2018 SBB/BB Exam Review

10/15/2018

Faculty Disclosures

The following faculty have no relevant financial relationships to disclose:

- Jayanna Slayten MS, MT(ASCP)SBBcm
- Lorraine Blagg MA, MLS (ASCP)SBB
- Katrina Billingsley MSTM, MT(ASCP)SBB
- Catherine (Kate) Hernandez MT(ASCP)SBB

AABB BOSTON

Learning Objectives

- Explain American Society of Clinical Pathologist (ASCP) SBB and BB exam requirements
- Explain the topics outlined on the ASCP BB/SBB Exam Content Outline
- Describe pertinent information which may be covered on these exams to aid in preparing for the BB or SBB exam
- Discuss helpful hints for studying for and taking these exams

AABB 18 BOSTON

2018
SBB/BB Exam Review

AABB Annual Meeting
Boston, MA

Outline of Presentation

Introduction	J.Slayten	825-830
Exam Requirements, Competencies and Content Outline	K. Hernandez	830-840
Immunology and Complement, Genetics and Lab Math	L. Blagg	840-925
Adverse Effects of Transfusion / Transfusion Reactions	K. Hernandez	925-940
Hemolytic Disease of the Fetus and Newborn	K. Hernandez	
Coagulation (topic falls under physiology/pathophysiology)	L. Blagg	940-1015

Outline of Presentation

Blood Groups **Methods** **DAT and Autoimmune Hemolytic Anemias**	K. Billingsley	1030-1120
Donors – Whole Blood and Apheresis **Component Preparation and Storage** **Component Quality Control** **Component Therapy** **Transfusion Transmitted Disease Testing & Re-entry**	J. Slayten	1120-1135
Lab Management **Education** **Quality Assurance/Quality Control**	K. Billingsley	1135-1145
BB/SBB Studying and Testing Strategies **Q and A**	J. Slayten	1145-1155 1155-1200

The SBB and BB Exams

Requirements
Competencies
Content

Kate Hernandez, MT(ASCP)SBB^{CM}

St. Mary Medical Center

Long Beach, CA

ASCP Website Information

- **Eligibility**
  - **Eligibility Assistant**
- **Required documentation**
- **Click on: *Board of Certification / Get Credentialed / Certification Exam Process / General Information / Procedures for Examination & Certification***
  - **36 page booklet – very helpful**
- **Scheduling exam / Studying for exam**
  - **Exam content guidelines**
  - **Reading list**
  - **Exam information**
- **Exam day**
- **Results and certificate**
- **US Military**

Application Information

- **Complete and submit application online via credit card or Paypal**
- **$240 for BB (Non-refundable)**
- **$290 for SBB (Non-refundable)**
- **All correspondence from BOC via email (keep email address current)**
- **Obtain all necessary documentation before applying**

Some of the Documents Required

- Academic education
  - Official transcript verifying date of degree
  - Evaluation of foreign transcripts
- Experience documentation
- Accredited program info
  - Program director, beginning/ending date, school number

Application Processing

- Application processed within 45 business days of receipt
- Review of documents may take up to 6 weeks
- Admission letter emailed with instructions for scheduling exam within 3 months
- All exams administered by computer at Pearson Professional Centers

SBB Exam Requirements

- **Route 1**
  - **Bachelor's degree with required courses**
  - **Successful completion of CAAHEP-accredited SBB program within last 5 years**
- **Route 2**
  - **MT/MLS(ASCP) or BB(ASCP)**
  - **Bachelor's degree**
  - **3 years FT BB experience within last 6 years after degree**
  - **Must be attained with pathologist oversight in accredited lab (AABB, CAP, COLA, DNV, TJC, JCI or under ISO 15189)**

SBB Exam Requirements

- **Route 3**
  - **Master's or doctorate degree**
  - **3 years FT BB experience in accredited lab within last 6 years after degree**
- **Route 4**
  - **Doctorate degree**
  - **2 years of post-doctoral fellowship in blood banking within last 5 years**

SBB Exam Requirements

- **Route 5**
  - **MT/MLS(ASCP) or BB(ASCP)**
  - **Bachelor's degree**
  - **3 years FT experience as an academic educator in clinical blood banking within last 6 years**

- **Route 6**
  - **Masters or Doctorate degree**
  - **3 years FT experience as an academic educator in clinical blood banking within last 6 years**

BB Exam Requirements

- **Route 1**
  - **MT/MLS(ASCP) and Bachelor's degree**
- **Route 2**
  - **Bachelor's degree in appropriate field with required courses**
  - **1 year full-time BB experience within last 5 years**
  - **Must be attained with pathologist oversight in accredited lab**

BB Exam Requirements

- **Route 3**
  - **Bachelor's degree in appropriate field with required courses**
  - **NAACLS Medical Laboratory Scientist Blood Banking Program within last 5 years**
- **Route 4**
  - **Master's or Doctorate degree**
  - **6 months FT BB experience in accredited lab within last 5 years after degree**

BB Exam Requirements

- **Route 5**
  - **Baccalaureate or post baccalaureate degree in Medical Lab Science or other appropriate degree**
  - **NAACLS Medical Laboratory Scientist Program within last 5 years**

Experience Required

- **Serologic Testing**
  - **ABO and Rh Typing**
  - **Antibody detection and identification**
  - **Crossmatching**
  - **Direct antiglobulin tests**
  - **Tests for other blood group antigens**

Experience Required (Cont.)

- **Routine Problem Solving**
  - **Transfusion reactions**
  - **Immune hemolytic anemias**
  - **Hemolytic disease of the fetus and newborn (HDFN)**
  - **Rh immune globulin evaluation**
  - **Indications for transfusion**

Experience Required (Cont.)

- **Quality Control / Quality Assurance**
  - **Reagents, equipment**
- **Laboratory Operations**

Experience Required (Cont.)

- **Donor Collection, Processing and Testing**
  - **Donor selection, preparation and collection**
  - **Processing and donor testing**
  - **Component preparation for storage and administration**

Certification Level Competencies

- Knowledge of Advanced Principles
- Technical Skills
- Problem Solving and Analytical Decision Making
- Communication
- Teaching and Training Responsibilities
- Supervision and Management

Competencies (Questions)

- **Theoretical - measure skills to:**
  - **Apply knowledge**
  - **Calculate results**
  - **Correlate results to disease states**
- **Procedural - measure skills to:**
  - **Perform lab techniques**
  - **Evaluate lab data**
  - **Follow QA protocols**

Competencies (Examples)

- **Knowledge of Advanced Principles**
  - **Ex: Know the underlying principles of lab testing, validity of results, causes of discrepant results**
- **Technical Skills**
  - **Ex: Know the immunohematology lab procedures (Methods section at the back of Technical Manual)**
  - **Ex: Test will measure your understanding of quality assurance and ability to monitor QC programs**

Competencies (Examples)

- **Problem Solving and Analytical Decision Making**
  - **Ex: Exam may assess your ability to develop and implement plans to correct and prevent problems**
- **Communication**
  - **Ex: Exam may assess your ability to communicate lab data and factors which can influence test results**
  - **Ex: Exam may test your ability to communicate lab policies and operations**

Competencies (Examples)

- **Teaching and Training Responsibilities**
  - **Ex: Exam may assess your ability to incorporate principles of educational methodology in the instruction of lab personnel and other health care providers**
- **Supervision and Management**
  - **Ex: Exam may assess your ability to give direction and guidance to technical and support personnel**

Exam Category Percentages

Subtest	BB (%)	SBB (%)
Blood Products	15-20	15-20
Blood Group Systems	15-20	15-20
Immunology	5-10	5-10
Laboratory Operations	5-10	15-20
Physiology / Pathophysiology	5-10	10-15
Serologic and Molecular Testing	20-25	20-25
Transfusion Practice	15-20	15-20

https://www.ascp.org/content/board-of-certification

Subtest Descriptions

Subtest	Description
Blood Products	Donors, processing, storage, blood components, product quality control
Blood Group Systems	Genetics, chemistry, antigens, role of blood groups in transfusion
Immunology	Immune response, immunoglobulins, antigen-antibody interactions, complement
Lab Operations	Development & evaluation of new technology, safety, training & education, administration & management, lab math, QA
Physiology / Pathophysiology	Physiology of blood, hemostasis & coagulation, HDFN, anemias, transplantation, HPC
Serologic and Molecular Testing	Routine tests, reagents, applications of special tests & reagents, leukocyte/platelet testing, QA
Transfusion Practice	Indications for transfusion, component therapy, adverse effects of transfusion, hemapheresis & extracorporeal circulation, blood administration & blood management

SBB/BB Exam Review

Immunology

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Baltimore, MD

Immunology

- Self vs. Non-self vs. Abnormal self
- Types of immune responses
  - **Innate** (nonspecific, present at birth, immediate action)
    - Physical barriers (skin, cilia, cough & sneeze reflex, mucus membranes)
    - Biochemical barriers (mucus, saliva, tears, sweat, pH)
    - Cellular (Phagocytic cells)
    - Humoral (complement, cytokines)
    - Inflammation (edema, vasodilation, cell migration)
  - **Adaptive/Acquired** (specific, memory, primary vs. secondary)
    - Cellular (lymphocytes & APCs)
    - Humoral (antibodies)

Harmening, D.M. (2012). Modern Blood Banking and Transfusion Practices (6th ed.). Philadelphia: F.A. Davis Company. Chapter 3

Immunology

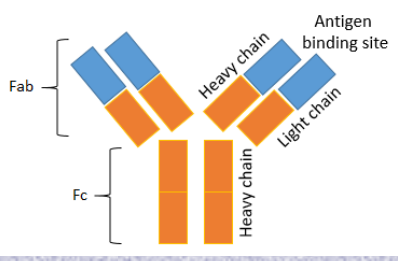
- Organs of the Immune System
  - **Primary** (Thymus & Bone Marrow)
    - Site of differentiation & maturation of T cells & B cells
  - **Secondary** (Lymph nodes, Spleen, MALT)
    - Site of cell function
- Cells of the Immune System
  - Hematopoietic Stem Cells (CD34) → self-renewal & differentiation
  - T Helper Cells (CD4) → MHC II → stimulate B & cytotoxic T cells
  - T Cytotoxic Cells (CD8) → MHC I → destroy tumor & infected cells
  - B cells (CD20) → Plasma cell → Make antibodies
  - NK cells (CD56) → Lyse tumor & virally infected cells
  - APCs (Monocytes, Macrophages, Dendritic cells…) → Phagocytize

Harmening, D.M. (2012). Modern Blood Banking and Transfusion Practices (6th ed.). Philadelphia: F.A. Davis Company. Chapter 3

Immunology

- Antigen Characteristics that affect immune response
  - Size (larger) & Density (more dense)
  - Charge
  - Accessibility (ability of immune system to see it)
  - Solubility (More soluble)
  - Digestibility
  - Degree of Foreignness
    - Chemical composition
    - Complexity
    - Conformation
- Relative Immunogenicity:
  - $D > K > c > E > k > e > Fy^a < C < Jk^a < S < Jk^b < s$

Harmening, D.M. (2012). Modern Blood Banking and Transfusion Practices (6th ed.). Philadelphia: F.A. Davis Company. Chapter 3



Immunology

- Antibody (Immunoglobulins) Characteristics
  - Two Heavy chains & two light chains
  - Variable (Idiotype), Constant (Allotype) & Hinge region
  - Fc domain & 2 Fab domain (papain)

Isotype	IgM	IgG	IgA	IgE	IgD
Structure	Monomer	Pentamer	Monomer or Dimer	Monomer	Monomer
Activate Complement	Yes, 1 IgM	Yes, 2 IgG	Alternative pathway	No	No
Cross Placenta	No	Yes, IgG2 weakly	No	No	No
Subclasses	No	Yes, 1-4	Yes, 1-2	No	No

Fung, M.K. et al. (Eds.). (2017). Technical Manual (19th ed.). Bethesda, MD: AABB Publications. Chapter 8

Immunology

- Cytokines Types:
  - Lymphokines – made by lymphocytes
  - Monokines – made by monocytes & macrophages
  - Chemokines – increase motility and migration of WBCs
  - Interleukins – made by WBCs to act on other WBCs
  - Effect:
    - Autocrine – affects itself
    - Paracrine – affects cells in close proximity
    - Endocrine – affects systemic activity
  - Function:
    - Growth factor – G-CSF, GM-CSF, M-CSF
    - HTR – IL-1, IL-6, IL-8, TNF-α, MCP-1
    - FNHTR – IL-1, IL-6, IL-8, TNF-α

Muylle, L. (1995). The role of cytokines in blood transfusion reactions. *Blood reviews,*9, 77-83.

