There Must Be a Reason Why It Is on the Hematology Printout: Exploration of RET-He (CHr)

Valerie Everard-Gigot, PhD, MLS(ASCP)

Wisconsin Clinical Laboratory Network Presentation

RETICULOCYTE HEMOGLOBIN

Presentation Goals

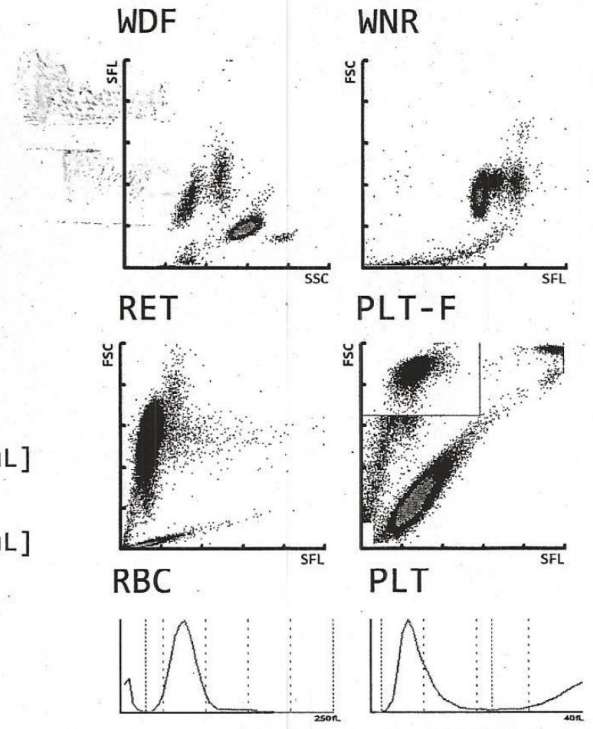
Overview:

- ✓ Definition
- ✓ Methodology
- ✓ Significance
- ✓ Limitations

Hematology Printout

Complete Blood Count (CBC)

WBC	7.90	[10^3/uL]			
RBC	3.44 -	[10^6/uL]			
HGB	7.8 -	[g/dL]			
HCT	25.7 -	[%]			
MCV	74.7 -	[fL]			
MCH	22.7 -	[pg]			
MCHC	30.4 -	[g/dL]			
RDW-SD	47.7	[fL]			
RDW-CV	17.7 +	[%]			
PLT &F	437 +	[10^3/uL]	No Clot		
MPV	8.8	[fL]			
NEUT	5.63	[10^3/uL]	71.2	[%]	
LYMPH	1.19	[10^3/uL]	15.1	[%]	
MONO	0.88	[10^3/uL]	11.1	[%]	
EO	0.10	[10^3/uL]	1.3	[%]	
BASO	0.04	[10^3/uL]	0.5	[%]	
IG	0.06	[10^3/uL]	0.8 +	[%]	
NRBC	0.00	[10^3/uL]	0.0	[%]	
RET	0.96	[%]	0.0330 +	[10^6/uL]	
IRF	8.9	[%]			
RET-He	23.8 -	[pg]			
IPF	0.7 -	[%]	3.1	[10^3/uL]	
WBC-BF		[10^3/uL]			
RBC-BF		[10^6/uL]			
MN		[10^3/uL]		[%]	
PMN		[10^3/uL]		[%]	
TC-BF#		[10^3/uL]			



NEUT	
BAND	
LYMPH	
MONO	
EOS	
BASO	

WBC IP Message

META	
MYELO	
PRO	
BLAST	
ATYP	
NRBC	
OTHER	

MACRO	
MICRO	
HYPO	
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OVAL	
TEAR	
ANISO	

RBC IP Message

TARGET	
B STIP	
SCHISTO	
BURR	
ACANTHO	
SPHERO	
PAPPEN	

DOEHLE	
TOXIC G	
PLT EST	
TECH:	

PLT IP Message

Hematology Printout

Red Blood Cell Indices

Platelet Indices

WBC	7.90	[10^3/uL]		
RBC	3.44 -	[10^6/uL]		
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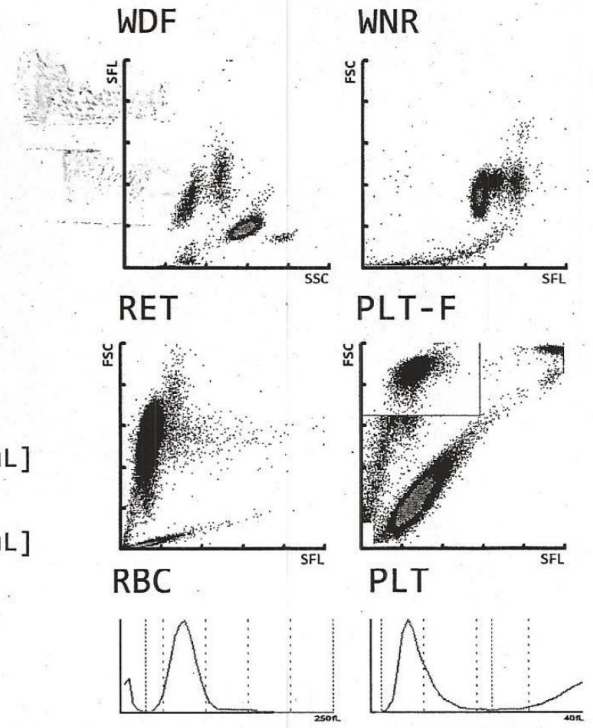
TC-BF#		[10^3/uL]		

No Clot



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Hematology Printout

Automated Differential

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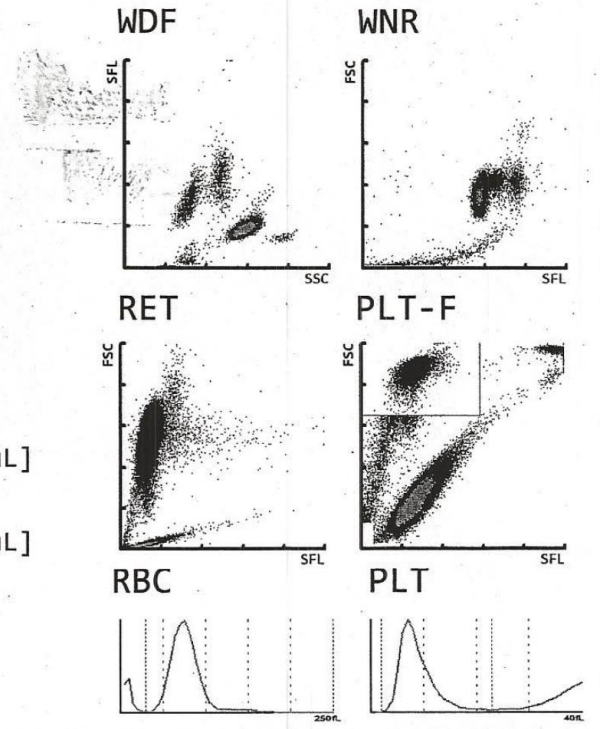

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Hematology Printout

Body Fluid Counts

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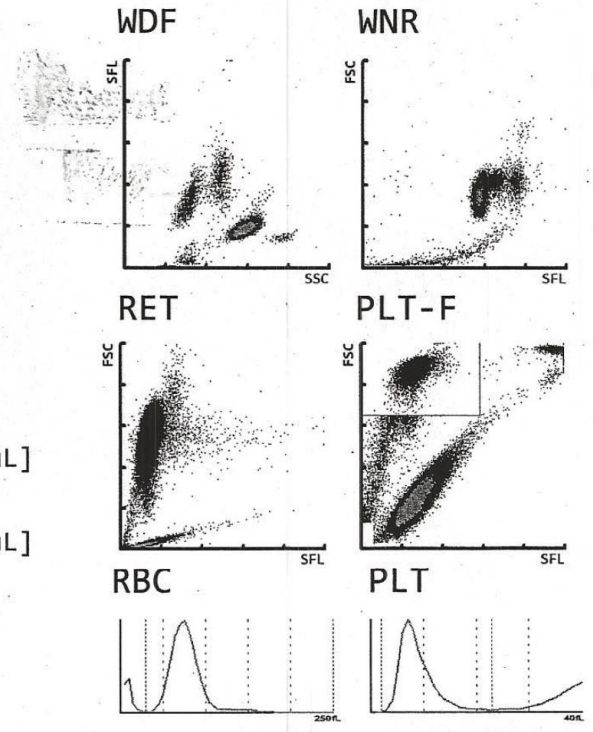

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PLT IP Message

Hematology Printout

IGNORE?!

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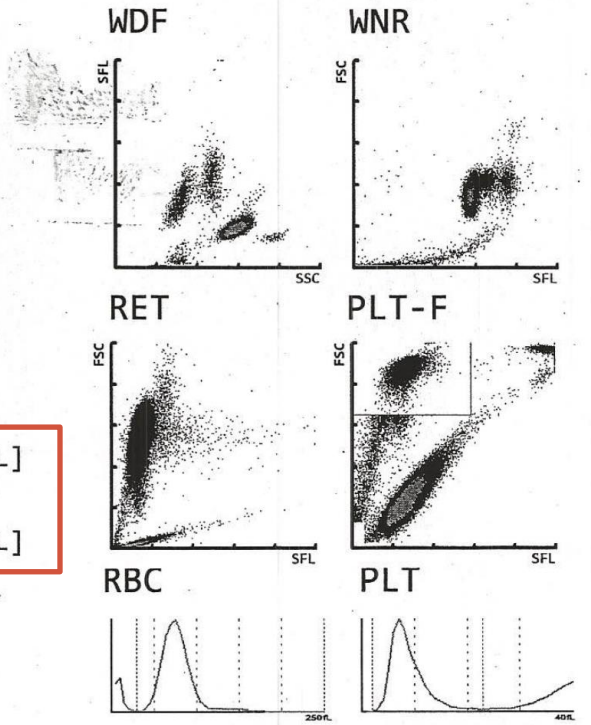

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Hematology Printout

Reticulocyte Count (#)

