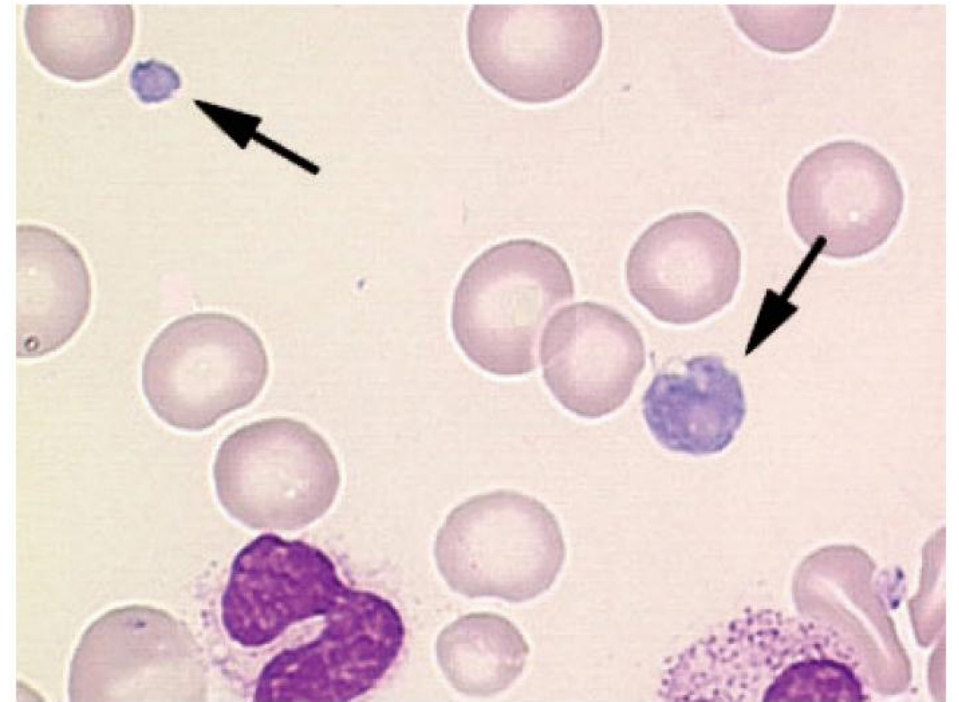
Other causes of falsely high automated platelet count

“Pseudoplatelets” in acute leukemia



van der Meer W, et al. J Clin Pathol 2003;56:772–4.