Immunology

- Immune-mediated diseases
  - Immunodeficiency diseases
    - Recurrent infections, risk of TA-GVHD
  - Autoimmune diseases
    - Antibodies form to self, positive DATs
  - Gammopathies
    - Abnormal production of Ig, Rouleaux
  - HDFN
- Immunotherapies
  - IVIg, RhIg, Monoclonal antibody therapy
    - Serologic test interference

Stevens, C.D. and Miller, L.E. (2017). Clinical Immunology and Serology: A Laboratory Perspective (4th ed.). Philadelphia: F.A. Davis Company. Chapter 15 & 19

Immunology

- Hypersensitivity
  - **Type I – Allergic**
    - IgE causes mast cells to release histamine
    - Rash, urticaria, anaphylaxis
  - **Type II – Cytotoxic**
    - Ag-Ab mediated
    - HDFN, Autoimmune disease
  - **Type III – Immune Complex**
    - Soluble Ag-Ab complexes
    - Drug Induced hemolytic anemia
  - **Type IV – Cell Mediated**
    - Antigen stimulates specific T cell mediated cellular damage
    - GVHD, Poison Ivy, Allograft rejection

Stevens, C.D. and Miller, L.E. (2017). Clinical Immunology and Serology: A Laboratory Perspective (4th ed.). Philadelphia: F.A. Davis Company. Chapter 14

Complement

- Role:
  - Lysis of cells, bacteria, and enveloped viruses
  - Opsonization of foreign material to enhance phagocytosis
  - Generation of minor proteins that mediate inflammation
- Pathways:
  - Classical – activated by 1 IgM or 2 IgG
  - Alternative – activated by cell walls (bacteria, viruses, etc)
  - Lectin – activated by mannose binding lectin on microbial cell walls
- Control of complement activation
  - Decay Accelerating Factor (DAF) – Cromer blood group
  - Complement receptor 1 (CR1)
- Deficiencies of complement components
  - PNH, SLE, RA

SBB/BB EXAM REVIEW

BLOOD GROUP GENETICS

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Mendel's Principles

- **Random Segregation**
  - Distinct units (genes) inherited
  - One from each parent
  - Random

	A	O
B	AB	BO
O	AO	OO

- **Independent Assortment**
  - Genes inherited independently if carried on different chromosomes
  - Combinations of genes are not dependent on other genes (Exception: linkage)
- **Linkage Disequilibrium**
  - genes on closely linked loci are inherited together as a haplotype

Fung, M.K. et al. (Eds.). (2017). Technical Manual (19th ed.). Bethesda, MD: AABB Publications. Chapter 9

Definitions

- Allele/ Locus/ Antithetical
- Cis/Trans
- Lyonization
- Genotype/Phenotype
- Dominant/Recessive
- Dosage
- Haplotype
- Homozygous/Heterozygous/Hemizygous
- Suppressor Gene
- Crossover
- Recombination
- Polymorphism
- Prevalence
- Frequency
- Linkage
- Chimera

ISBT Terminology

- Allele
  - *JK*01* or *JK*A*
  - *N* demotes null (*RHD*01N.01* – D negative)
- Genotype/haplotype
  - *JK*01/JK*01* or *JK*A/JK*A*
- Phenotype
  - JK:1,-2 (traditionally Jk(a+b-)
- Antigen
  - Jk1 or 0009001 or 9.1 (traditionally Jk^a)

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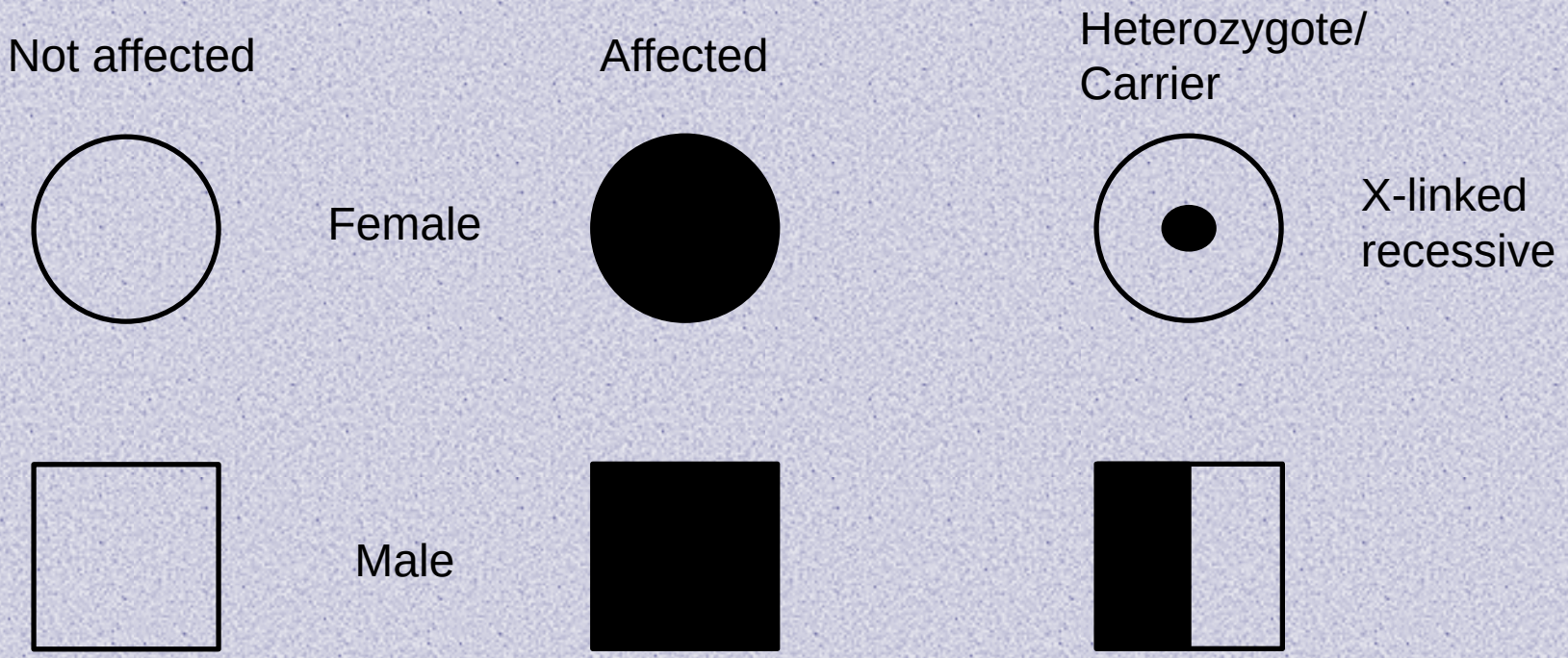
Genetic Symbols

- See Technical Manual...



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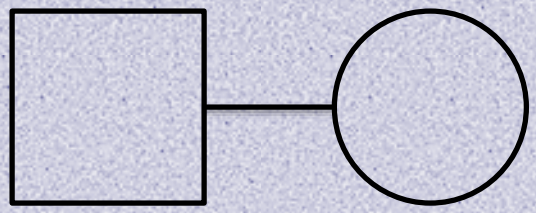
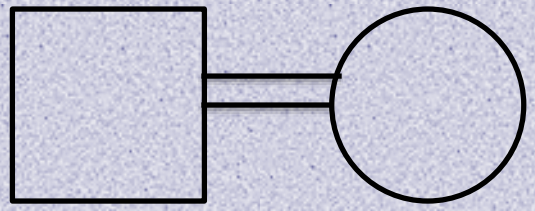
Genetic Symbols

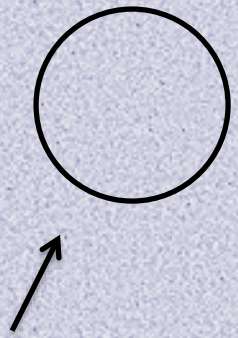
- See Technical Manual...



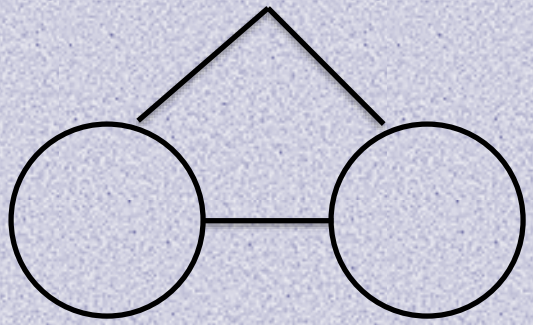
Mating



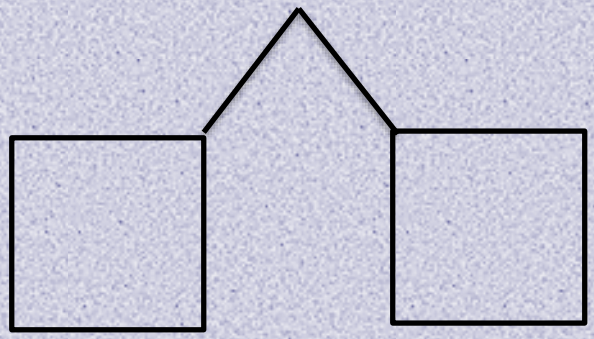
Consanguineous Mating



Proband



Monozygotic Twins



Dizygotic Twins

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Types of Inheritance

- Autosomal Dominant
  - Trait appears in **every** generation; no “skipping”
  - Trait is transmitted by an affected person to **half** his children on the average
  - Unaffected persons do **not** transmit the trait to their children
  - Occurrence and transmission of the trait are not influenced by sex; **equally likely in both males and females**

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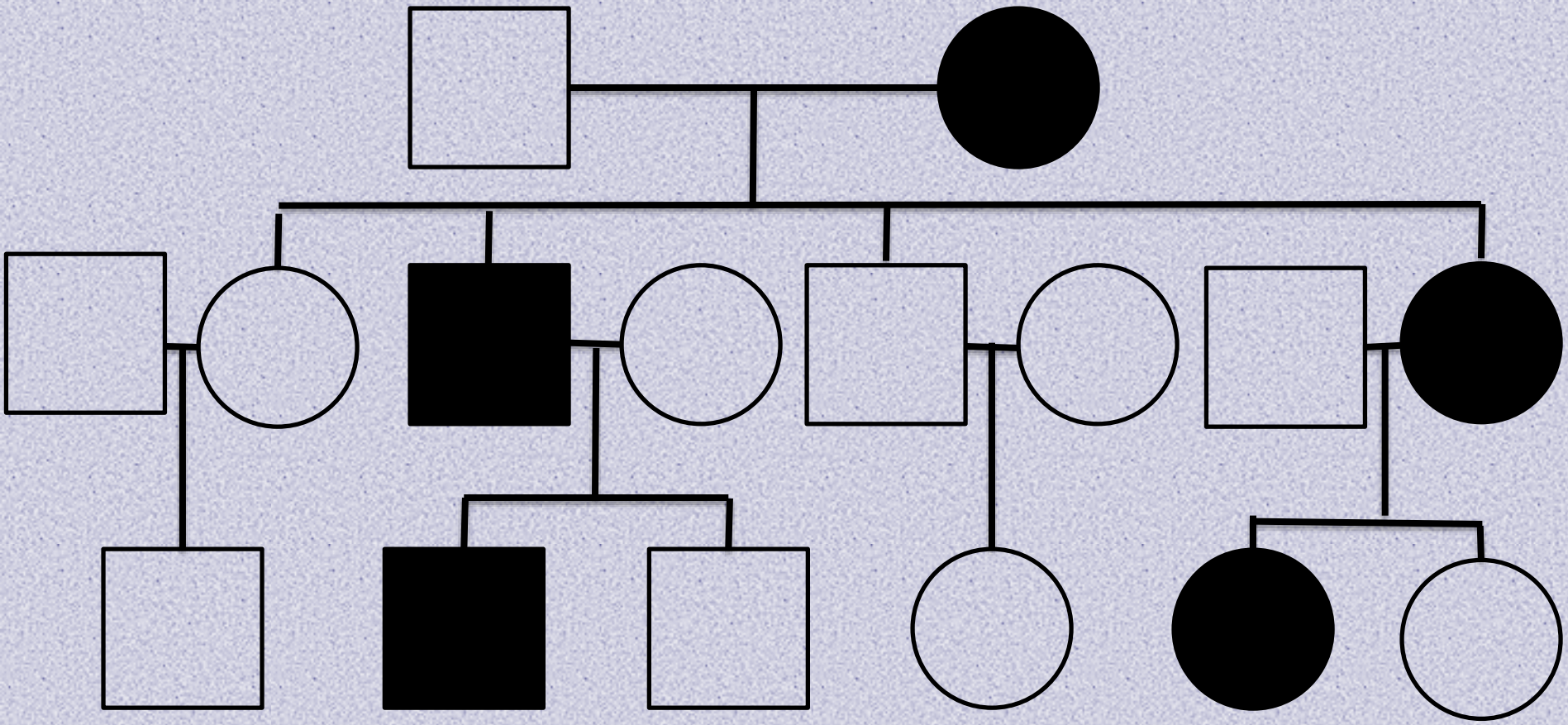
Autosomal Dominant