Immature Reticulocyte Fraction

Immature Platelet Fraction

WBC	7.90	[10^3/uL]			
RBC	3.44 -	[10^6/uL]			
HGB	7.8 -	[g/dL]			
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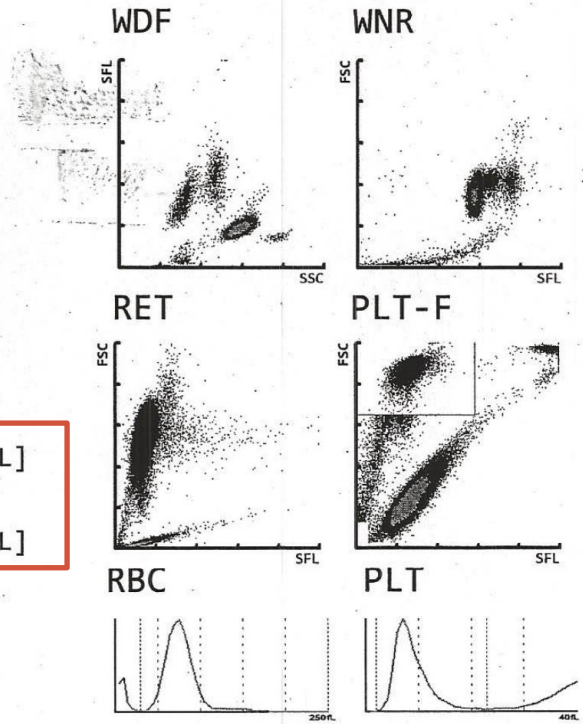

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WBC IP Message

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PLT IP Message

Hematology Printout

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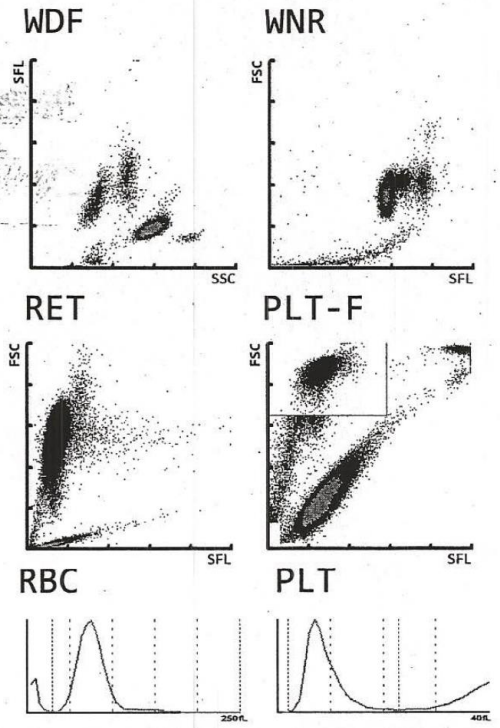

Reticulocyte Hemoglobin

NEUT	
BAND	
LYMPH	
MONO	
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WBC IP Message

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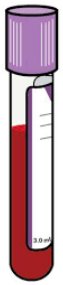
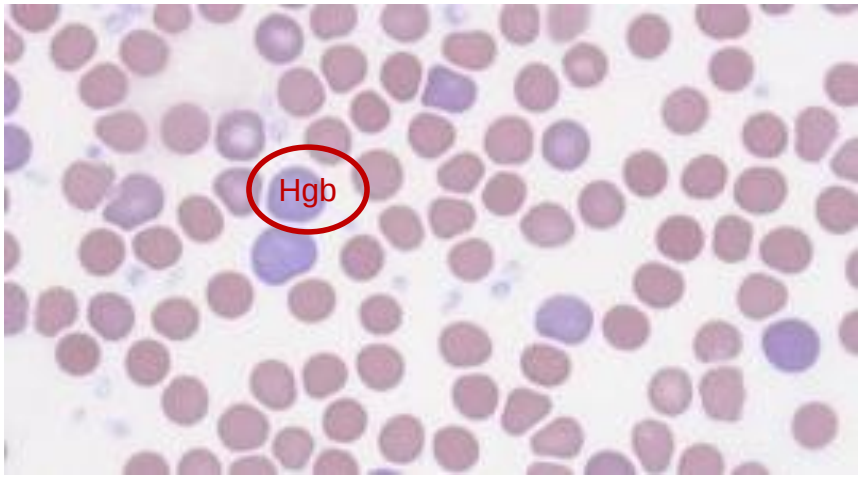
Blood Collection



Automated analysis of blood

Measurement of hemoglobin in reticulocytes (immature red blood cells)



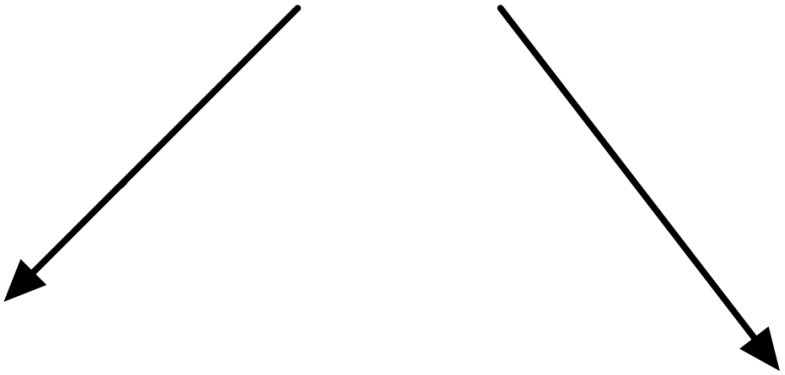
Why?

?

Image Sources: ASH Image Bank, Shutterstock

Reticulocyte Hemoglobin

INSTRUMENTATION



SIEMENS

CHr

- Advia
- Cellular hemoglobin mean for reticulocyte population
- ~1997

SYSMEX

RET-He

- XN Series
- Reticulocyte hemoglobin equivalent
- ~2005

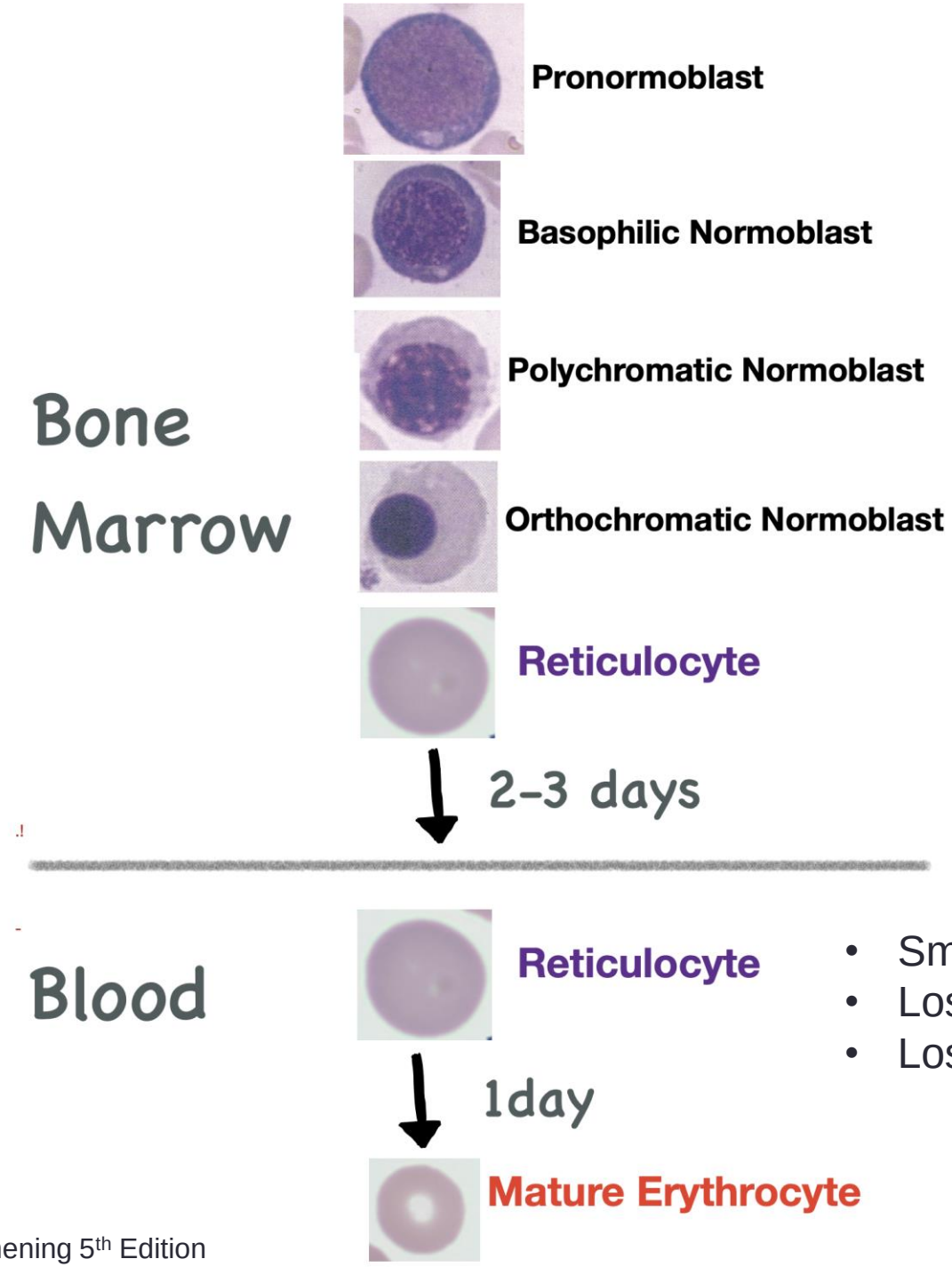
Results Correlate

RETICULOCYTE HEMOGLOBIN

Reticulocyte Production

Reticulocyte Hemoglobin Definition

Erythropoiesis

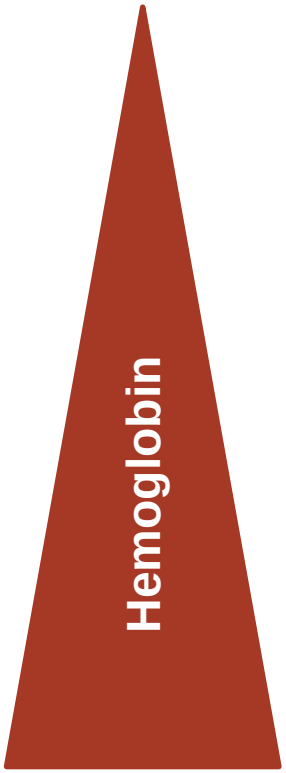


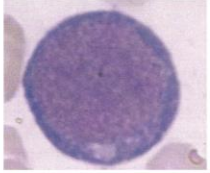
- Smaller
- Condensed chromatin
- Decreased RNA
- Decreased organelle
- Decreased DNA
- Increased Hgb