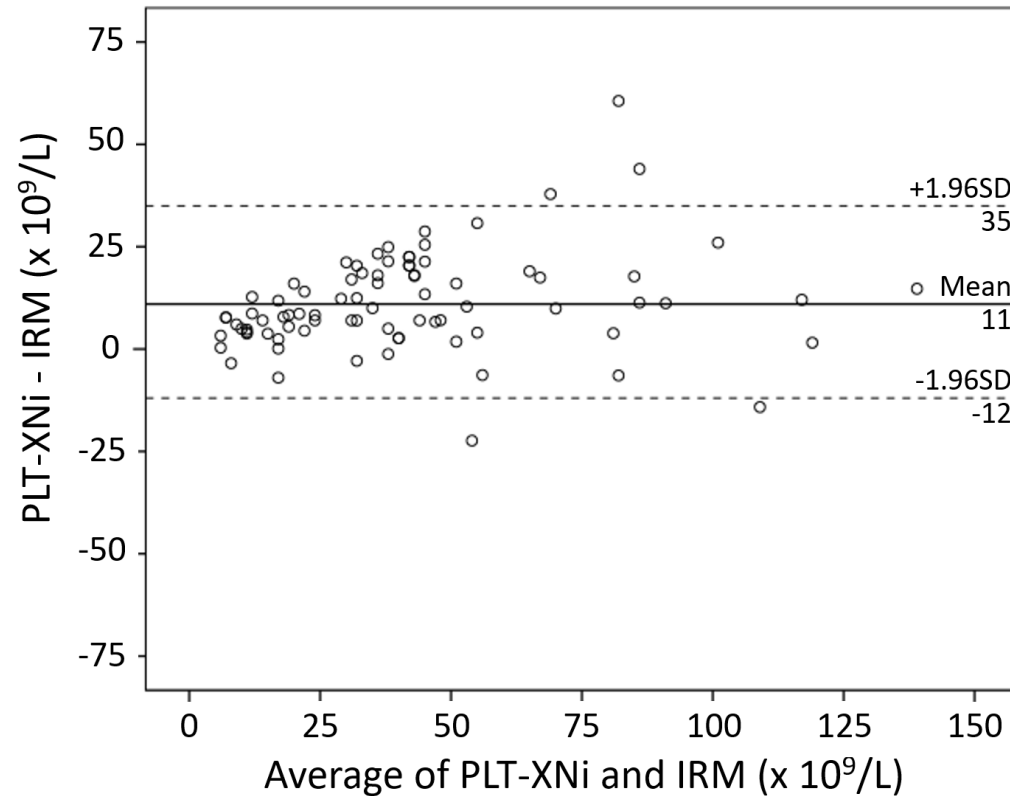
Fragments of lymphoma cells



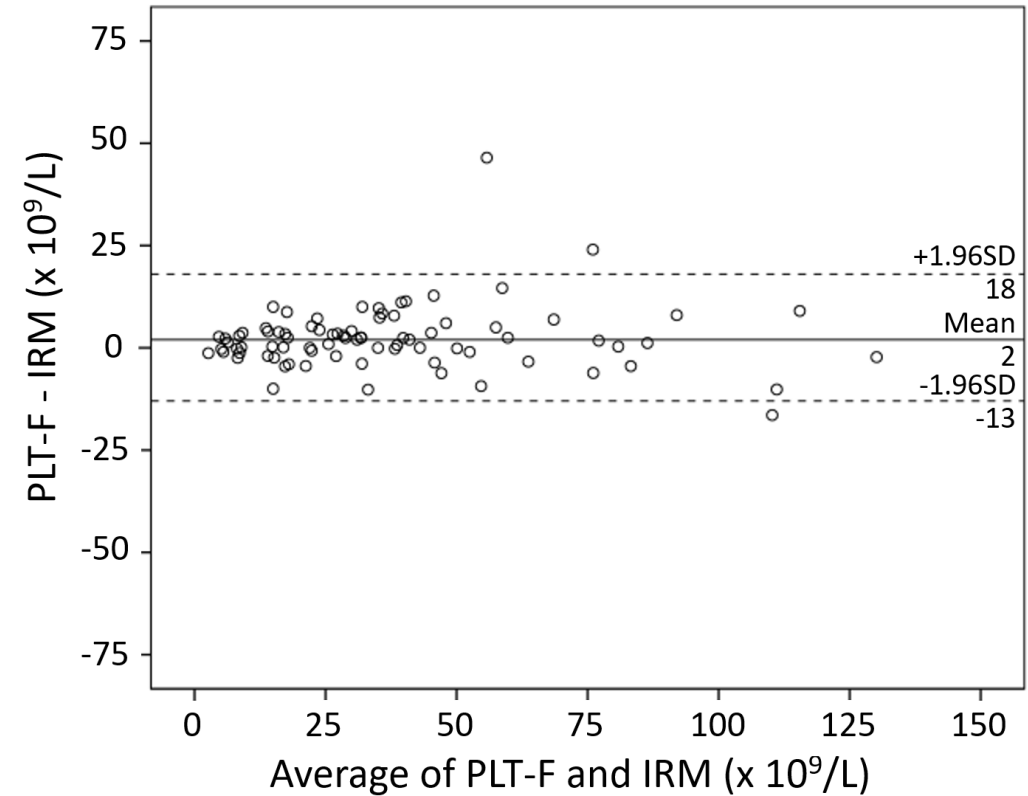
Zandecki M, et al. Int J Lab Hematol 2007; 29: 4-20.