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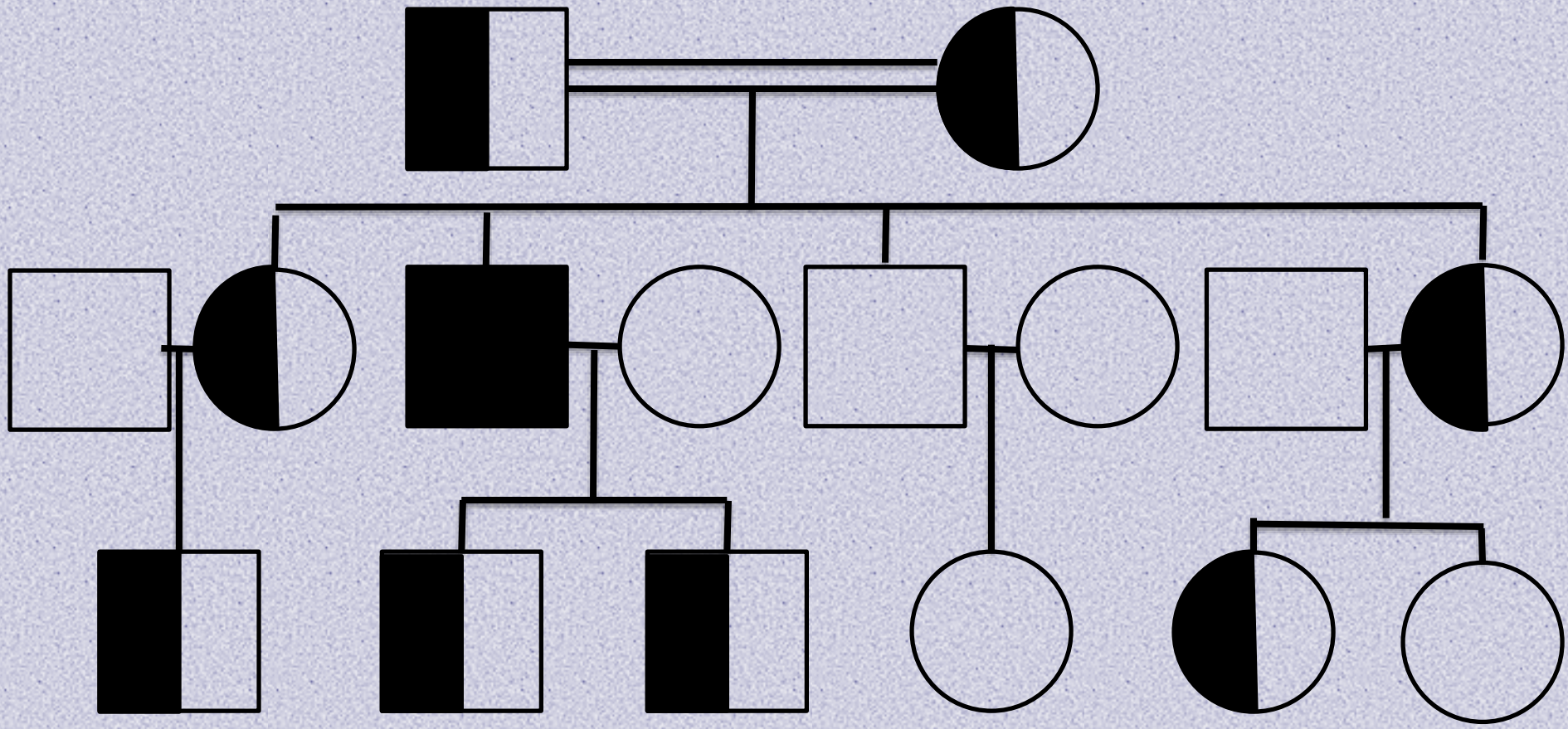
Types of Inheritance

- Autosomal recessive
  - Trait appears in siblings, not in their parents or offspring (**not in every generation**)
  - On the average, **one-fourth** of sibs of propositus are affected
  - Parents of the affected child may be **consanguineous**
  - **Males and females equally likely to be affected**

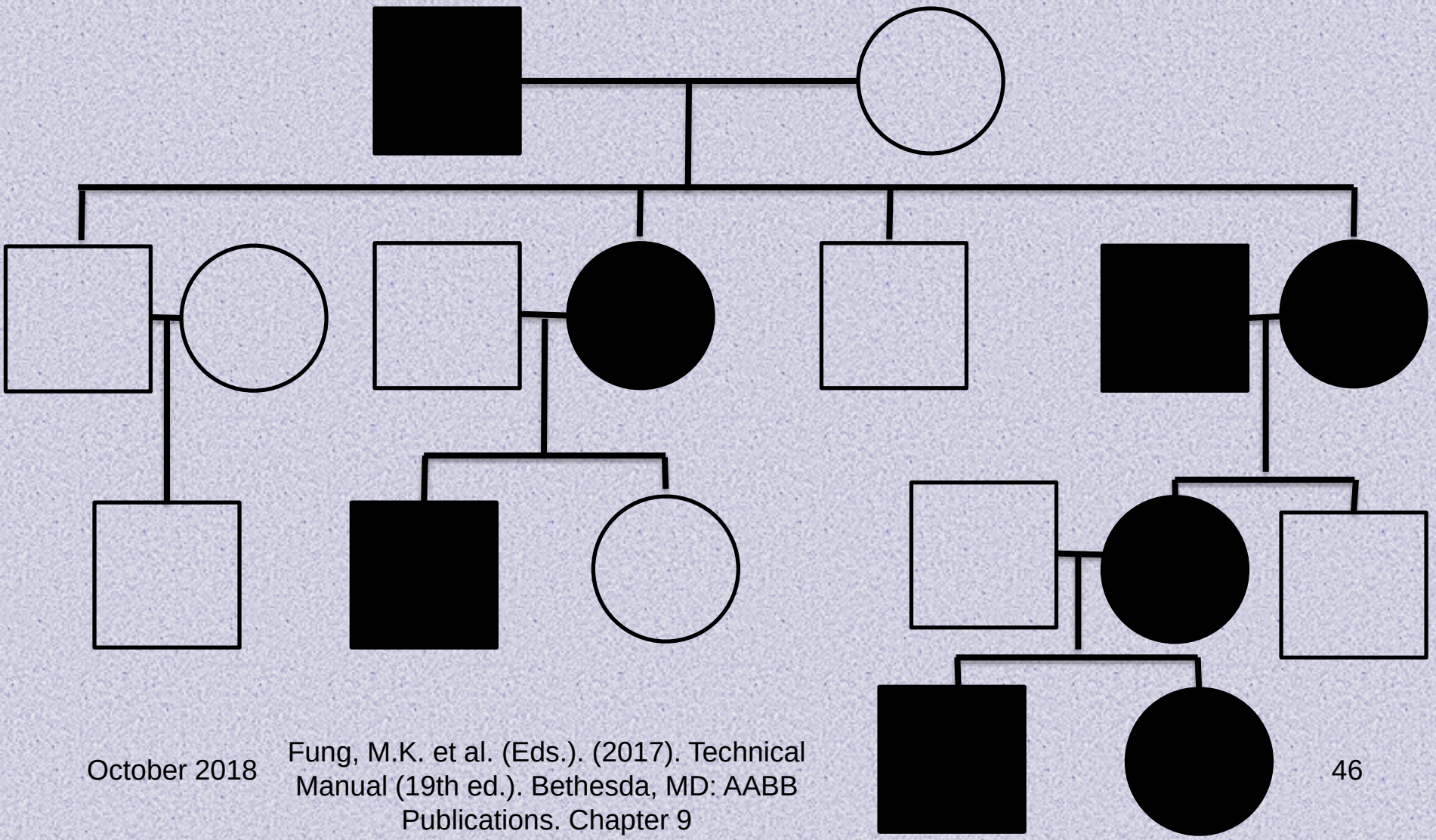
Autosomal Recessive



Types of Inheritance

- Sex-linked dominant
  - Affected **Males** (**XY**) transmit the trait to **ALL** daughters and to **NO** sons
  - Affected **Females** (**heterozygous** (**XX**)) transmit to **half** of their children of either sex.
  - **Homozygous females** (**XX**) transmit to **ALL** their children
  - Distinguished from autosomal dominant only by offspring of affected males

Sex-Linked Dominant



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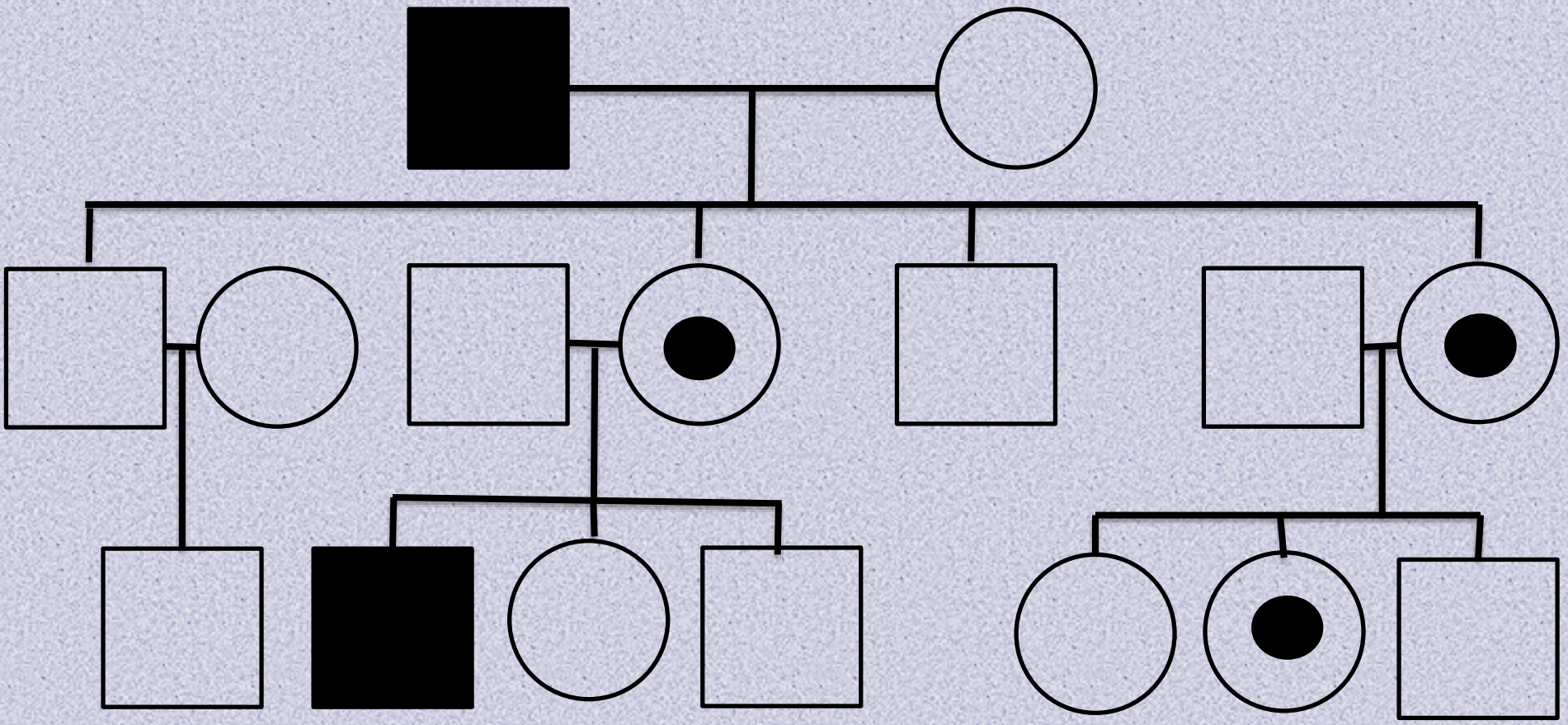
Types of Inheritance

- Sex-linked recessive
  - Incidence of the trait is much **higher in males than females**
  - Trait passed from **affected man through all daughters to half of sons**
  - Trait is **never** transmitted directly from father to son
  - Trait may be transmitted through a series of female carriers