- Smaller
- Loss of residual RNA
- Loss of residual organelles

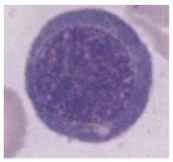
Images Source: Harmening 5th Edition

Erythropoiesis

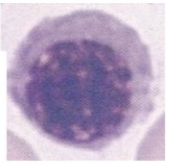




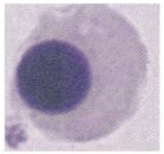
Pronormoblast



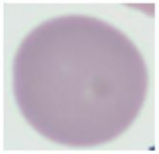
Basophilic Normoblast



Polychromatic Normoblast

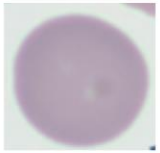


Orthochromatic Normoblast



Reticulocyte 8-10µm + Residual RNA

2–3 days

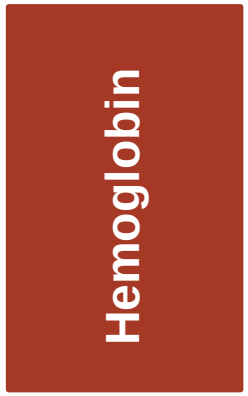


Reticulocyte 8-10µm + Residual RNA

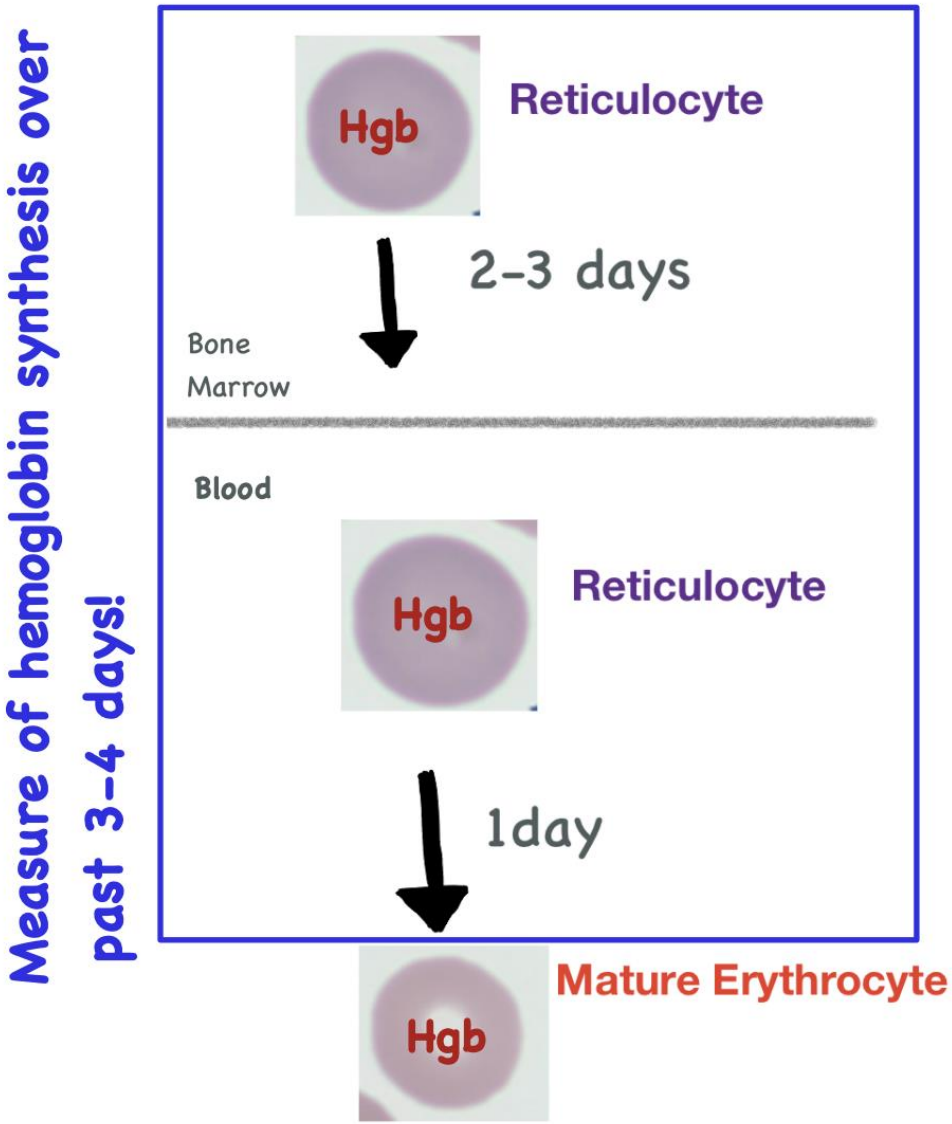
1day

Mature Erythrocyte 6-8µm

120 Day Lifespan

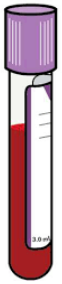


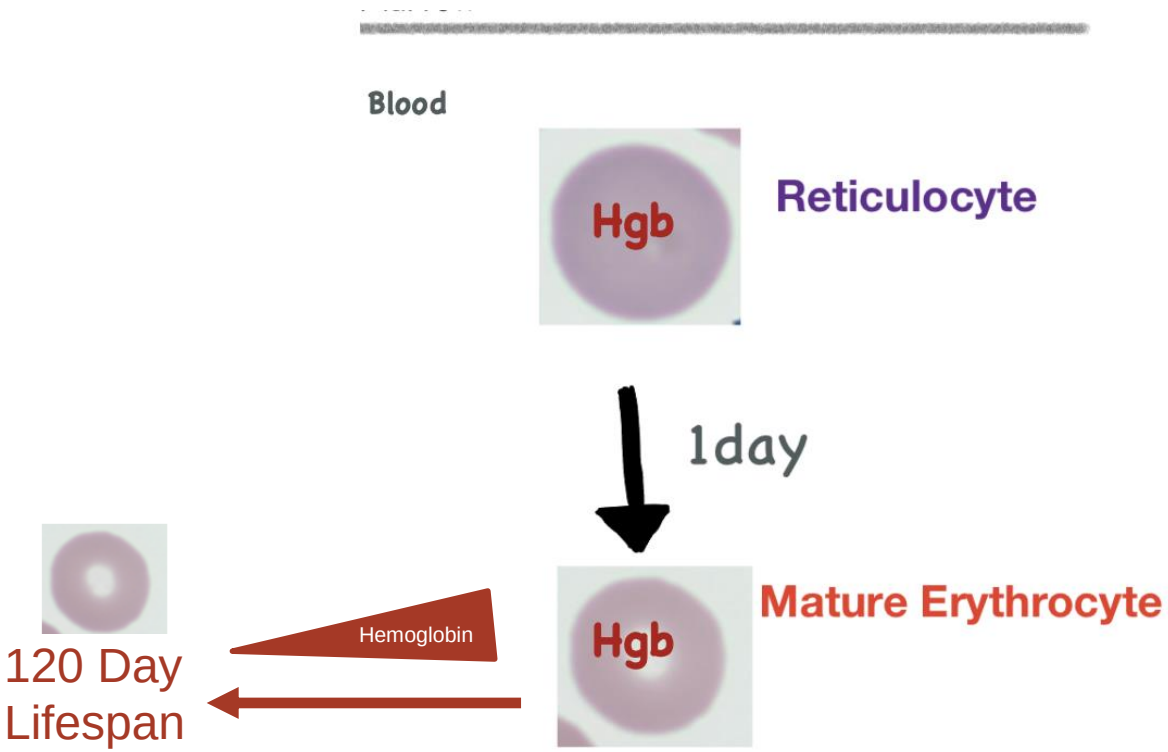
Reticulocyte Hemoglobin: Definition



Reticulocyte Hemoglobin: Definition

Why measure hemoglobin in reticulocytes?



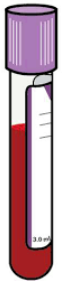


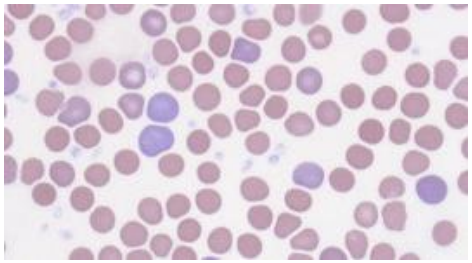
RETICULOCYTE HEMOGLOBIN

Methodology

Methodology

SIEMENS





ADD REAGENT

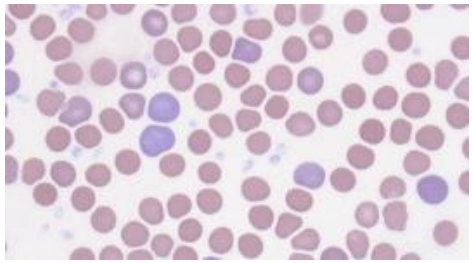
- Oxazine 750
- Buffer
- Zwitterionic Surfactant

MIXED + INCUBATED

Image Source: ASH Image Bank

Methodology

SIEMENS



Mature Erythrocyte Reticulocyte

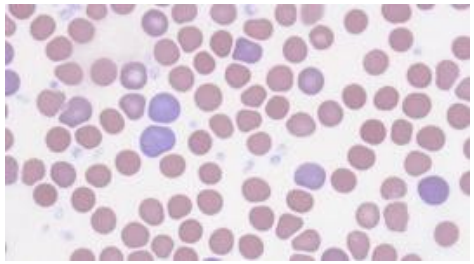
Red blood cells are spheroidized

Mature Erythrocyte Reticulocyte

Image Source: Shutterstock

Methodology

SIEMENS



Mature Erythrocyte

Reticulocyte

Surfactant permeabilizes cell membrane →
Oxazine 750 dye enters through pores →
Nucleic acid is stained blue-green

