MUST TO KNOW IN HEMATOLOGY	
Hematology	Greek: -Haima = Blood -Logos = Study/science
EDTA (Lavender top)	Chelates calcium <u>Inversion: 8x</u> Anticoagulant of choice for hematology cell counts and cell morphology Blood smear: prepare <u>w/in 2 hrs</u> Preferred anticoagulant for platelet count: = In some patients w/ EDTA anticoagulated blood – platelet satellitism = Platelet satellitism: platelets adhere to neutrophils ♫ Effect to automated platelet count → Decreased ♫ Remedy: Repeat platelet count using citrate (Rodak: Platelet count x 1.1) ↑ EDTA = Shrinkage of cells = ↓ Hct = ↓ ESR Not for coagulation tests: = Inhibits fibrinogen-thrombin reaction = Factor V is not stable in EDTA
Modified Westergren ESR (Black top tube)	2mL EDTA + 0.5mL NSS/Citrate Ratio = 1:4 (Anticoagulant-to-Blood)
Citrate (Light blue top tube)	For coagulation and platelet studies = Preserves labile factors V and VIII = Buffered 3.2% (0.109M) citrate <u>Inversion: 3-4x</u> Ratio = 1:9 (Anticoagulant-to-Blood)
Polycythemic patients	↑ Hct ↑ Excess Citrate = ↑ PT, ↑ APTT Remedy: Reduce the volume of citrate Amount of citrate = [(100-Hct)/(595-Hct)] x mL WB
Oxalate	Double/balanced oxalate (Ratio = 2:3): Maintained cell structures a. Potassium oxalate (Paul-Heller's) = shrink cells b. Ammonium oxalate (Wintrobe's) = swell cells
Heparin	Inactivation of thrombin Anticoagulant for osmotic fragility test <u>Inversion: 3-4x</u> Not for blood film preparation: = Distorts cells = Produces bluish background on Romanowsky's stain Not for coagulation = Inhibits thrombin and all stages of coagulation
Order of Draw (Henry 21st Edition)	Evacuated tube: 1. Sterile blood culture tube 2. Citrate (blue) 3. Nonadditive tube (red) 4. Heparin (green) 5. EDTA (lavender) 6. Fluoride (gray)
Order of Draw (Syringe method)	1. EDTA 2. Other anticoagulated tubes 3. Nonadditive tube
EDTA containing tubes	Lavender Pink White

Morula cell Berry cell	♫ Multiple myeloma
Russell bodies	Individual globules of immunoglobulin
Dutcher's bodies	Intranuclear protein inclusions
Platelet Abnormalities (Morphologic)	
Giant platelet	♫ Bernard-Soulier syndrome ♫ May-Hegglin anomaly
Small/micromegakaryocyte Large megakaryocyte Mononuclear megakaryocyte Vacuolated megakaryocyte	♫ Myelodysplastic syndromes
Leukemia	
Leukemia	Abnormal, uncontrolled proliferation and accumulation of one or more of the hematopoietic cells Symptoms: Fever, weight loss, ↑ sweating; hepatosplenomegaly, enlarged lymph nodes (chronic leukemia) ↑ BMR
Acute leukemia	Days to 6 months Predominantly immature cells (blasts and "pro" stages)
Subacute leukemia	2 to 6 months
Chronic leukemia	Variable Minimum of 1 or 2 years Predominantly mature cells
Leukemic leukemia	WBC >15,000/µL
Subleukemic leukemia	WBC <15,000/µL (+) Abnormal and immature cells in PB
Aleukemic leukemia	WBC <15,000/µL (-) Abnormal and immature cells in PB