Sex-Linked Recessive



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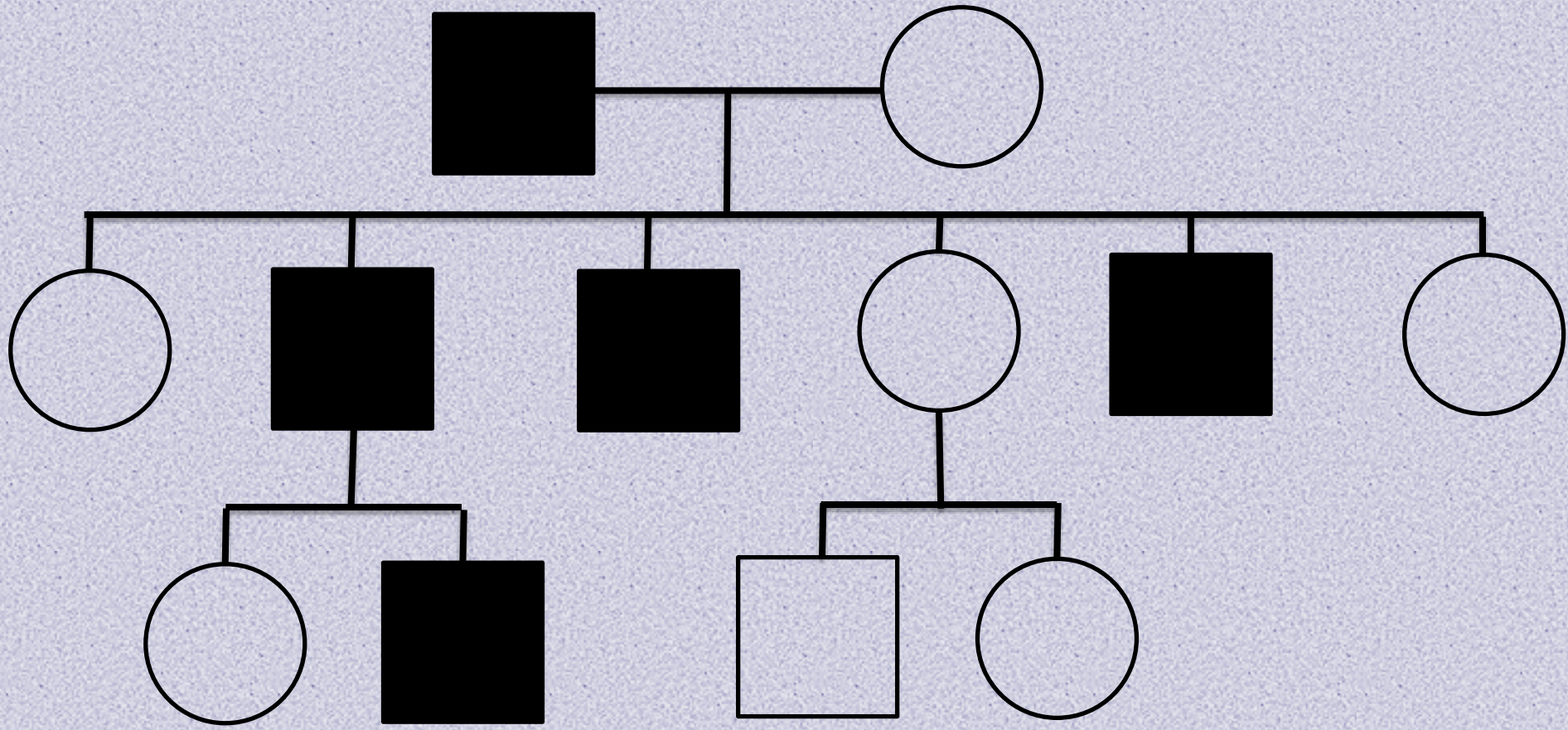
Types of Inheritance

- Y-linked
  - Resembles X-linked
  - Trait is transmitted only from **father to son**, never to daughter
  - **ALL** sons will be affected

Y-Linked



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Blood Group Chromosomes

Chromosome	Blood Group
1	Rh, Duffy, Scianna, Cromer, Knops, Vel
2	Gerbich, Lan
3	Globoside
4	MNS, JR
6	Chido/Rodgers, I, RHAG, HLA, Augustine
7	Kell, Yt, Colton
9	ABO, Gill, FORS
11	Indian, Raph, CD59
12	Dombrock
15	JMH
17	Diego
18	Kidd
19	Lutheran, Lewis, LW, H, Ok,
22	P1Pk
X	Xg, Kx

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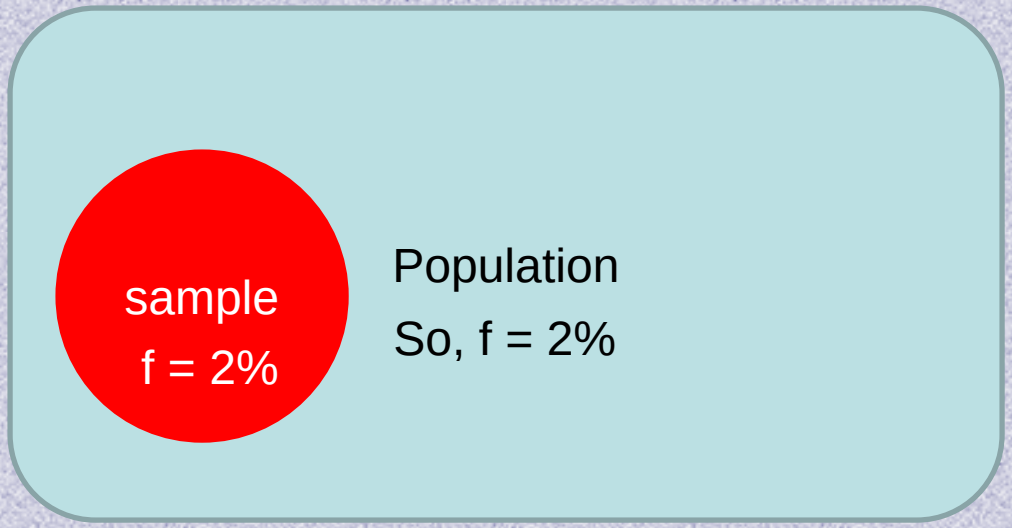
Population Genetics

- Gene and phenotype frequencies are based on probability



- To determine the frequency of any two (or more) unrelated traits, simply multiply the frequencies of each trait.

Jorde, L.B., Carey, J.C., Bamshad, M.J., & White, R.L. Medical Genetics, 4th ed. St. Louis, MO: Mosby, 2009. Chapter 3

Population Genetics

- Gene frequency changes:
  - **Selection** - One gene makes organism more efficient in reproduction, gene increases in frequency
    - Sickle Cell Disease
  - **Genetic drift** – random change in gene frequency by chance, seen more in small populations
    - Ellis–van Creveld syndrome in PA Amish
  - **Migration/Gene flow** – movement of population and breeding with other populations
  - **Mutation** – change in genetic material
  - **Meiotic drive** – more genes for one allele produced during meiosis

Jorde, L.B., Carey, J.C., Bamshad, M.J., & White, R.L. Medical Genetics, 4th ed. St. Louis, MO: Mosby, 2009. Chapter 3

Population Genetics

- Hardy/Weinberg Equation
- Basic Formula: $(a + b)^2$
  - Two heterozygous parents: (Aa x Aa)
  - Offspring: 1 AA + 2 Aa + 1aa

	Mom Aa	
Dad Aa	AA	Aa
	Aa	aa

Fung, M.K. et al. (Eds.). (2017). Technical Manual (19th ed.). Bethesda, MD: AABB Publications. Chapter 9

Hardy-Weinberg Equation

- Gene Frequencies

  p and q (2 allele)

  p, q, and r (3 allele)

- Phenotype
  - 2 allele

    p^2 and q^2 (Homozygous)

    2pq (Heterozygous)
  - 3 allele

    p^2 , q^2, r^2 (Homozygous)

    2pq, 2pr, 2qr (Heterozygous)

Hardy-Weinberg Equation

- Generalized equation:
  - $(p + q)^2 = p^2 + 2pq + q^2 = 1$
  - For 2 alleles (gene frequencies):
    - $p + q = 1$ or $q = 1 - p$
  - Expanded (phenotype frequencies):
    - $p^2 + 2p(1 - p) + (1 - p)^2 = 1$

Assumption for Hardy-Weinberg Equation

- Individuals of each genotype must be as reproductively fit as individuals of any other genotype (no infertility or mortality)
- Population must have large number of individuals
- Random mating must occur
- No mutations
- No migration

Genetics Problem 1

- Assume that in a given population 84% of the individuals are D positive and 16% are D negative (d):
  - p = gene frequency of D
  - q = gene frequency of d
  - Then:

Homozygous (DD) = p^2

Heterozygous (Dd) = 2pq

= 0.84

D negative (dd) = q^2

= 0.16

1.00

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Genetics Problem 1, cont.

- $q^2 = 0.16$,
  - so q = square root of 0.16 = 0.4
- p + q = 1 and p = 1 - q = 1 - 0.4 = 0.6
  - p = 0.6
  - q = 0.4
- Therefore:
  - $DD = p^2 = (0.6)^2 = 0.36$
  - $Dd = 2pq = (2)(0.6)(0.4) = 0.48$
  - $dd = q^2 = (0.4)^2 = 0.16$

  1.00

Genetics Problem 2 (2 allele system):

- Trait is autosomal dominant; occurs in 51% of the population. What is the gene frequency?

  $p^2 + 2pq + q^2 = 1$; $p + q = 1$

  p = GF of dominant trait

  q = GF of recessive allele

  $p^2 + 2pq = 0.51$; $q^2 = 0.49$

  q = 0.7

  p = 1 - 0.7 = **0.3**

Genetics Problem 3, (2 allele blood group system):

- Population studies reveal that 27% type Jk^a negative. What are the gene frequencies of Jk^a and Jk^b in this population? What percentage are Jk(a+b+)?

  $p^2 + 2pq + q^2 = 1$; $p + q = 1$

  p = gene freq. of Jk^a; q = gene freq. of Jk^b

  $q^2 = 0.27$; **q = 0.52 (Jk^b)**

  p = 0.48 (Jk^a)

  2pq = 0.50 - Jk(a+b+)

Genetics Problem 4 (3 allele blood group system):

- 3 Genes: $p + q + r = 1$
- Phenotypes:

$p^2 + 2pq + q^2 + 2qr + r^2 + 2pr = 1$

Homozygous:

p^2 = AA; q^2 = BB; r^2 = OO

Heterozygous:

$2pq$ = AB; $2qr$ = BO; $2pr$ = AO

Genetics Problem 4, cont.
(3 allele blood group system):

- Our population has:
  - 28% A (AA, AO) - 53% O (OO)
  - 15% B (BB, BO) - 4% AB (AB)

- O's (OO): $r^2 = 0.53$

$$\sqrt{r} = \sqrt{0.53} = \mathbf{0.73}$$

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Genetics Problem 4, cont.
(3 allele blood group system):

- Our population has:
  - 28% A (AA, AO) - 53% O (OO)
  - 15% B (BB, BO) - 4% AB (AB)

- A's & O's (AA, AO, OO): .28 + .53 = 0.81

$$p^2 + 2pr + r^2 = 0.81$$
$$(p + r)^2 = 0.81$$
$$\sqrt{(p + r)^2} = \sqrt{0.81}$$
$$p + r = 0.90$$
$$p = 0.90 - 0.73$$
$$\mathbf{p = 0.17}$$

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Genetics Problem 4, cont. (3 allele blood group system):

- Our population has:
  - 28% A (AA, AO) - 53% O (OO)
  - 15% B (BB, BO) - 4% AB (AB)

- q = B gene frequency

  $p + q + r = 1$

  $q = 1 - (p+r)$; $q = 1 - 0.90$

  $q = 0.10$

Genetics Problem 4, cont.
(3 allele blood group system):

- G.F. (*A [p]*) = 0.17
- G.F. (*B [q]*) = 0.10
- G.F. (*O [r]*) = 0.73

- What is percentage of BO individuals in this population?

  2qr = 2 (0.10)(0.73) = 0.146 or **14.6%**

Genetics Problem 5:

- A patient has an anti-E and anti-K. How many units of ABO compatible RBCs must be tested to find three compatible units?
  - Approx. 30% of population E positive
    - (70% E negative)
  - Approx. 10% of population K positive
    - (90% K negative)
  - 0.7 X 0.9 = 0.63 (or 63% of population is negative for E and K)
- Therefore, approximately 3 out of 5 random units would be negative for K and E.

Caution!

- Beware of ethnic differences
  - (i.e. Duffy antigens in African ethnicity).

Genetics Problem 6

- A father's genotype is BO and the mother is OO? What is the probability that they will have 3 OO children in a row?

  Each time the probability is 50% (or 0.5).

  0.5 x 0.5 x 0.5 = **0.125 or 1/8**

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One Last Genetics Problem

- Given the following phenotype information determine the most likely genotype for the child?

Father: D-, C-, E-, c+, e+
Mother: D+, C+, E+, c+, e+
Paternal Grandfather: D+,C+,E-,c+, e+
Paternal Grandmother: D+, C-, E-, c+, e+
Maternal Grandmother: D-,C+,E-,c+,e+

a. R_0r'
b. R_1R_2
c. rr'
d. rr

One Last Genetics Problem

- Given the following phenotype information determine the most likely genotype for the child?

Father: D-, C-, E-, c+, e+ (rr)
Paternal Grandfather: D+,C+,E-,c+,e+ (R_0r' or R_1r)
Paternal Grandmother: D+, C-, E-, c+, e+ (R_0r)

	Grandmother R_0r	
Grand father R_0r'	R_0R_0	R_0r
	R_0r'	rr'

	Grandmother R_0r	
Grand father R_1r	R_1R_0	R_1r'
	R_0r	rr

One Last Genetics Problem

- Given the following phenotype information determine the most likely genotype for the child?

Mother: D+, C+, E+, c+, e+ (R_2r' or R_1r'')

Maternal Grandmother: D-,C+,E-,c+,e+ (rr')

One Last Genetics Problem

- Given the following phenotype information determine the most likely genotype for the child?

Father: D-, C-, E-, c+, e+ (rr)
Mother: D+, C+, E+, c+, e+ (R_2r')

	Mom R_2r'	
DAD rr	R_2r	rr'
	R_2r	rr'

One Last Genetics Problem

- Given the following phenotype information determine the most likely genotype for the child?