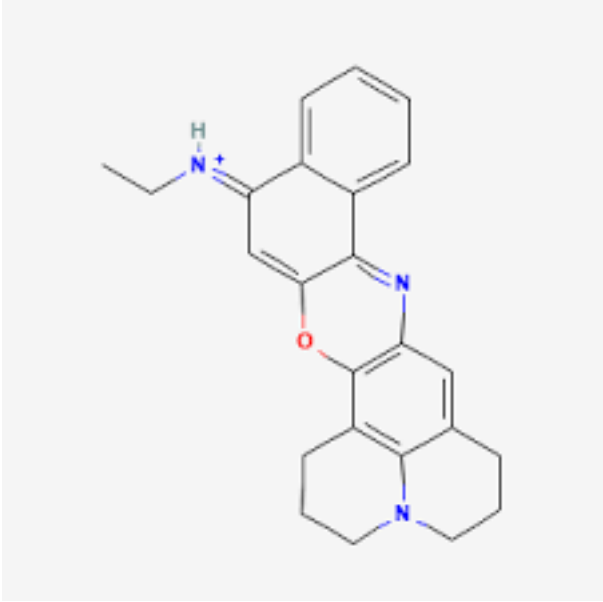
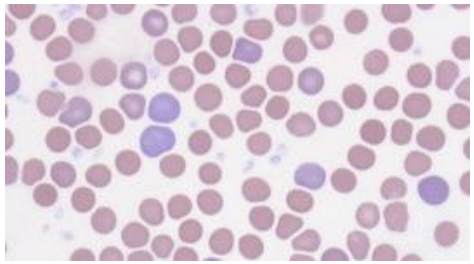
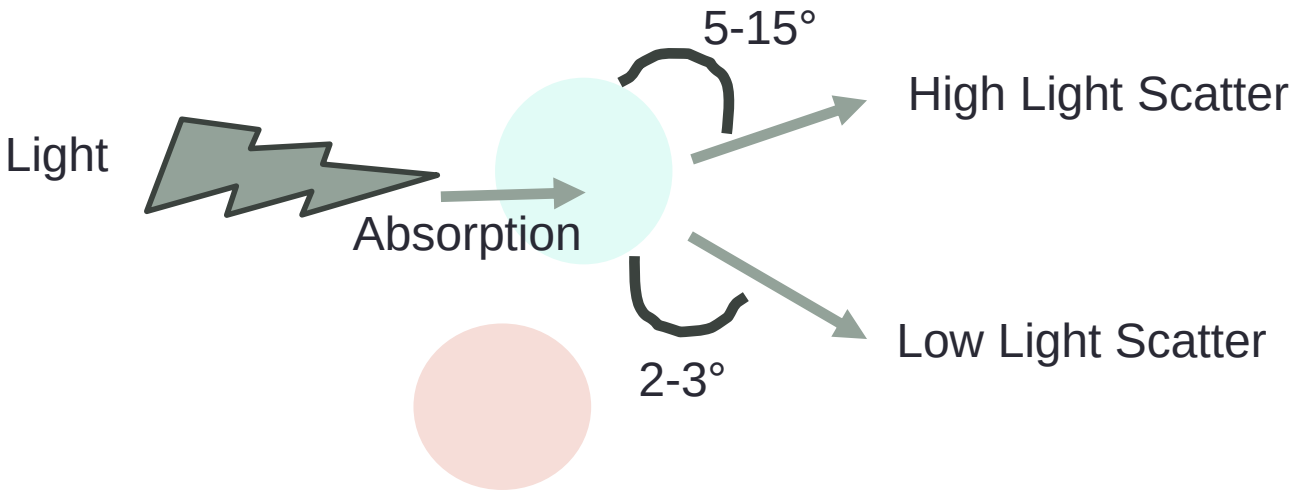


Image Sources: Pubchem; Shutterstock

Methodology

SIEMENS





Light Scatter

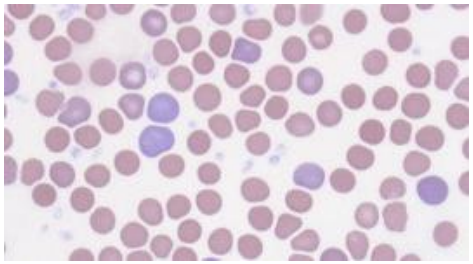
Proportional to cell size and hemoglobin concentration

Absorption

Proportional to RNA content

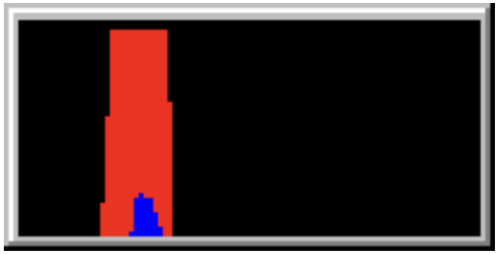
Methodology

SIEMENS



GRAPHED

RETIC-CH Histogram
(hemoglobin concentration per cell volume or hemoglobin mass)



Mature RBC population (red)

Reticulocyte population (blue)

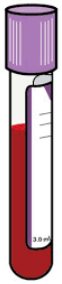
0 to 100pg

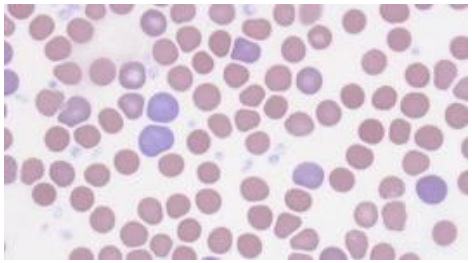
CALCULATED

CHr
Mean of the RETIC-CH Histogram for the reticulocyte population

Methodology

SYSMEX





ADD REAGENT

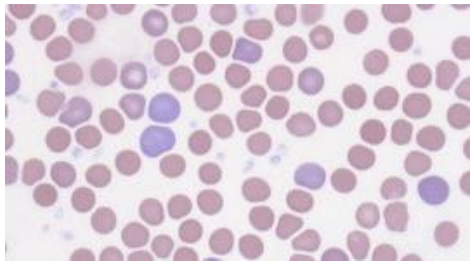
- Fluorescent Dye (Fluorocell RET; RET Search II)

MIXED + INCUBATED

Image Source: ASH Image Bank

Methodology

SYSMEX



Platelet

Mature Erythrocyte

Reticulocyte

Dye perforates cell membranes of intact cells →
Nucleic acids are fluorescently stained

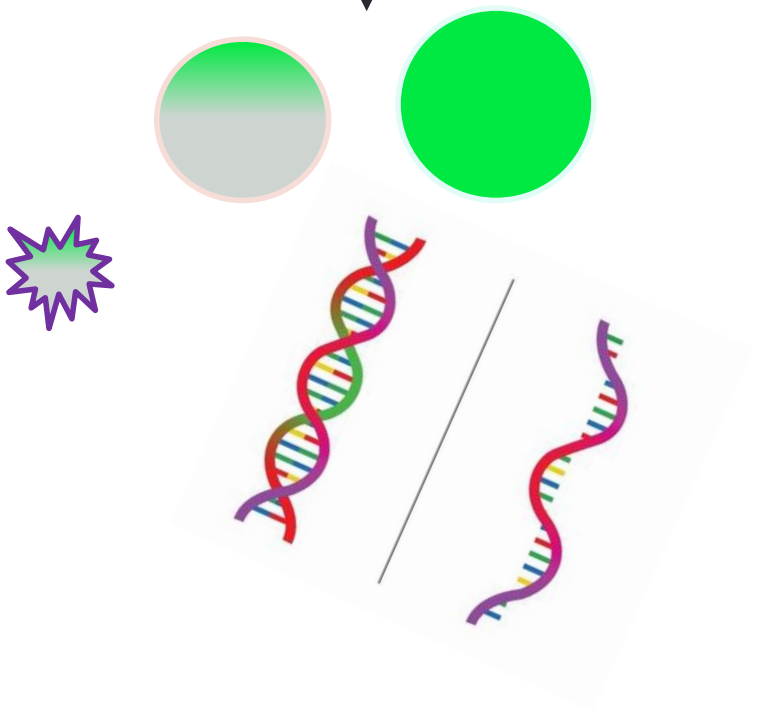
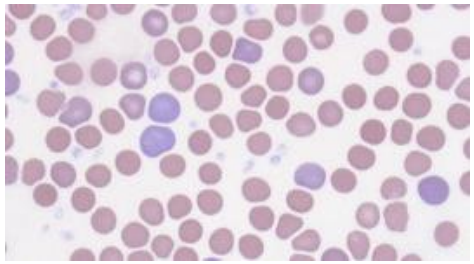
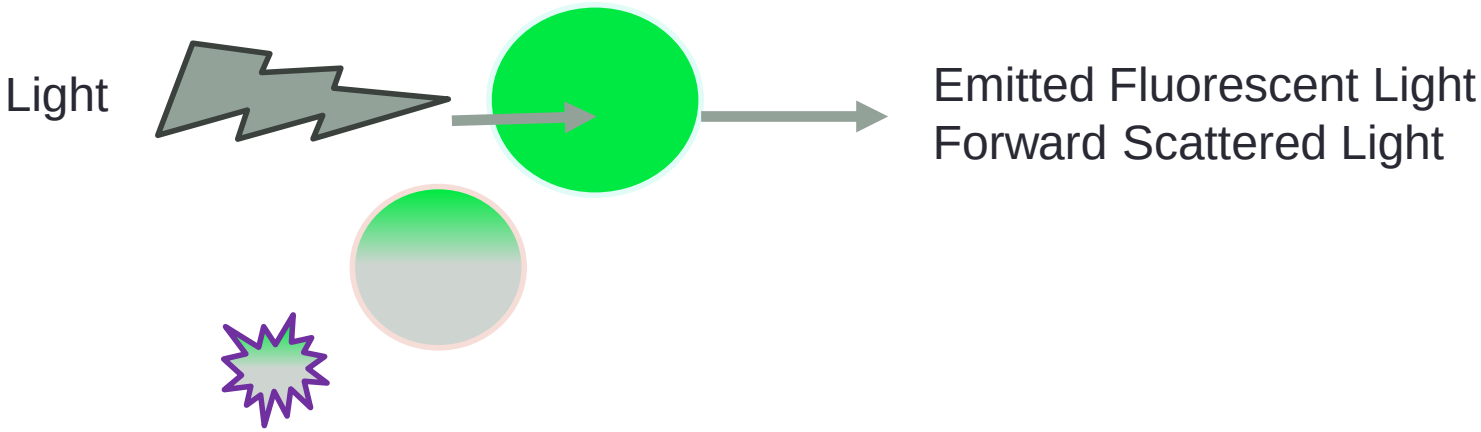