French-American-British (FAB) Classification of Acute Leukemias	Divides acute leukemias into lymphoblastic and monoblastic = Subdivided according to cellular morphology, cytochemical staining results, cytogenetic studies and T & B lymphocytes marker results
Acute leukemia	Normocytic, normochromic RBCs FAB = ≥30% blasts Henry: WHO (Now the standard for diagnosis) = ≥20%
Acute leukemia in children	80% ALL 20% ANLL
Tests to differentiate ALL from ANLL	1. MPO: Myeloperoxidase = (+) AML = (-) ALL 2. SBB: Sudan Black B = (+) AML = (-) ALL 3. TdT: Terminal Deoxyribonucleotidyltransferase = Marker for immature lymphocyte = (+) ALL = (-) ANLL
Acute Lymphoblastic Leukemias (ALL)	
L1	Lymphoblasts are <u>small</u> and <u>homogeneous</u> (vary little in size) Childhood ALL
L2	Lymphoblasts are <u>large</u> and <u>heterogeneous</u> (vary in size) Adult ALL

L3	Burkitt-type Rare Lymphoblasts are large but homogeneous, and vacuolated
Acute Nonlymphocytic Leukemias (ANLL)	
M1	Acute myeloblastic leukemia w/o maturation (AML w/o mat) BM: >30% blasts <10% granulocytic cells
M2	Acute myeloblastic leukemia w/ maturation (AML w/ mat) BM: >30% blasts >10% granulocytic cells
M3	Acute promyelocytic leukemia (APL) >30% blasts >10% granulocytic cells ≥30% or >50% promyelocytes (+) Faggot cells = Associated w/ DIC
M4	Acute myelomonocytic leukemia (AMML) Naegeli's leukemia 20% to <80% monocytic cells
M5a	Acute monoblastic leukemia w/o maturation Schilling's leukemia >80% monocytic cells (>80% monoblasts)
M5b	Acute monoblastic leukemia w/ maturation >80% monocytic cells (<80% monoblasts)
M6	Erythroleukemia Erythremic myelosis DiGuglielmo's syndrome >30% blasts >50% erythrocytic precursors
M7	Acute megakaryocytic leukemia >30% blasts >30% megakaryocytic cells
Chronic Myeloproliferative Disorders	
MPD	Proliferation of abnormal pluripotential stem cell Stem cell differentiates into the granulocytic (myeloid stem cell), megakaryocytic and erythroid cell lines
1. Chronic Myelogenous Leukemia (CML)	(+) Philadelphia chromosome: t($9^{+};22^{-}$) - both long arms If (-) Ph' chromosome = poor prognosis Similar to leukomoid reaction, to differentiate: a. Chromosome studies b. LAP = (↑ in Leukomoid reaction, ↓ in CML)
2. Myelofibrosis w/ myeloid metaplasia (MMM)	Fibrosis and granulocytic hyperplasia of BM, w/ granulocytic and megakaryocytic proliferation in the liver and spleen (extramedullary) (+) Dacryocytes ↑ LAP BM aspirate = impossible (dry tap) BM biopsy = appropriate
3. Essential Thrombocythemia (ET)	Thrombocytosis: 1,000 x 10^{9}/L Functionally abnormal platelets
4. Polycythemia Vera (PV)	BM: Panmyelosis PB: Pancytosis/Pancythemia

	↑RBCs, ↑WBCs, ↑Plts ↑ LAP (Other polycythemia: N-LAP)		
Polycythemia			
1' Absolute polycythemia	Other names: Polycythemia Vera, Polycythemia Rubravera, Vaquez Osler disease, Panmyelosis ↑ RBC mass (↑ Hct) ↑ RBCs, ↑ WBCs, ↑ Platelets ↓ Erythropoietin (EPO)		
2' Absolute polycythemia w/ appropriate production of EPO	In response to hypoxia In patients w/ pulmonary/cardiac disease ↑ RBCs, ↑ WBCs, ↑ Platelets ↑ EPO		