Father: D-, C-, E-, c+, e+

Mother: D+, C+, E+, c+, e+

Paternal Grandfather: D+,C+,E-,c+,e+

Paternal Grandmother: D+, C-, E-, c+, e+

Maternal Grandmother: D-,C+,E-,c+,e+

a. R_0r'

b. R_1R_2

c. rr'

d. rr

SBB/BB Exam Review

Lab Math Problems

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Lab Math

- Scientific Notation (x 10^6)
  - Add, subtract, multiply, & divide
- Conversion Factors
  - Weight: 1lb = 0.45kg; 1kg = 2.2 lbs
  - Length: 1in = 2.54cm; 1cm = 0.39in
  - Volume: 1qt = .95L; 1L = 1.06qt
- Solution preparation (Technical Manual: Method 1-4)
- Concentration (V1C1 = V2C2)
- Dilutions (Technical Manual: Method 1-5 & 1-6)
- Statistics
  - Central tendency, Variability, Probability, Accuracy vs. Precision

Doucette, L. J. Mathematics for the Clinical Laboratory, 3rd ed.
Philadelphia: W.B. Saunders Company, 2016.

Lab Math

- Component Dosing
  - RBCs
    - Hct decreases at a rate of 1% per day
    - One unit of RBCs increase Hgb by 1 g/dl & Hct by 3%
    - 1ml of RBCs = 1 mg of iron
  - Plasma
    - One unit of plasma increase coag factors by 10%
  - Platelets
    - One WB derived plt increases 5,000-10,000/µl in an adult
    - One apheresis plt increases 50,000-60,000/µl in an adult
    - One WB derived plt increases 75,000-100,000/µl in a term infant

Lab Math Problem 1

- A 200 lb patient is admitted to the ER with a Hematocrit of 38% and fibrinogen level of 95 mg/dL. The physician decides that the Fibrinogen level should be close to 150 mg/dL

- How many bags of Cryoprecipitate is needed?

- First you need to determine the patient's weight in kg

$$200 \text{ lb} \times \frac{1 \text{ kg}}{2.2 \text{ lb}} = 90.909 \text{ kg}$$

Lab Math Problem 1, cont.

- Next, we need to determine the Plasma Volume
  - Approximate Total Blood Volume (BV)

    Adult: 60-66 ml/kg

    Term infant: 85-88 ml/kg

    Premature infant: 108 ml/kg

  - Total Blood Volume (ml) = Wt (kg) x BV (ml/kg)

    90.909 kg x 66 ml/kg = 6000 ml

  - Plasma Volume formula (PV) (ml) =

    Total Blood Volume (ml) x (1.0 - Hct)

    6000 ml x (1 - 0.38) = 3720 ml

Fung, M.K. et al. (Eds.). (2017). Technical Manual (19th ed.). Bethesda, MD: AABB Publications. Appendix 5.

Lab Math Problem 1, cont.

- Now, we can determine the amount of Fibrinogen needed

  – Amount of fibrinogen (mg) required =

PV (ml) x (desired fibrinogen – initial fibrinogen) x 1dl/100ml

3720 ml x (150 mg/dl – 95 mg/dl) x 1dl/100ml

3720ml x 55 mg/dL x 1dl/100ml

2046 mg

Lab Math Problem 1, cont.

- Now, we can determine the amount of Cryoprecipitate
  - Assume amount of Fibrinogen in Cryo at 250mg
  - Bags of Cryo needed =

    fibrinogen needed (mg) / 250 (mg/bag)

$$\frac{2046\ \cancel{mg}}{250\ \cancel{mg}/bag}$$

= 8.184 or 8 bags

Lab Math

- If Cryo is requested for FVIII dosing, calculation is similar
  1. Determine plasma volume
  2. Determine desired increment of FVIII (IU/ml)

     (desired FVIII – initial FVIII)
  3. Desired FVIII (IU)

     PV (ml) x desire increment FVIII (IU/ml)
  4. Bags of Cryo

     Assume 80 IU FVIII per bag of cryo

     Desired FVIII (IU)/80 IU/bag

Lab Math Problem 2

- A 160 cm patient weighing 82 kg was transfused with 6 x 10^{11} platelets. The patients pre-transfusion platelet count was 15,000 platelets/µL and 1 hour post transfusion count was 45,000 platelets/µL. What is the Corrected platelet count increment (CCI)?

- First, What is the patients Body Surface Area (BSA)?

$$BSA\ (m^2) = \sqrt{[Ht\ (cm) \times Wt\ (kg)/3600]}$$

$$\sqrt{\frac{160\ cm \times 82\ kg}{3600}}$$

BSA = 1.91 m^2

Fung, M.K. et al. (Eds.). (2017). Technical Manual (19th ed.). Bethesda, MD: AABB Publications. Appendix 5.

Lab Math Problem 2 , cont.

- Platelet Count Increment = 45,000-15,000 = 30,000

$$CCI = \frac{BSA(m^2) \times Plt\ increment}{\#\ platelets\ transfused}$$

$$CCI = \frac{1.91m^2 \times 30{,}000 \times 10^{11}}{6 \times 10^{11}}$$

$$CCI = 9550$$

Refractory = CCI less than 5000 (two consecutive transfusions)

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Lab Math Problem 3

- A 73-lb female is donating an autologous Whole Blood unit for her surgery in two weeks. How much blood can be drawn from this patient?

Allowable amount (ml) = Donor wt (lb)/110 lb x 450 ml

73 lb/110 lb x 450ml

298 ml of blood allowed to be collected

Lab Math Problem 3 , cont.

- How much anticoagulant must be removed from the primary container?

405-495ml collected in a 450ml bag = 63 ml anticoagulant

450-550ml collected in a 500 ml bag = 70 ml anticoagulant

$$\frac{\text{Anticoagulant in bag (ml)}}{110\text{ lb}} = \frac{\text{amount anticoagulant needed (ml)}}{\text{donor weight (lb)}}$$

$$\frac{63\text{ml}}{110\text{ lbs}} = \frac{x}{73\text{ lbs}}$$

x = 41.8 ml anticoagulant needed

63ml - 41.8ml = 21.2 ml anticoagulant to remove

Lab Math Problem 4

- A test is performed on patients having a disease and a control group to determine the sensitivity and specificity of the test.

True Positive (TP) = patients who test positive

True Negative (TN)= controls who test negative

False Positive (FP)= controls who test positive

False Negative (FN)= patients who test negative

$$\text{Sensitivity} = \frac{TP}{TP + FN} \times 100$$

$$\text{Specificity} = \frac{TN}{TN + FP} \times 100$$

Lab Math Problem 4 , cont.

	Positive	Negative
Patient	285	15
Control	50	450

- Sensitivity = 285/285+15 x100 = 95%
- Specificity = 450/450+50 x100 = 90%
- When looking for a screening test which is most important Sensitivity or Specificity?

Lab Math Problem 4 , cont.

	Positive	Negative
Patient	285	15
Control	50	450

- Positive Predictive Value (PPV)

$$\frac{TP}{TP + FP} \times 100 \qquad \frac{285}{285 + 50} \times 100$$

PPV = 85%

- Negative Predictive Value (NPV)

$$\frac{TN}{TN + FN} \times 100 \qquad \frac{450}{450 + 15} \times 100$$

NPV = 97%

Lab Math Problem 5

- It is determined that a 30 week gestation fetus that weighs 1300 grams with a hematocrit of 25% requires an IUT (RBC with a hematocrit of 85%) due to maternal anti-c. Desired final hematocrit is 45%.
  - Fetal placental total volume (FV) (ml) =
    fetal wt (g) x 0.14 ml/g
    1300 ~~g~~ x 0.14 ml/~~g~~ = 182 ml
  - Hematocrit increment desired =
    desired post Hct – pre Hct
    45% - 25% = 20% = 0.20
  - Volume to transfuse (ml) =
    FV (ml) x Hct increment / Hct of RBCs
    182 ml x 0.20 / 0.80 = 45.5 ml

Bonus Question:
What type of RBCs would you transfuse?

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Lab Math Problem 6

- Given the following information, determine the number of FTEs required for the workload:

  - Vacation/year: 2 weeks
  - Ave. sick leave/year: 5 days
  - Holidays/year: 5 days
  - Productivity: 75% (45min/hr)
  - Annual Workload: 400,000 units (minutes/year)

Lab Math Problem 6 , cont.

- # hours worked/year:
  - 52 wks/yr – Vac/Sick/Holiday (4 wks) = 48 wks/yr
  - 48 wks/yr x 40 hrs/wk = 1920 hrs/yr
- # productive minutes/year:
  - 1920 hrs/yr x 45 min/hr = 86,400 min/yr
- # FTEs
  - 400,000 units / 86,400 min/yr = 4.63 FTE

Lab Math Problem 7

- Your laboratory is deciding whether to keep a test currently on your test menu. You decide to do a break even analysis.
  - Net income = revenue – total cost
  - Volume of tests needed to break even =

(Fixed cost/test + net income) / (expected revenue/test – Variable costs/test)

Fixed cost/test (reagents, labor, etc.) = $12.50

Variable cost/test = $2.65

Total cost = $5,000

Revenue = $7,000

Expected revenue per test = $6.25

Net income = 7,000 – 5,000 = 2,000

tests to break even =
(12.50 + 2,000) / (6.25 – 2.65)
559 tests

Lab Math

- Additional Formulas:
  - Relative Centrifugal Force $11.17(r)(n/1000)^2$
    - r = radius in cm
    - n = rotor speed in rpm
  - Volume Fetal maternal hemorrhage (ml)

(# fetal cells counted x maternal blood volume) / # maternal cells counted

Or

% fetal cells x 50

  - RhIg dose (vials) =

Volume FMH whole blood (ml) / 30 ml/vial

Volume FMH pRBC (ml) / 15 ml/vial

- Round up or down + 1 vial

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Lab Math

- Additional Formulas:
  - Percent Yield (Cryo or WB platelets) =

$$\frac{\text{count x ml final product}}{\text{count x ml original product}} \times 100$$

  - Relative Risk (RR) HLA disease association

$$\frac{\text{(\% patients with HLA antigen) x (\% controls without HLA antigen)}}{\text{(\% controls with HLA antigen) x (\% patients without HLA antigen)}}$$

  - Neonatal RBC exchange transfusion

Volume of RBCs to transfuse (ml) =

BV (ml) x (Observed Hct – Desired Hct) / Observed Hct

Fung, M.K. et al. (Eds.). (2017). Technical Manual (19th ed.). Bethesda, MD: AABB Publications. Chapter 16 & 24

SBB/BB Exam Review

Transfusion Reactions

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Transfusion Reaction Categories

- Acute (<24hrs) or Delayed (>24 hrs)
- Immunologic (Ag-Ab) or Non-immunologic
- Intravascular or Extravascular Hemolysis

Recognize signs and symptoms as summarized in the Table provided in the Technical Manual

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Acute Hemolytic Transfusion Reaction (HTR) - Immune

- Occurs within minutes of start of infusion
  - Fever, chills/rigors, hemoglobinemia, hemoglobinuria, excessive pain and/or bleeding at infusion site, facial flushing
  - IgM or complement-fixing IgG
  - Activation of Complement, Kinin and Coagulation systems. Phagocyte activation
  - Systemic inflammatory response
  - Hypotension, renal failure, DIC

Fung, M.K. et al. (Eds.). (2017). Technical Manual (19th ed.). Bethesda, MD: AABB Publications.

Acute HTR – (Cont.)