Image Source: Shutterstock

Methodology

SYSMEX



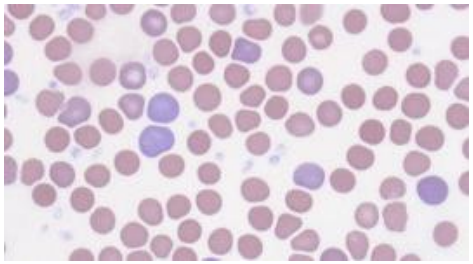


Emitted Light
Proportional to nucleic acid concentration

Forward Scatter
Proportional to size

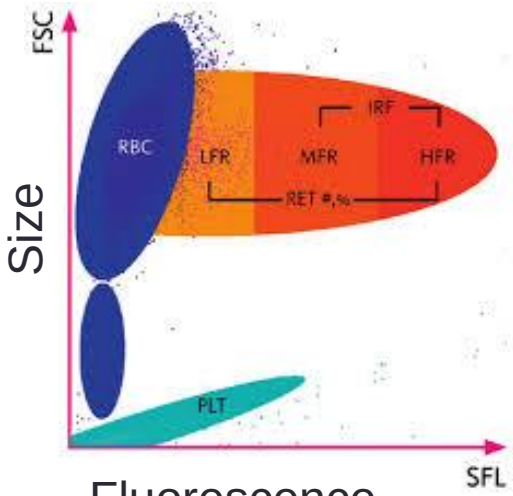
Methodology

SYSMEX



GRAPHED

RETIC CHANNEL



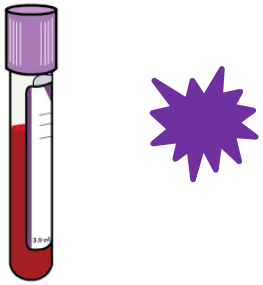
CALCULATED

RET-He

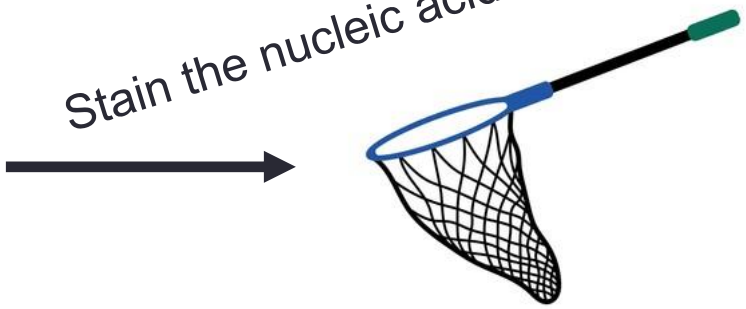
Algorithm estimates hemoglobin concentration based on light scatter

Reticulocyte Hemoglobin Equivalent Fact Sheet, (2025), *Sysmex*
Derived from *Sysmex* materials

Methodology



Stain the nucleic acids



Isolate the reticulocytes

Calculate hemoglobin concentration based on characteristics (light scatter, etc.)

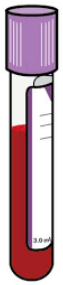
MCHC of Reticulocyte!!

Image Source: Shutterstock

RETICULOCYTE HEMOGLOBIN

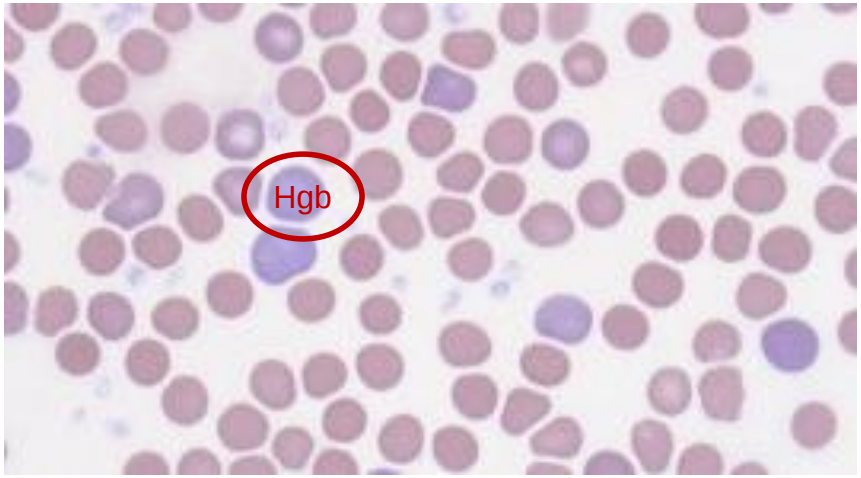
Significance

Blood Collection



Automated analysis of blood

Measurement of hemoglobin in reticulocytes (immature red blood cells)



Why?

?

Image Source: ASH Image Bank

Significance

1. Screen for iron deficiency

(which often leads to iron deficiency anemia → Screen for iron deficiency anemia)

2. Monitor iron treatment response in iron-deficient patients

Often better detects iron deficiency than traditional tests!

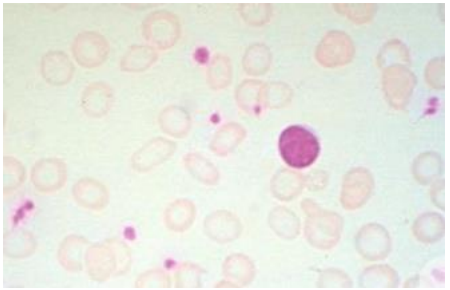
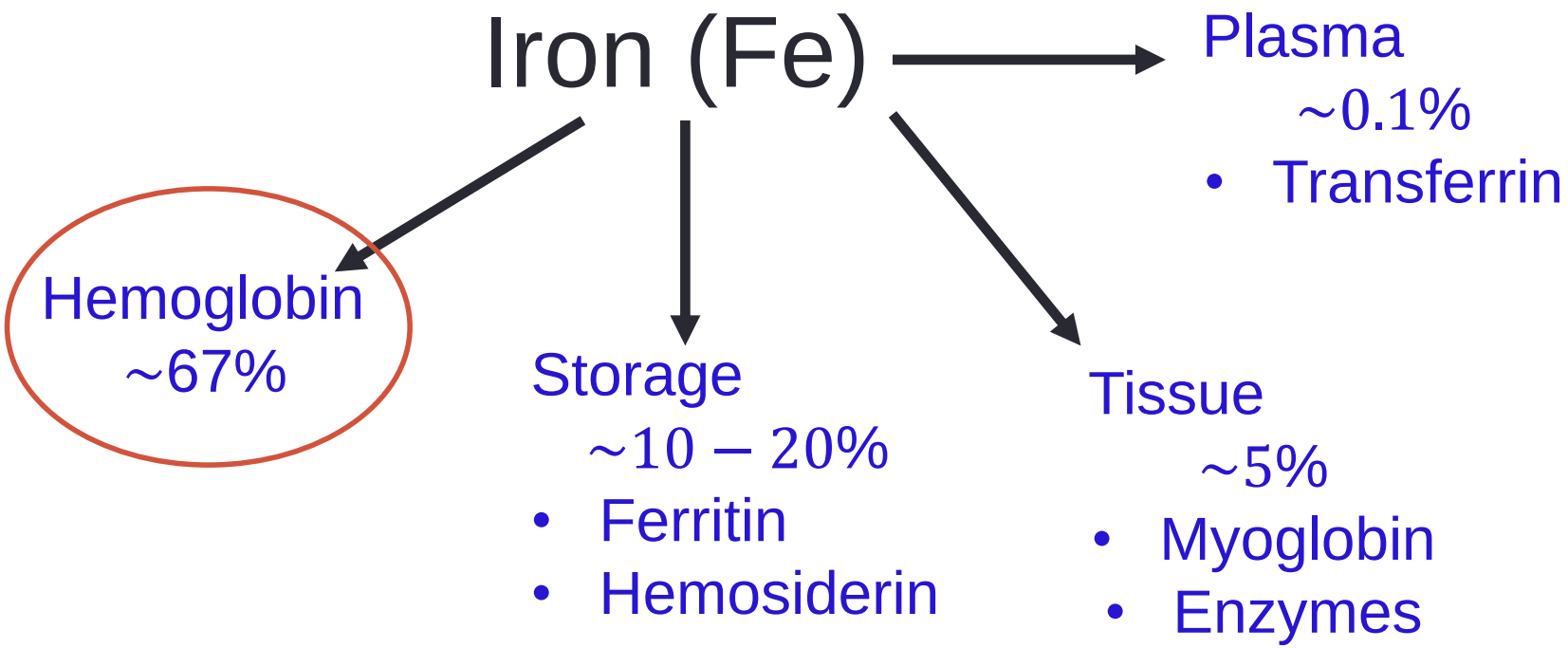