2' Absolute polycythemia w/ inappropriate production of EPO	In patients w/ tumors of kidney, liver, brain, adrenal and pituitary gland ↑ RBCs, N-WBCs, N-Platelets ↑ EPO		
Relative polycythemia	Spurious/Gaisböck polycythemia Associated w/ stress and anxiety N-RBC mass ↑ Hct because of decreased plasma volume		
RBC mass	Differentiate absolute from relative polycythemia ↑ RBC mass = Absolute polycythemia N-RBC mass = Relative polycythemia		
Myelodysplastic Syndrome/Dysmyelopoietic Syndrome			
MDS	Clonal abnormalities in hematopoietic cells "Pre-leukemia": can progress to AML if not treated <30% blast		
Differentiation	% Blasts in PB	% Blasts in BM	Comments
Refractory anemia (RA)	<1%	<5%	Mildest type
RA w/ ringed sideroblast (RARS)	<1%	<5% >15% RS	RS: Ringed sideroblast
RA w/ excess blast (RAEB)	<5%	5-20%	
RAEB in transformation (RAEBt)	>5%	20-30%	
Chronic Myelomonocytic Leukemia (CMML)	<5%	5-20%	Persistent monocytosis
Lymphoproliferative disorders			
LPD	Proliferation of cells derived from lymphoid stem cell → T/B cells		
1. T/B cell leukemia	--		
2. Lymphoma	Malignancy involving lymphoid tissue a. Non-Hodgkin's lymphoma = proliferation of neoplastic lymphocytes = Rappaport classification b. Hodgkin's lymphoma = proliferation of cells reacting to neoplasm = (+) Reed-Sternberg cell: large cell w/ large nucleoli (Owl's eye) ♫ Diagnosis: Lymph node biopsy = Rye classification: based on histologic appearance of lymph node biopsy = Ann-Arbor: staging based on the extent of tissue involved		
3. Hairy cell leukemia	Leukemic reticuloendotheliosis Originally B cells w/ hairlike projections		

Laboratory Tests for Primary Hemostasis	
Platelet count	1. Direct (Neubauer counting chamber) Plt. count/mm^3 = # plt x AF x DCF x DF = # plt x 1 x 10 x 200 = <u># plt x 2,000</u> (RBC pipette = 1:200 dilution) ♫ <u>1 platelet uncounted = -2,000 plt/mm^3</u> a. Reese-Ecker - Sodium citrate - Formaldehyde - Brilliant cresyl blue b. Guy and Leake - Sodium oxalate - Formaldehyde - Crystal violet c. Brecker-Cronkite - 1% ammonium oxalate (phase contrast microscopy) --- d. Unopette 2. Indirect (smear) Platelet count = (# of plts ÷ 1000 RBC) x RBC count a. Dameshek b. Fonio's c. Olef's
Unopette	Diluent: 1% Ammonium oxalate Stand moist chamber for 15-20mins to allow platelets to settle 1.98mL diluent 0.02mL blood TV = 2mL Dilution = 1:100 Plt. count/mm^3 = # plt x ACF x DCF x DF = # plt x 1 x 10 x 100 = <u># plt x 1,000</u> ♫ 1 platelet uncounted = -1,000 plt/mm^3
Platelet estimate (Wedge smear)	8-20 platelets/OIO Factor: 20,000
WBC estimate (HPF)	Factor: 2,000
Platelet Estimates (Smear)	
Platelet Estimate of	**Report Platelet Estimate as**
0-49,000/µL	Markedly decreased
50,000-100,000/µL	Moderately decreased
100,000-150,000/µL	Slightly decreased
150,000-199,000/µL	Low normal
200,000-400,000/µL	Normal
401,000-599,000/µL	Slightly increased
600,000-799,000/µL	Moderately increased
>800,000µL	Markedly increased