- Treatment
  - Stop transfusion
  - Treat hypotension - maintain adequate renal blood flow with fluids and diuretics
    - Furosemide
  - Monitor for/support DIC (Plt, plasma, cryo)
  - Medical management may be complicated and require aggressive interventions (exchange transfusion)

Delayed Hemolytic Txn Rx

- Usually only causes delayed serologic reaction (no clinical symptoms) occasionally, may see hemolysis
- Most common antibodies
  - Jk^a, K, Fy^a, E, c, D
- If DHTR suspected, test for unexpected alloantibody on RBCs and in serum.
  - Compare to previous results or patient history

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Delayed Serologic Tx Rx: Alloimmunization

- Immune response to foreign antigens on RBC, or WBC and platelets (HLA)
  - Weeks to months after transfusion
  - Antibody may fall to undetectable levels (Kidd)
  - Anamnestic response (within hours to days)
- DAT will become positive first
  - May need to elute Ab off RBCs to identify
- Prior to antibody being detected in serum, crossmatch may be compatible

Passively Transfused Antibodies

- In plasma, platelets, IVIG
  - Group O high titer anti-A, anti-B given to group A or B patient

- Transplantation
  - Lymphocytes engraft, produce antibody to recipient RBCs - hemolysis

Types of Transfusion Reactions

- Urticarial
  - Only reaction where the transfusion can be stopped and restarted
- Anaphylactic
  - Causes -IgA deficiency, Anti-IgA
  - Prevention
    - IgA-deficient components
    - Washed RBCs and platelets
    - Autologous
  - Other triggers, antibodies against haptoglobin or C4
- ACE Inhibitor hypotension
  - Inhibited metabolism of Bradykinin

Know causes, treatment and prevention

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Types of Transfusion Reactions

- Acute Non-immune Mediated Hemolysis
  - Heating, Freezing, IV solutions
- Transfusion-Associated Sepsis
  - Platelet testing / Pathogen reduction
- Febrile Nonhemolytic Reactions
  - Common, but initial symptom is Fever

Other Acute Complications

- **TRALI - Acute onset (within 6 hrs)**
  - Hypoxemia, respiratory failure, hypotension, fever
  - Bilateral lung infiltrates (white out on chest x-ray)
  - No circulatory overload
- **Transfusion-Associated Circulatory Overload**
  - Similar symptoms to TRALI, but responds to diuretics
  - Pulmonary edema is cardiogenic (TRALI noncardiogenic)
- **Metabolic Reactions**
  - Citrate toxicity
  - Hypothermia
  - Hyperkalemia / hypokalemia
- **Air embolism**

Transfusion-Associated Graft-vs-Host Disease (TA-GVHD)

- Rare, usually fatal - no effective treatment
- Donor lymphocytes engraft in the recipient, proliferate, and attack host tissue.
- Symptoms usually appear within 8-10 days of transfusion: maculopapular rash, fever, enterocolitis, elevated liver function tests
- Usually see refractory pancytopenia with bleeding and infectious complications
- Prevention – Irradiation

Post-transfusion Purpura

- Abrupt onset of severe thrombocytopenia (<10,000/μL) following blood transfusion in a previously pregnant or transfused patient
- Most patient cases have platelets that lack the HPA-1a (PI^{A1}) antigen, and form an antibody directed to this antigen
- Antibody destroys HPA-1a positive donor platelets, but also the patient's own HPA-1a negative platelets (mechanism unknown)
- Random platelet transfusions are contraindicated, treatment is IVIG

Evaluation of Transfusion Reaction

- Role of clinician: stop transfusion, clerical check, notify MD & BB, observe/document signs, collect samples for BB, return product to BB
- Role of Blood Bank: clerical check, post specimen check for hemolysis, repeat ABO on post specimen, DAT on post specimen
  - DAT will be negative if cells were destroyed
  - DAT will be + if incompatible cells coated with antibody, mf not always seen, DAT can remain pos. for months, sometimes autoantibody / mimicking antibody formed
  - Non-immune hemolysis causes hemoglobinemia, but DAT will be negative
- Additional testing for suspected HTR

Records of Transfusion Complications

- Interpretation of the evaluation shall be recorded in the patient's medical record
- Maintain records indefinitely
- Notification to collecting facility
- Fatalities – report to FDA

SBB/BB Exam Review

Hemolytic Disease of the Fetus and Newborn (HDFN)

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HDFN Prerequisites

- Mom lacks antigen
  - Exposed through pregnancy or transfusion
- Fetus possesses antigen; paternal inheritance
- Mom has formed an IgG
  - Sensitization depends on:
    - Recognition of antigen
    - Responder
    - Antigen is immunogenic
    - Amount of bleed
    - ABO compatibility
- Stillbirth, hydrops, kernicterus

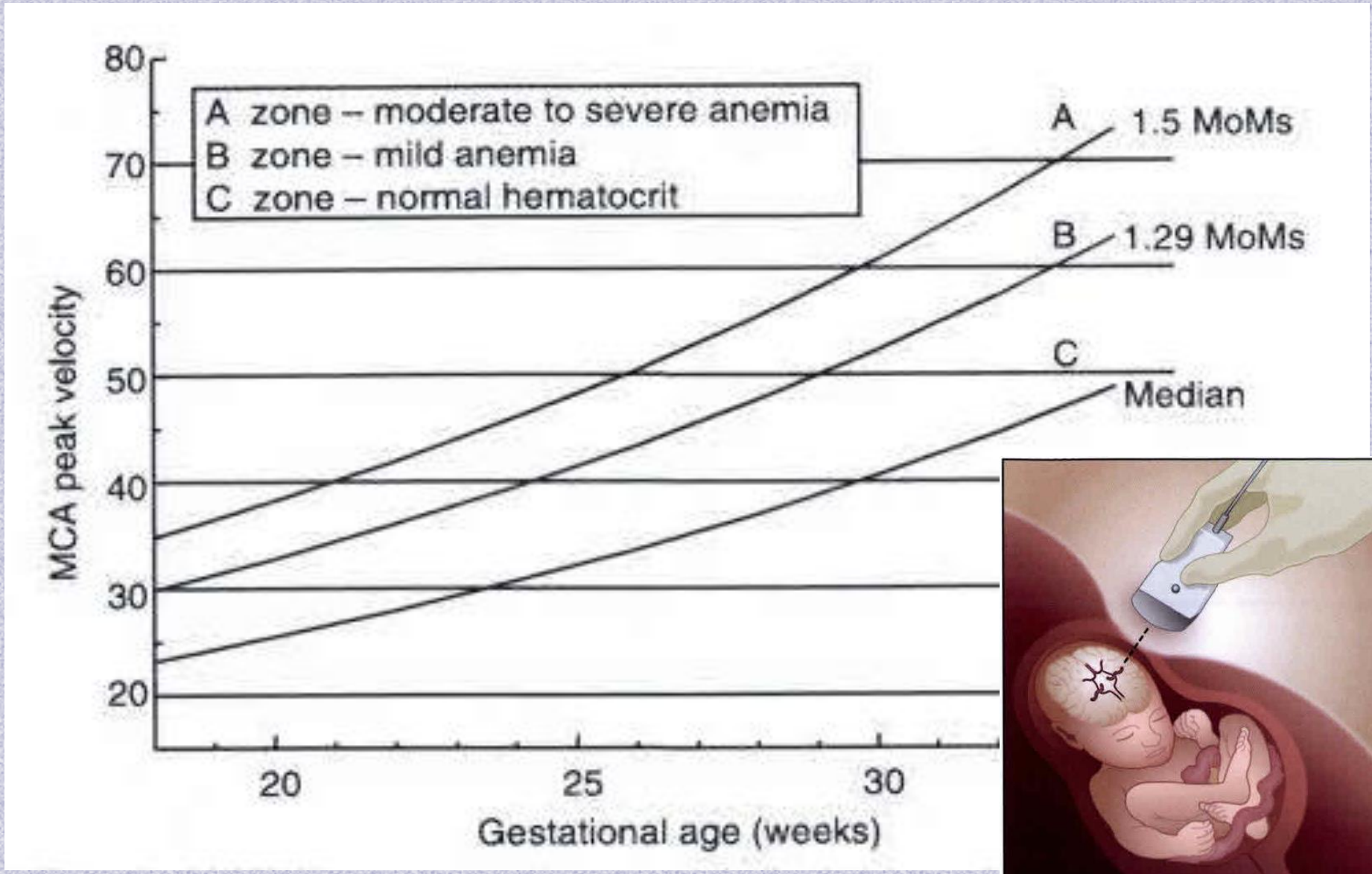
HDFN Severity

- 40-50% mild, 25% moderate, 20% severe
- Class/subclass of antibody
- Strength/quantity of antibody
- Presence/quantity of antigen on fetal RBC
- Efficiency of placental transfer
- Efficiency of fetal RES/macrophages
- Maternal antibodies to fetal macrophages (HLA-DR)
- Competition effect of antigen in fetal body fluids and tissues

HDFN Pathophysiology

- Antibody coated RBC removed by macrophages in spleen causing anemia
- Immature BM RBC release – erythroblasts
- Extramedullary hematopoiesis, large liver & spleen
- Hypoxia, ↑Cardiac output, ↓blood viscosity, ↑blood flow (cardiac failure, pleural & paracardial effusion)
  - Measured on Doppler Ultrasonography (MCA-PSV)
  - > **1.5 Multiples of the Median (MoM)** indicates moderate/severe anemia
  - Non-invasive, preferred over amniocentesis

Doppler Ultrasonography



After Birth – Bilirubin Problem

- Maternal liver processes bilirubin before birth
- Infant liver immature at birth
  - ↓Glucuronyl transferase, can't adequately conjugate bilirubin from RBC destruction
- Unconjugated bilirubin is toxic to CNS
  - Kernicterus

Fung, M.K. et al. (Eds.). (2017). Technical Manual (19th ed.). Bethesda, MD: AABB Publications.

Rh vs. ABO HDFN

	Rh	ABO
Mother	Neg	Group O
Infant	Pos	Group A or B
1st Born	5%	40-50%
Stillbirth/hydrops	Frequent	Rare
Severe anemia	Frequent	Rare
DAT	Pos	Pos or Neg
Spherocytes	None	Present
Ex Transfusion	Frequent	Very rare
Phototherapy	Adjunct to Exch.	Often only treatment

HDFN Testing & Treatment

- Prenatal testing & testing on neonates
- Titrations
- Rosette test, Kleihauer-Betke, Flow Cytometry – FMH calculations
- RHIG – timing, calculations & dosing
- Treatment – phototherapy / transfusions
  - IUT – intraperitoneal, intravascular
- Exchange transfusions
  - Indications, beneficial effects, component requirements, calculations

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SBB/BB Exam Review

Coagulation

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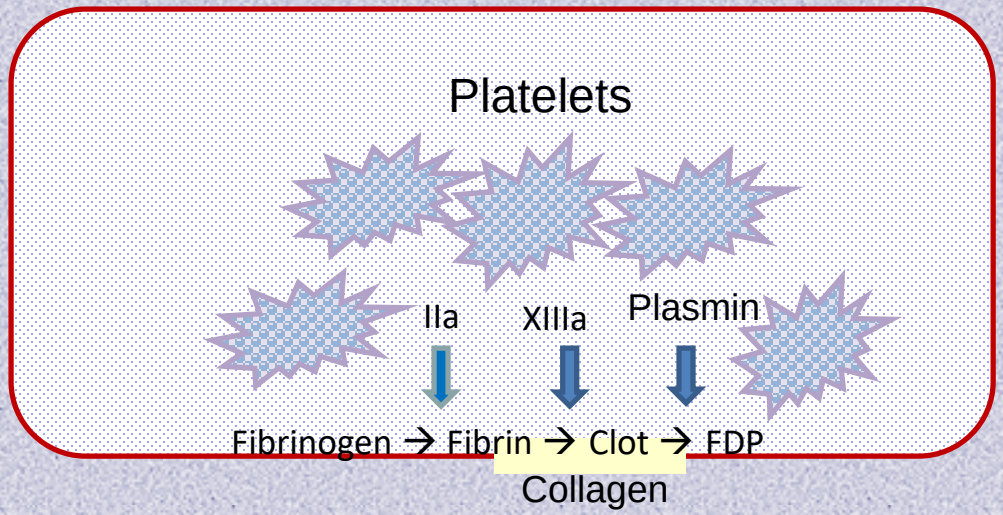
Hemostasis

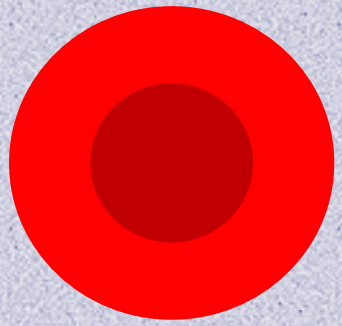
- Simultaneous action of:
  - Vascular System
  - Platelets
  - Coagulation Cascade
  - Fibrinolysis



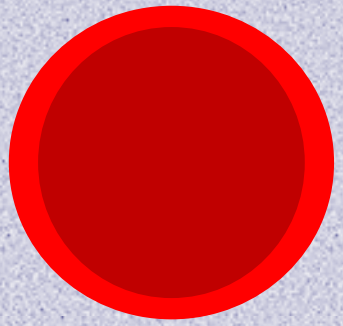
DeLoughery, T. *Hemostasis and thrombosis, 2nd ed. 2004*

Vascular System

- When the blood vessel is damaged
  - Tissue Factor is released
  - Collagen and Laminin are exposed
  - Vasoconstriction of the Blood vessel to slow bleeding
  - Diverting Blood Flow



Vasoconstriction



Vasodilation

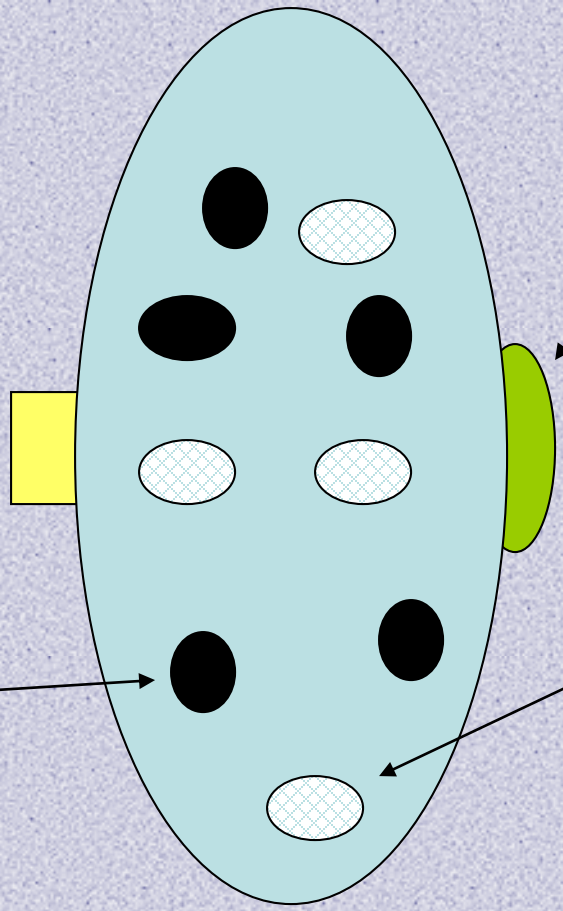
Platelets

- Produced in bone marrow
- Megakaryocyte – precursor
  - One megakaryocyte can produce 2,000 platelets
  - Platelets bud off edge (no nucleus)
  - Megakaryocyte eventually perishes
- Platelet lifespan is 9-10 days
- Platelets circulate freely or are sequestered in spleen
  - 1/3 of platelets are usually located in spleen