Image Sources: ASH Image Bank; Shutterstock

RETICULOCYTE HEMOGLOBIN

Iron Deficiency

Iron Metabolism



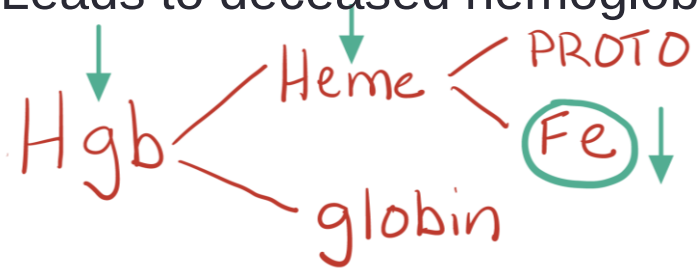
Iron Deficiency

- Characteristics

✓Major Causes

- Dietary
- Chronic Bleed

✓Leads to deceased hemoglobin production



✓Iron Deficiency Anemia

✓ Microcytic Hypochromic

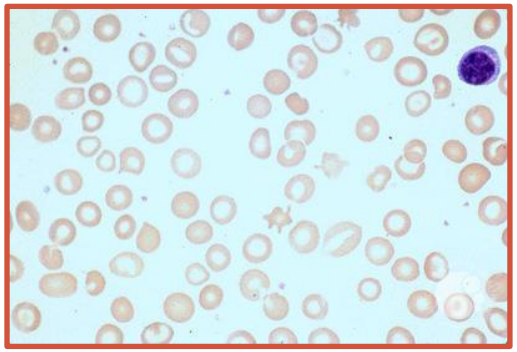
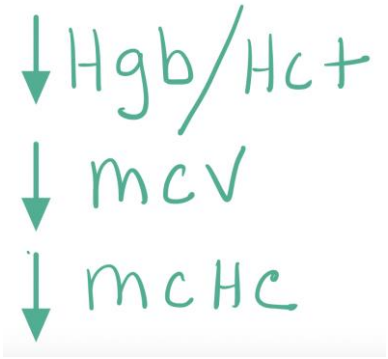


Image Source: ASH Image Bank

Iron Deficiency

- Traditional Laboratory Data

✓Microcytic Hypochromic Anemia



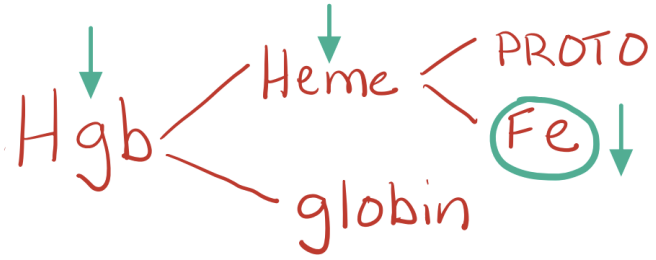
✓Altered Iron Panel

Serum Iron	TIBC	%SAT (TSAT)	Ferritin
↓	↑	↓	↓

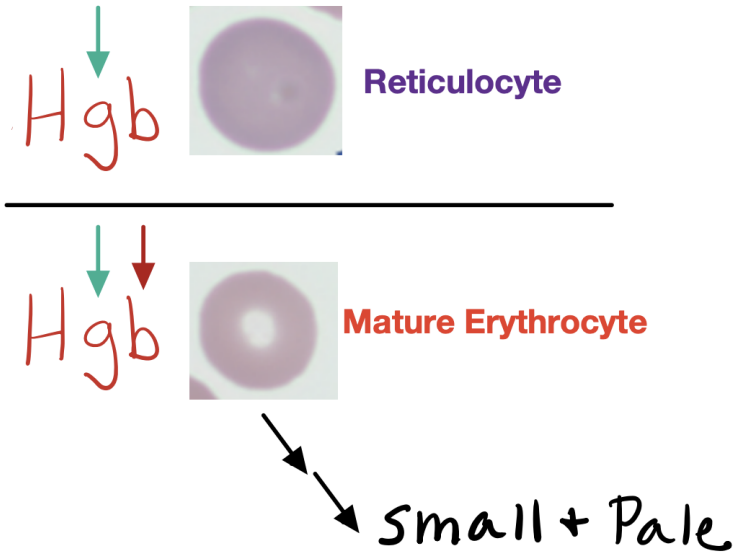
Iron Deficiency

- Newer Laboratory Data

✓Decreased reticulocyte hemoglobin (RET-He or CHr)



Download



Iron Deficiency

- Newer Laboratory Data – cont -

✓Decreased reticulocyte hemoglobin (RET-He or CHr)

↓ RET-He

↓ CHr

Normal	
29.3 – 35.4pg	Reticulocyte Hemoglobin Equivalent Fact Sheet, (2025), *Sysmex*
Mean 28.9pg	Advia 2120/2120i Operators Manual, (2010), *Advia*
Value associated with iron deficiency	
< 28pg (adult) < 26.0/27.5pg (infant)	Reviewed by Mast, A., et al, (2007) Reticulocyte Hemoglobin Content, *American Journal of Hematology*
< 32pg (cancer patients)	Peerschke, E., et al, (2014), Using Hemoglobin Content of Reticulocytes (RET-He) to Evaluate Anemia in Patients with Cancer, *Am J Clin Pathol*

< 28pg

RETICULOCYTE HEMOGLOBIN

Significance:

1. Screen for iron deficiency (iron deficiency anemia)

DATA ANALYSIS #1

Question

Which better detects iron deficiency:
reticulocyte hemoglobin or traditional iron panel testing?

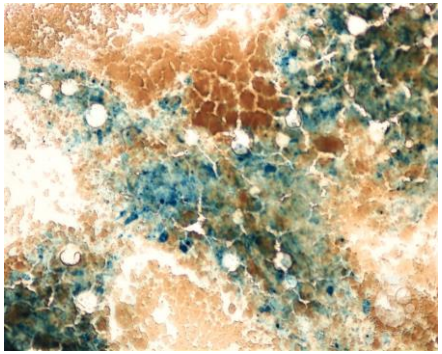
Answer

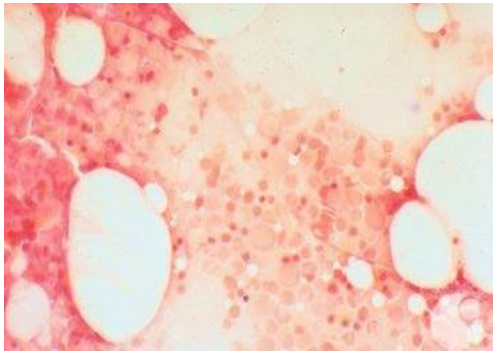
Reticulocyte hemoglobin

Reticulocyte Hemoglobin vs. Traditional Iron Panel Testing

Iron deficiency anemia

- Confirmed by bone marrow aspirate iron staining





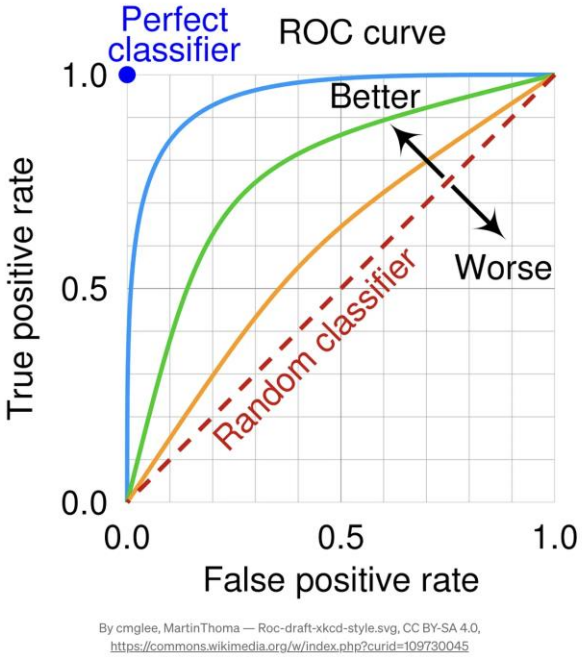
Comparison to serum ferritin, transferrin saturation, CHr

Images Sources: ASH Image Bank

Reticulocyte Hemoglobin vs. Traditional Iron Panel Testing

Statistics

AUC; Area under the curve



By cmglee, MartinThoma — Roc-draft-xkcd-style.svg, CC BY-SA 4.0, https://commons.wikimedia.org/w/index.php?curid=109730045

Sensitivity

$$\frac{TP}{TP + FN}$$

Specificity

$$\frac{TN}{TN + FP}$$

AUC 0.5	Random
0.5 < AUC < 1	Closer to 1, the better
AUC = 1	Perfect

Song, M., (2023) Understanding the ROC-AUC Curve, *Medium*

Reticulocyte Hemoglobin vs. Traditional Iron Panel Testing

↓CHr, ↓ Ferritin, ↓ $TSAT$

Iron deficiency threshold values

Ability to detect iron deficiency

Table 1
The studies of determing the parameters to diagnose iron deficiency.

Auther	Cut point	Sensitivity (%)	Specificity (%)	ROC area	Ref
Mast AE	CHr < 28 pg	73.9	73.3	0.735	14
	s-ft < 50 ng/mL	52.4	92.9	0.69	
	TSAT < 13%	65	70.3	0.637	
Karlsson T	CHr < 30.5 pg	93	69	0.84	15
	s-ft < 30 ng/mL	87	95	0.92	
	TSAT < 12%	93	57	0.84	
Cai J	CHr < 27.2 pg	78	71	0.929	16
	s-ft < 12.9 ng/mL	38	53	0.887	

CHr; reticulocyte hemoglobin content, s-ft; serum ferritin, TSAT; transferrin saturation.
Hematology analyzer for CHr; ADVIA.

Comparison to iron staining	AUC (ROC)
Serum Ferritin (s-ft)	0.69 – 0.89
Transferrin Saturation (TSAT)	0.64 – 0.84
Reticulocyte Hemoglobin (CHr)	0.74 – 0.93

Reviewed by Ogawa, C., et al (2020) Reticulocyte Hemoglobin Content, *Clinica Chimica Acta*

DATA ANALYSIS #2

Question

Which better detects iron deficiency:
reticulocyte hemoglobin or traditional iron panel testing?