N-Plt count, ↑ BT	Qualitative platelet abnormality Primary vascular abnormality vWD
↓ Plt count, N-BT	Autoimmune thrombocytopenia
↓ Plt count, ↑↑ BT	Simultaneous quantitative and qualitative platelet deficiency
Platelet aggregation test	Aggregating agents:

Protamine sulfate test	Detects the presence of fibrin monomers (2'-DIC) in the plasma Plasma + Protamine sulfate + ETOH → (+) gel-like clot (paracoagulation)	
Ethanol gelation test	Detects the presence of fibrin monomers (2'-DIC) in the plasma Plasma + NaOH (↑pH) + ETOH → (+) pptn/gel	
Latex D-dimer assay	Most specific test for DIC	
Test	**Primary Fibrinolysis**	**Secondary Fibrinolysis**
WBCLT	< 48 hrs	< 48 hrs
Euglobulin clot lysis	< 2 hrs	< 2 hrs
Ethanol gelation	-	+
Protamine sulfate	-	+
D-dimer	-	+
Heparin	Injected Action: inhibits thrombin Monitoring: APTT = sensitive, method of choice (CAP) Neutralize w/ protamine sulfate	
Warfarin Coumarin Coumadin	Oral WARF: Wisconsin Alumni Research Foundation Action: Vit. K antagonist, inhibits II, VII, IX, X Monitoring: PT (reported in INR) Neutralize w/ Vitamin K, FFP	
INR	International normalized ratio INR = (Patient PT ÷ Normal PT)^{ISI} ♫ INR = 2-3 - Prevents MI, embolism & thrombosis ♫ INR = 2.5-3.5 - For patients w/ mechanical heart valves	
ISI	International Sensitivity Index (PT rgt) PT rgt is calibrated w/ Manchester rgt = from human brain thromboplastin	

RBC Count	
RBCs	↑AM, ↓PM
RBC diluting fluids	Isotonic solutions 1.) NSS 2.) 3.8% Sodium citrate 3.) Dacies or formol citrate 4.) Hayem's 5.) Toisson's 6.) Bethell's 7.) Gower's Dilution (RBC pipette) = 0.5:100 (Blood: Diluent) = 1:200
RBC count	RBC/mm^3 = # RBC x AF x DCF x DF = # RBC x 5 x 10 x 200 = # RBC x 10,000
WBC Count	
WBCs	↓AM, ↑PM
WBC Diluting fluids	Hypotonic solutions: lyse non-nucleated RBCs 1.) 1-3% acetic acid 2.) 1% HCl 3.) Turk's diluting fluid: Gentian violet + glacial acetic acid (solid at 17'C) + H_2O Mix = 3 mins (To allow lysis of RBCs) Dilution (WBC pipette) = 0.5:10 (Blood: Diluent) = 1:20 Leukocytosis = Use RBC pipette (1:100 or 1:200)
WBC count	WBC/mm^3 = # WBC x AF x DCF x DF
Counting Chamber	
Fuch's Rosenthal	2 counting areas Each CA w/ 16 1mm^2 squares Depth = 0.2mm Depth factor = 5 Volume = Area x Depth x # CA = 16mm^2 x 0.2mm x 2 = 6.4mm^3 Volume/counting chamber = 3.2mm^3
Speir's Levy	4 counting areas Each CA w/ 10 1mm^2 squares Depth = 0.2mm Depth factor = 5 Volume = Area x Depth x # CA = 10mm^2 x 0.2mm x 4 = 8mm^3 Volume/counting chamber = 2mm^3
Improved Neubauer	2 primary squares Each 1' square w/ 9 1mm^2 2' squares Depth = 0.1mm Depth factor = 10 Volume = Area x Depth x # CA = 9mm^2 x 0.1mm x 2 = 1.8mm^3 Volume/counting chamber = 0.9mm^3
RBC count	Center square: w/ 25 3' square Each 3' square w/ 16 small squares 25 x 16 = 400 5 (counted) x 16 = 80 small squares
WBC count	4 corners: Each 2' square w/ 16 3' squares 4 x 16 = 64 3' squares
Nucleated RBCs	Not lysed by WBC diluents Falsely counted as WBCs