Function of Platelets

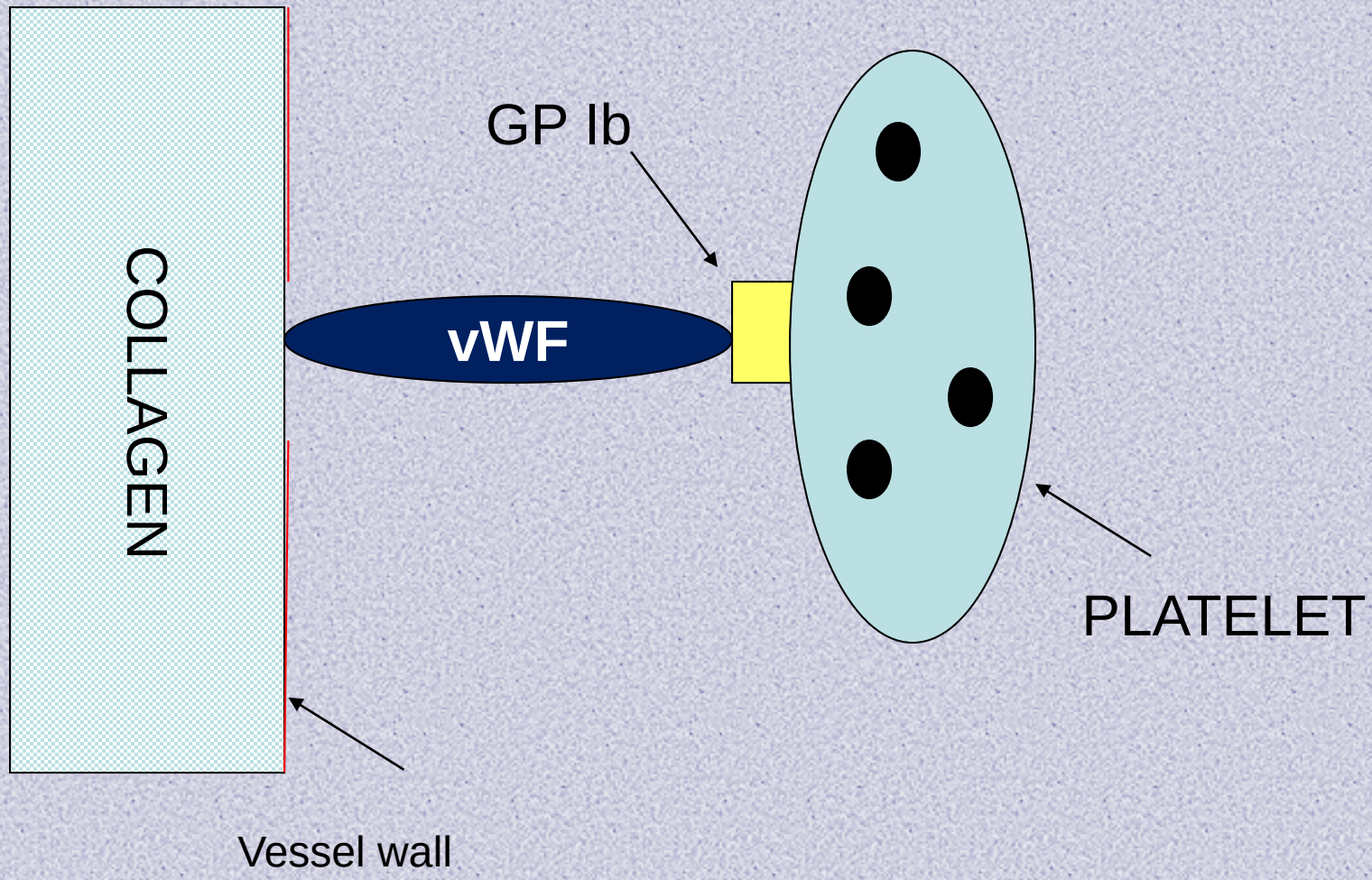
- **Storage** of ADP and proteins
- **Adhesion** to damaged endothelium
- **Aggregation** with other platelets
- Provide **surface** for coagulation reactions

Platelet Structure - Storage



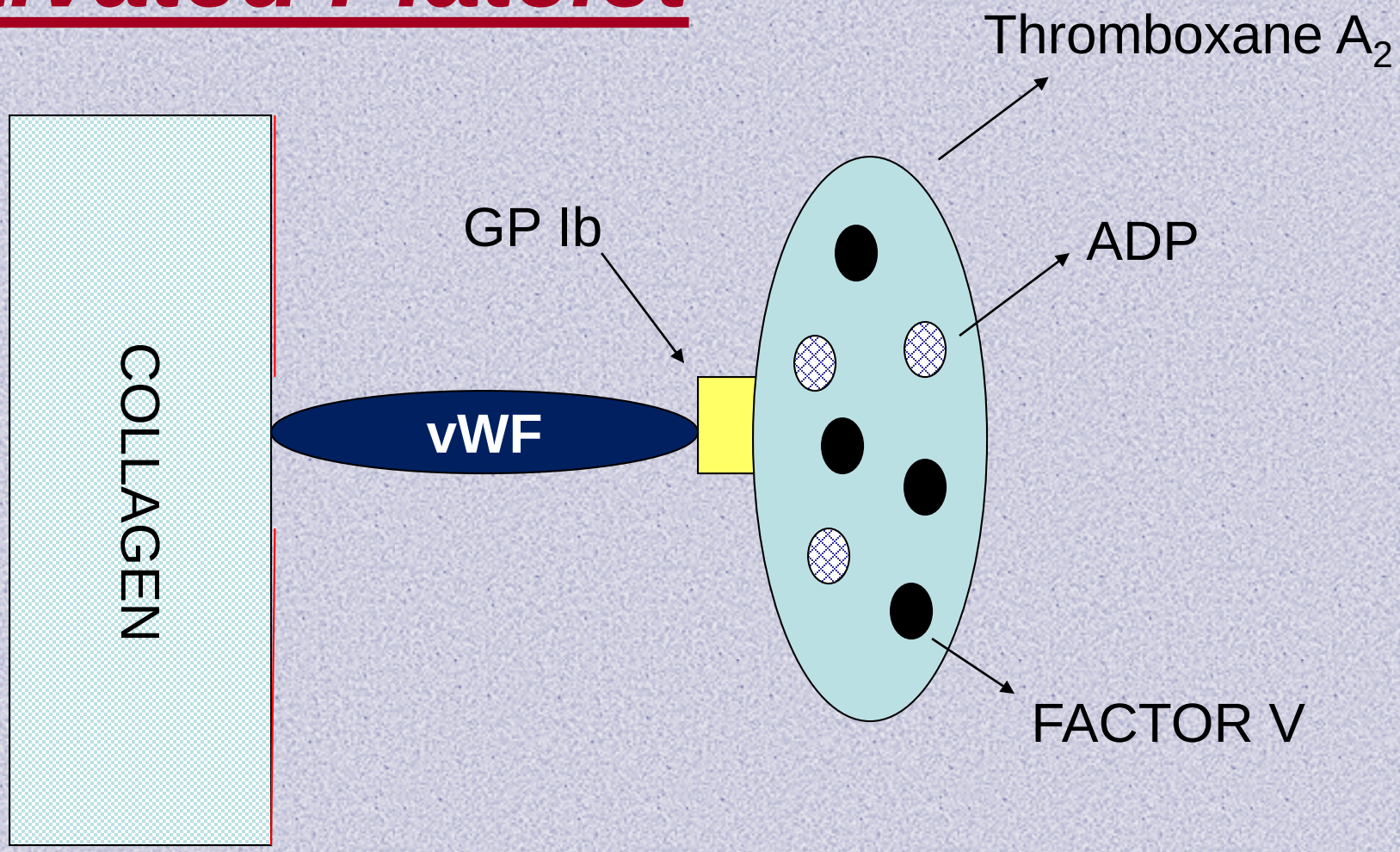
DeLoughery, T. *Hemostasis and thrombosis, 2nd ed. 2004*

Platelet Adhesion



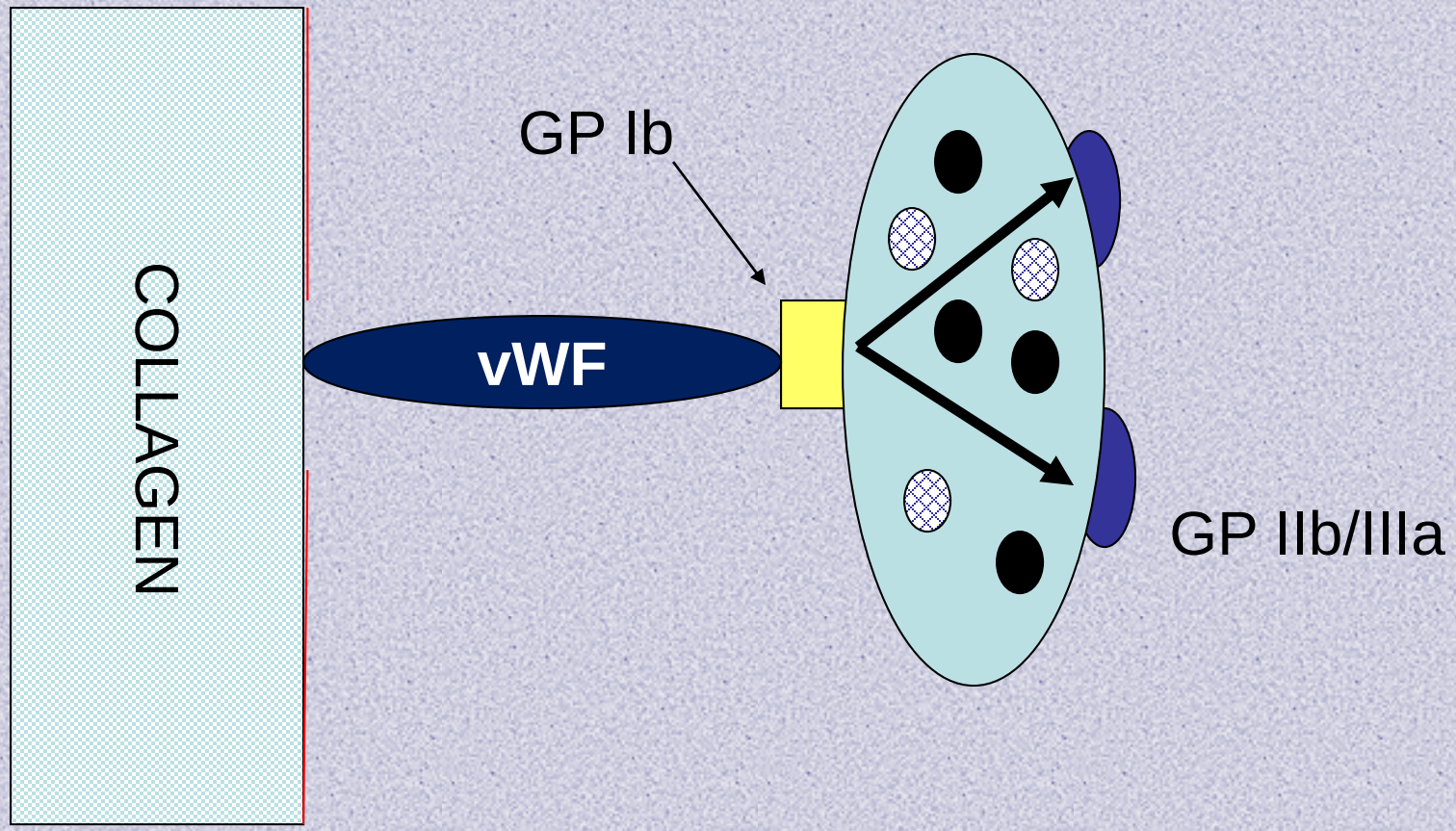
DeLoughery, T. *Hemostasis and thrombosis, 2nd ed. 2004*