Answer

Reticulocyte hemoglobin

Reticulocyte Hemoglobin vs. Traditional Iron Panel Testing

556 anemic patients

↓

RET-He of < 30.7pg used to identify iron deficient anemic patients

↓

Lowest quartile of iron deficient patients
RET-He < 28.6pg

	% of IDA patients
↓ Ferritin	25%
↓ TSAT	37.2%

Auerbach, M., et al (2021) Using Reticulocyte Hemoglobin Equivalent as a Marker for Iron Deficiency and Responsiveness to Iron Therapy, *Mayo Clinic*

RETICULOCYTE HEMOGLOBIN

Significance:

2. Monitor iron treatment response in iron-deficient patients

Reticulocyte Hemoglobin to Monitor Iron Treatment

556 anemic patients

↓

RET-He, iron panel, hemoglobin results used to identify iron deficient anemic patients

↓

Given intravenous iron treatment (1000mg over 1-2 visits)

↓

Reanalyzed
- RET-He
- Hgb

Auerbach, M., et al (2021) Using Reticulocyte Hemoglobin Equivalent as a Marker for Iron Deficiency and Responsiveness to Iron Therapy, *Mayo Clinic*

Reticulocyte Hemoglobin to Monitor Iron Treatment

Reanalyzed
- RET-He
- Hgb

Iron Deficiency

RET-He < 28.6pg
+
Hgb < 10.3g/dL

Mild – Severe Anemia

1000 mg IV IRON →

Predicted anemia compensation

↑ Hgb by + 1.0 g/dL

↑ Unit of RET-HE →

Predicted anemia compensation

↑ Hgb by + 0.21 g/dL

Auerbach, M., et al (2021) Using Reticulocyte Hemoglobin Equivalent as a Marker for Iron Deficiency and Responsiveness to Iron Therapy, *Mayo Clinic*

RETICULOCYTE HEMOGLOBIN

Benefits

Benefits

- Shown to better identify iron deficiency vs. iron panel

- Rapidly rule out iron deficiency
  - Outpatient setting
  - No need for chemistry testing

Test Used to Identify Iron Deficiency	Expected Reduction in Iron Studies
RET-He	66%
RET-He + Anemia (Hgb < 11g/dL)	80%

Peerschke, E., et al (2014) Using the Hemoglobin Content of Reticulocytes (RET-He) to Evaluate Anemia in Patients with Cancer, *Am J Clin Pathol*

Benefits

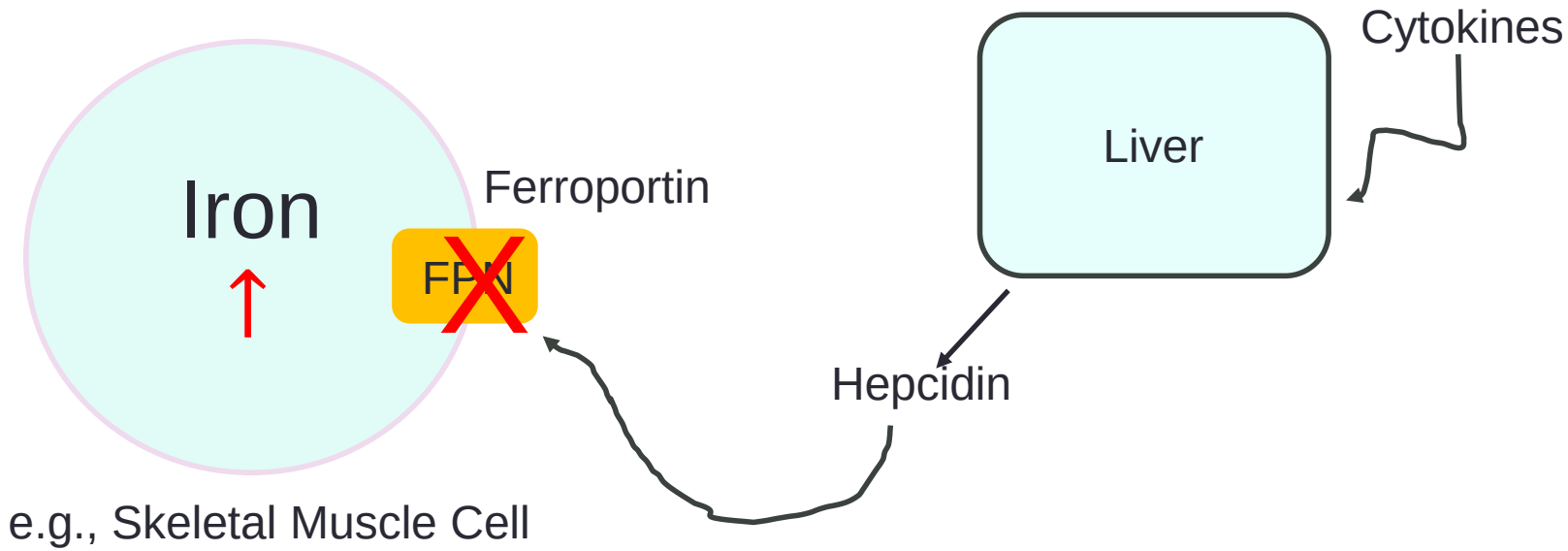
- Can aid in the interpretation of inconclusive iron panel results
- Assesses ability to USE iron to make hemoglobin
  - Functionality of iron
- Reticulocyte hemoglobin has fewer limitations/interferences vs. iron panel

Iron Panel Limitations

Ferritin

- Increases with inflammation

(inflammatory cytokines increases signal → breakdown iron transporter → iron stays inside cell)

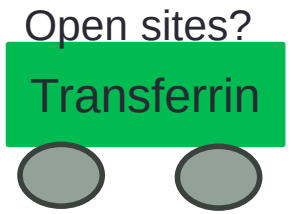


Iron Panel Limitations

Serum Iron

- Falsely ↑ with diet
- Diurnal variation (high in am; low in pm)

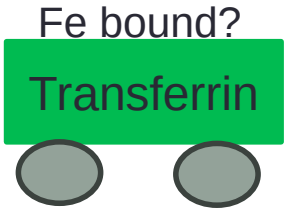
Total Iron Binding Capacity (TIBC)



- Falsely ↓ with medications
- Falsely ↓ with liver disease

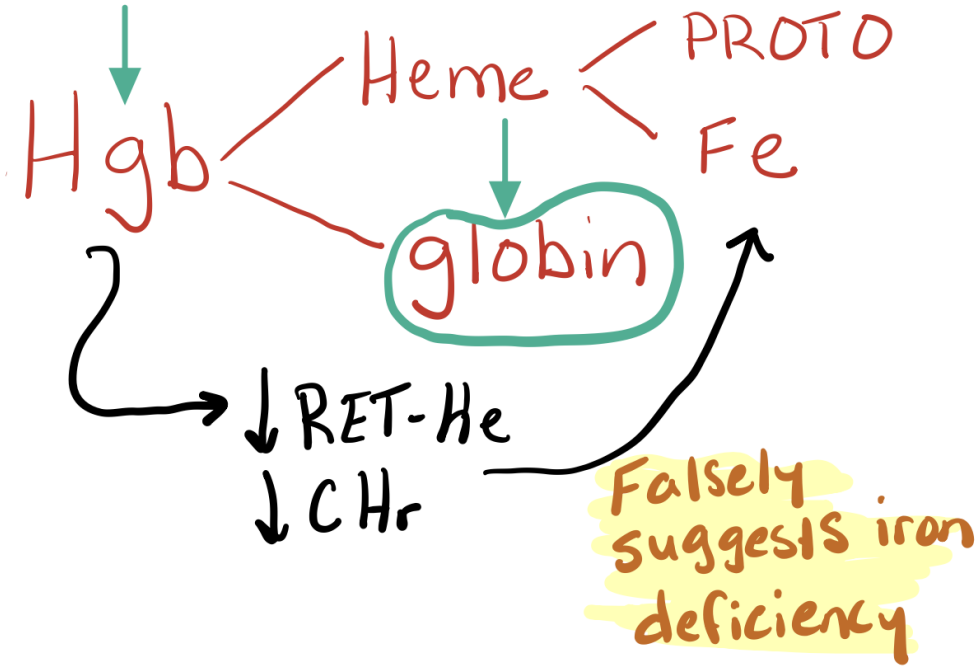
TSAT (Transferrin Saturation)

- Affected due to calculation with serum iron + TIBC



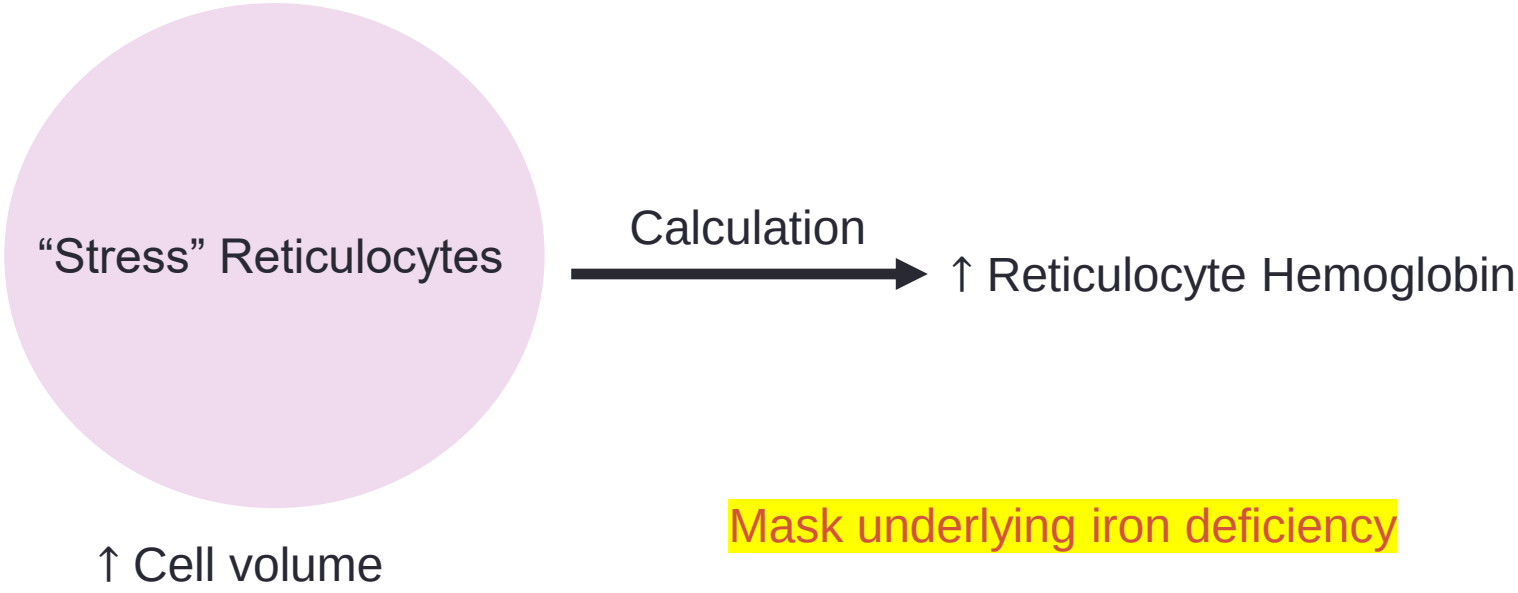
Reticulocyte Hemoglobin Limitations

Thalassemia (Hemoglobinopathy)