Spurious platelet count in acute leukemia

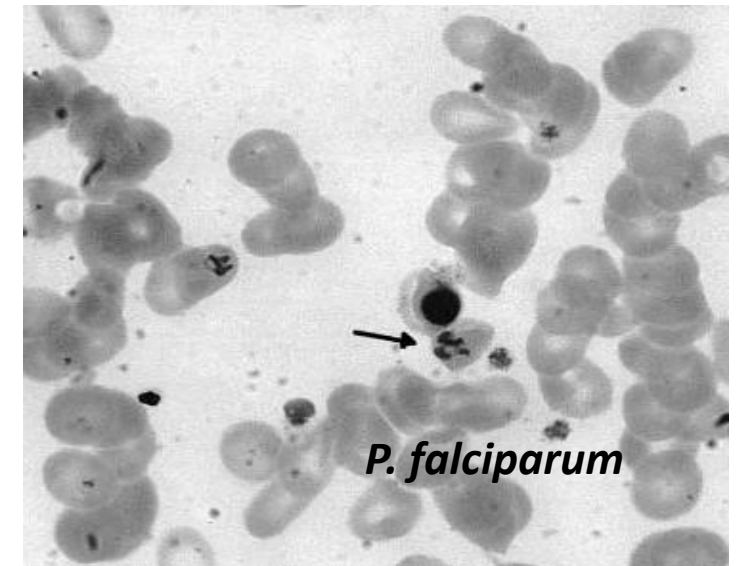
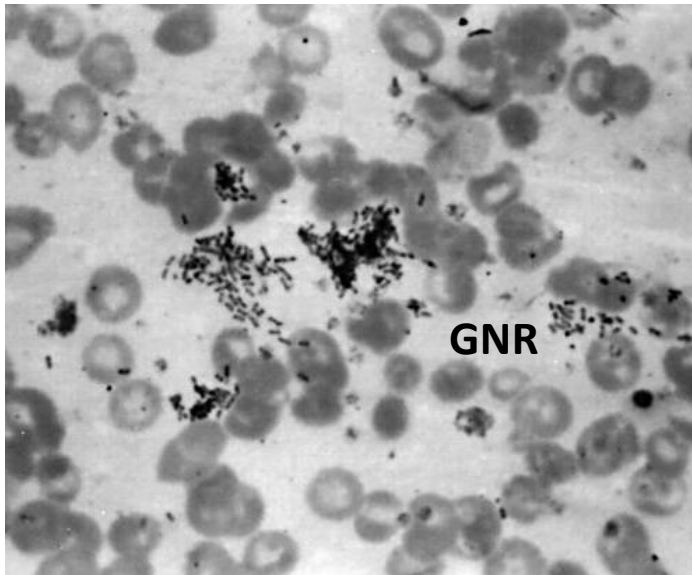
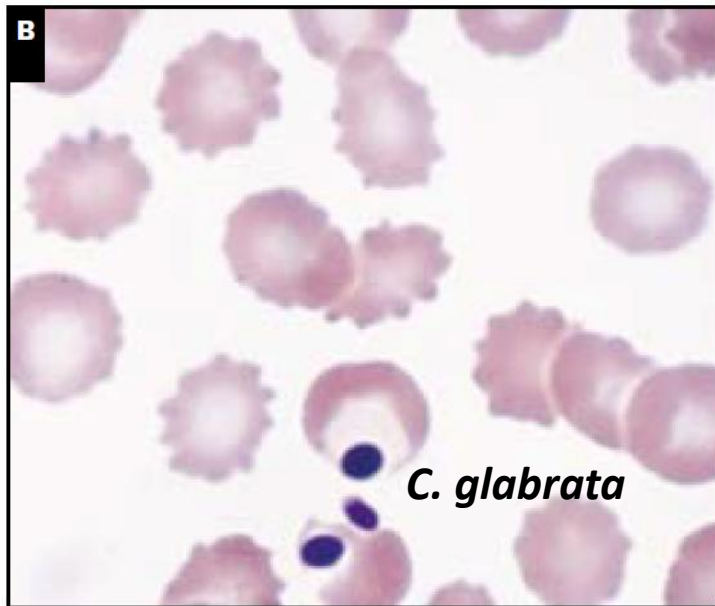
Impedance



Optical fluorescence



Other causes of falsely high automated platelet count



Latif S, et al. Am J Clin Pathol 2003;120:882-5.
Kakkar N. J Clin Pathol 2004;57:1096-7.
Crabbe G et al. Clin Lab Haematol 2002;24:179-82.

Take Home Message

Although methods of platelet enumeration has been continuously developed, spurious results still occur

Careful blood smear examination is essential

Improved novel technology and algorithm of analysis may reduce an inaccurate platelet measurement

Discussion