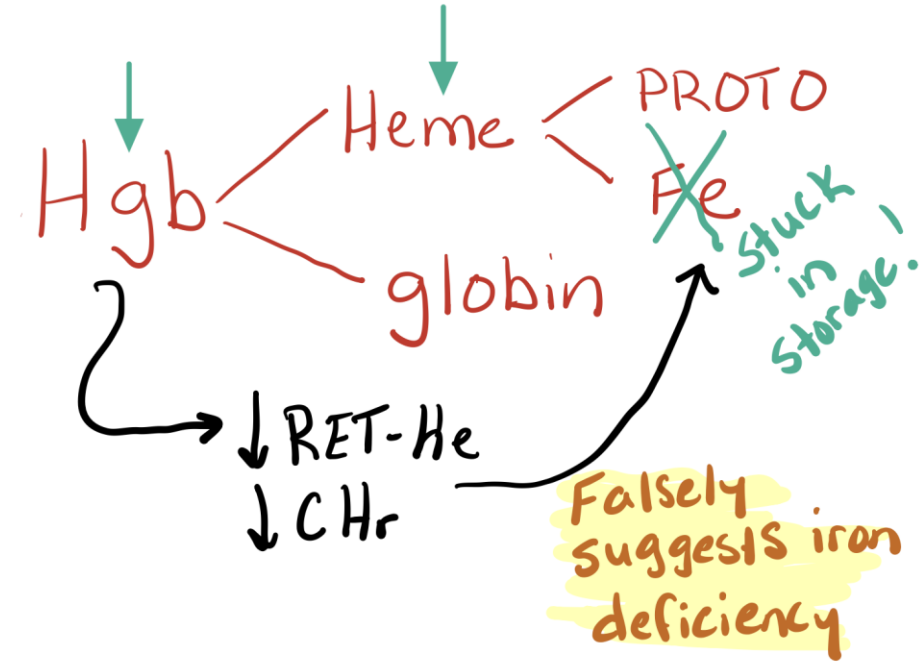
Reticulocyte Hemoglobin Limitations

Increased immature reticulocytes (bone marrow compensation)



Reticulocyte Hemoglobin Limitations

Anemia of Chronic Disease (ACD)



IDA vs. ACD?!

↓ RET-He
↓ CHr

↓/N MCV
↓/N MCHC
History
SL ↓ RET-He
CHr

CONCLUSION

Reticulocyte hemoglobin can assess hemoglobin production over the past 3-4 days

Better indicator for iron deficiency vs. iron panel

- Screen for iron deficiency (< 28pg)

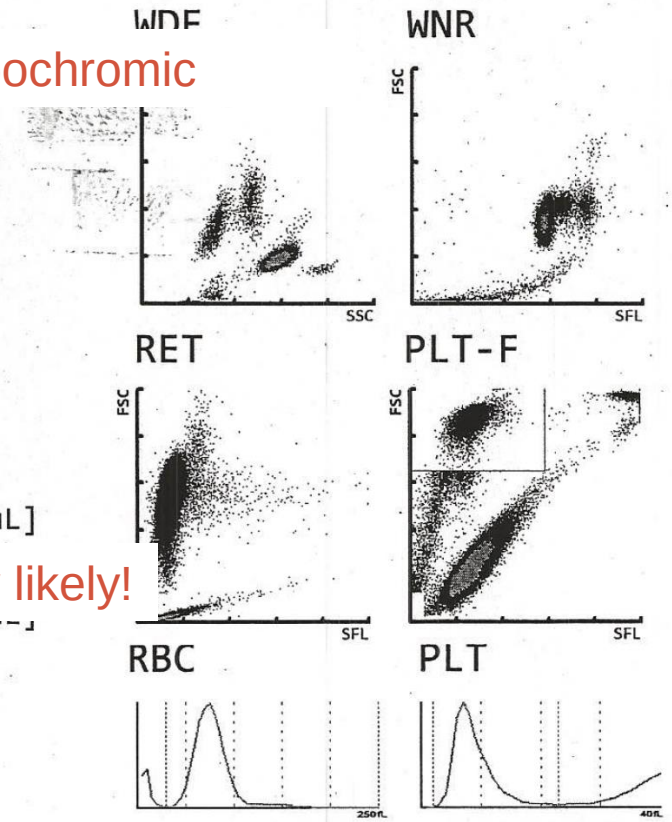
WBC	7.90	[10^3/uL]		
RBC	3.44 -	[10^6/uL]		
HGB	7.8 -	[g/dL]		
HCT	25.7 -	[%]		
MCV	74.7 -	[fL]		
MCH	22.7 -	[pg]		
MCHC	30.4 -	[g/dL]		
RDW-SD	47.7	[fL]		
RDW-CV	17.7 +	[%]		
PLT &F	437 +	[10^3/uL]		
MPV	8.8	[fL]		
NEUT	5.63	[10^3/uL]	71.2	[%]
LYMPH	1.19	[10^3/uL]	15.1	[%]
MONO	0.88	[10^3/uL]	11.1	[%]
EO	0.10	[10^3/uL]	1.3	[%]
BASO	0.04	[10^3/uL]	0.5	[%]
IG	0.06	[10^3/uL]	0.8 +	[%]
NRBC	0.00	[10^3/uL]	0.0	[%]
RET	0.96	[%]	0.0330 +	[10^6/uL]
IRF	8.9	[%]		
RET-He	23.8 -	[pg]		
IPF	0.7 -	[%]		
WBC-BF		[10^3/uL]		
RBC-BF		[10^6/uL]		
MN		[10^3/uL]		[%]
PMN		[10^3/uL]		[%]
TC-BF#		[10^3/uL]		

Anemic

Microcytic/Hypochromic

No Clot

Iron deficiency likely!



NEUT	
BAND	
LYMPH	
MONO	
EOS	
BASO	

WBC IP Message

META	
MYELO	
PRO	
BLAST	
ATYP	
NRBC	
OTHER	

MACRO	
MICRO	
HYPO	
POLY	
OVAL	
TEAR	
ANISO	

RBC IP Message

TARGET	
B STIP	
SCHISTO	
BURR	
ACANTHO	
SPHERO	
PAPPEN	

DOEHLE	
TOXIC G	
PLT EST	
TECH:	

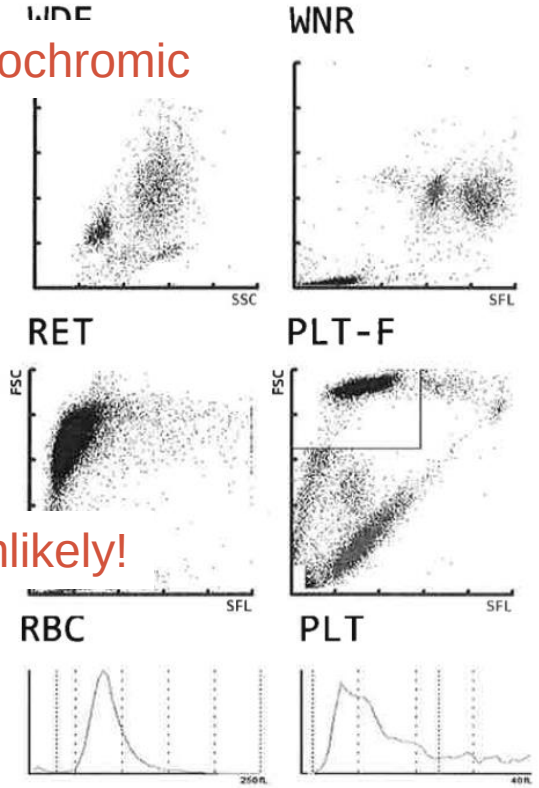
PLT IP Message

WBC	2.63	-	[10^3/uL]		
RBC	2.49	-	[10^6/uL]		
HGB	7.7	-	[g/dL]		
HCT	21.3	-	[%]		
MCV	85.5	-	[fL]		
MCH	30.9		[pg]		
MCHC	36.2		[g/dL]		
RDW-SD	53.1		[fL]		
RDW-CV	18.5	+	[%]		
PLT &F	48	-	[10^3/uL]		
MPV	12.0		[fL]		
NEUT	0.52	*	[10^3/uL]	19.8 *	[%]
LYMPH	0.79	*	[10^3/uL]	30.0 *	[%]
MONO	0.45	*	[10^3/uL]	17.1 *	[%]
EO	0.01	*	[10^3/uL]	0.4 *	[%]
BASO	0.00		[10^3/uL]	0.0	[%]
IG	0.86	*	[10^3/uL]	32.7 *	[%]
NRBC	0.11		[10^3/uL]	4.2	[%]
RET	2.75		[%]	68.5	[10^9/L]
IRF	19.0		[%]		
RET-He	37.5		[pg]		
IPF	4.1		[%]		
WBC-BF			[10^3/uL]		
RBC-BF			[10^6/uL]		
MN			[10^3/uL]		[%]
PMN			[10^3/uL]		[%]
TC-BF#			[10^3/uL]		

Anemic

Normocytic/Normochromic

Iron deficiency unlikely!



NEUT	
BAND	
LYMPH	
MONO	
EOS	
BASO	

META	
MYELO	
PRO	
BLAST	
ATYP	
NRBC	

MACRO	
MICRO	
HYPO	
POLY	
OVAL	
FRAG	

PLT	
OTHER	
TECH:	

Normal 29.3 – 35.4pg

WBC IP Message
NRBC Present
IG Present
Blasts/Abn Lympho?
Left Shift?

RBC IP Message
Fragments?

PLT IP Message
Thrombocytopenia

Thank You