Activated Platelet



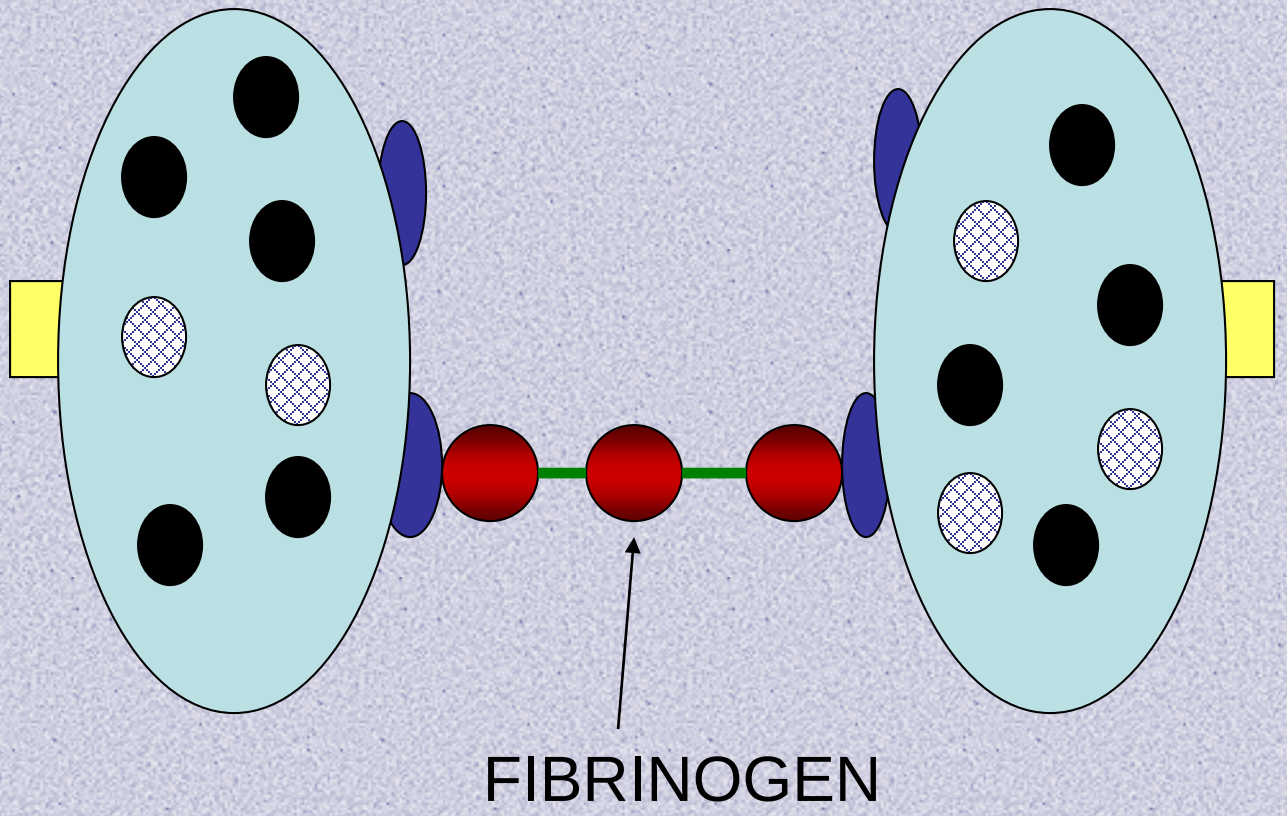
DeLoughery, T. *Hemostasis and thrombosis, 2nd ed. 2004*

GP IIb/IIIa Receptors



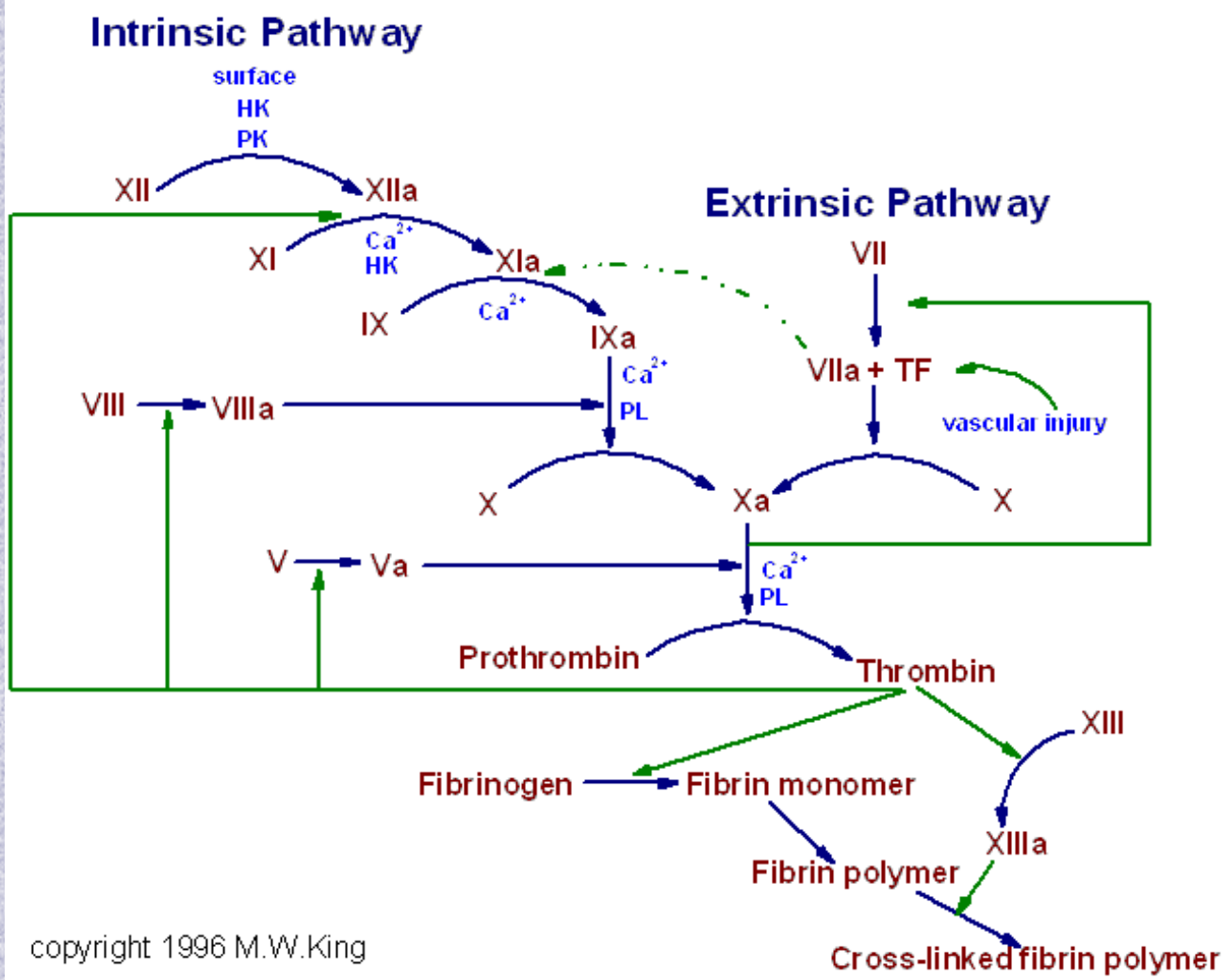
DeLoughery, T. *Hemostasis and thrombosis, 2nd ed. 2004*

Platelet Aggregation



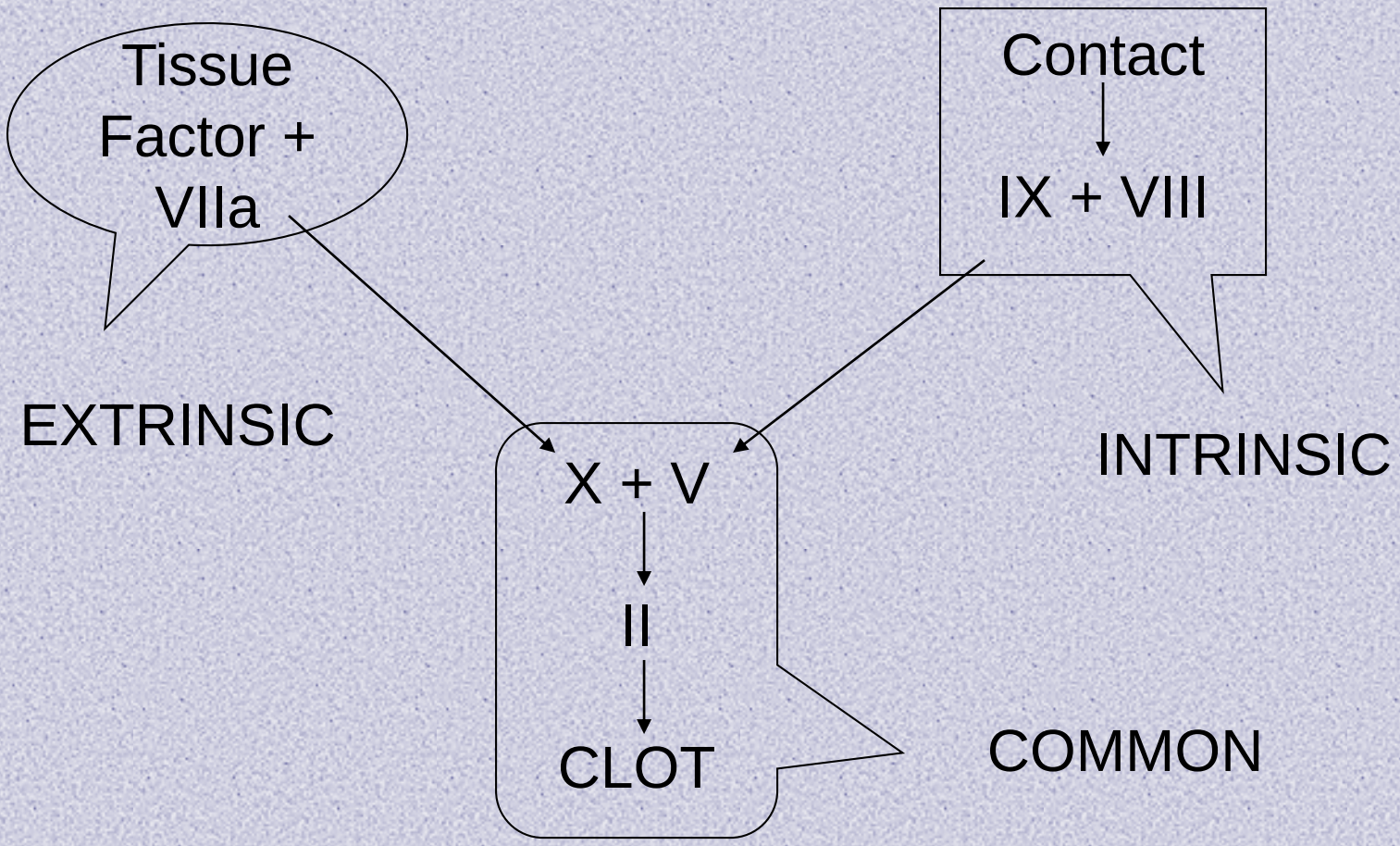
DeLoughery, T. *Hemostasis and thrombosis, 2nd ed. 2004*

Traditional Coagulation Pathway



From http://web.indstate.edu/thcme/mwking/blood-coagulation.html#image. Accessed 7/6/05.

"The old pathways…"



The Players

- Most of the coagulation proteins are either enzymes (serine proteases) or cofactors

Enzymes	Cofactors	Miscellaneous
Factor IIa	Tissue factor	Fibrinogen
Factor VIIa	Factor V	Factor XIII
Factor IXa	Factor VIII	$Alpha_2$ antiplasmin
Factor Xa	Protein S	PAI-1
Protein C		Antithrombin
TPA		
Plasmin		

Enzymes

- Factors II, VII, IX, X, protein C and protein S
  - Become activated are work on other enzymes or cofactors
  - Vitamin-K dependent
    - Without vitamin K, dysfunctional proteins are produced
      - Bleeding can occur
- **Warfarin blocks recycling of vitamin K**

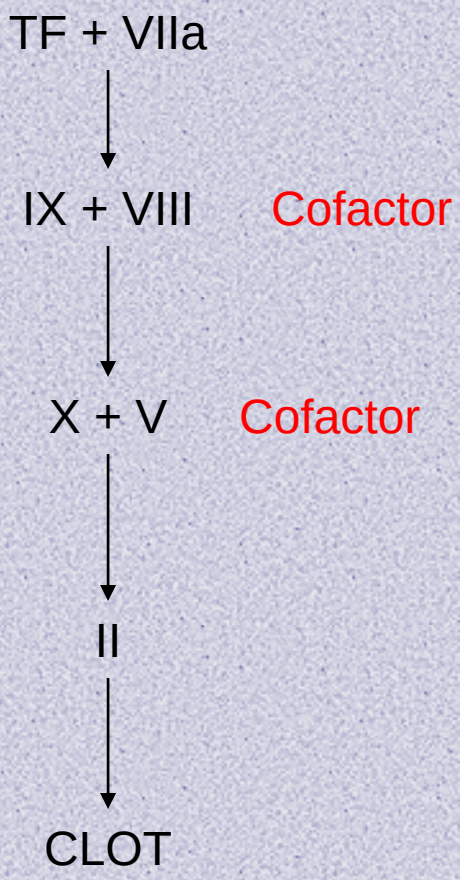
Cofactors

- Cofactors V and VIII
  - Similar molecules
  - Require activation by thrombin
  - Enhances efficiency of coagulation factors by at least 100,000-fold
  - Defects in both proteins result in common hemostatic problems

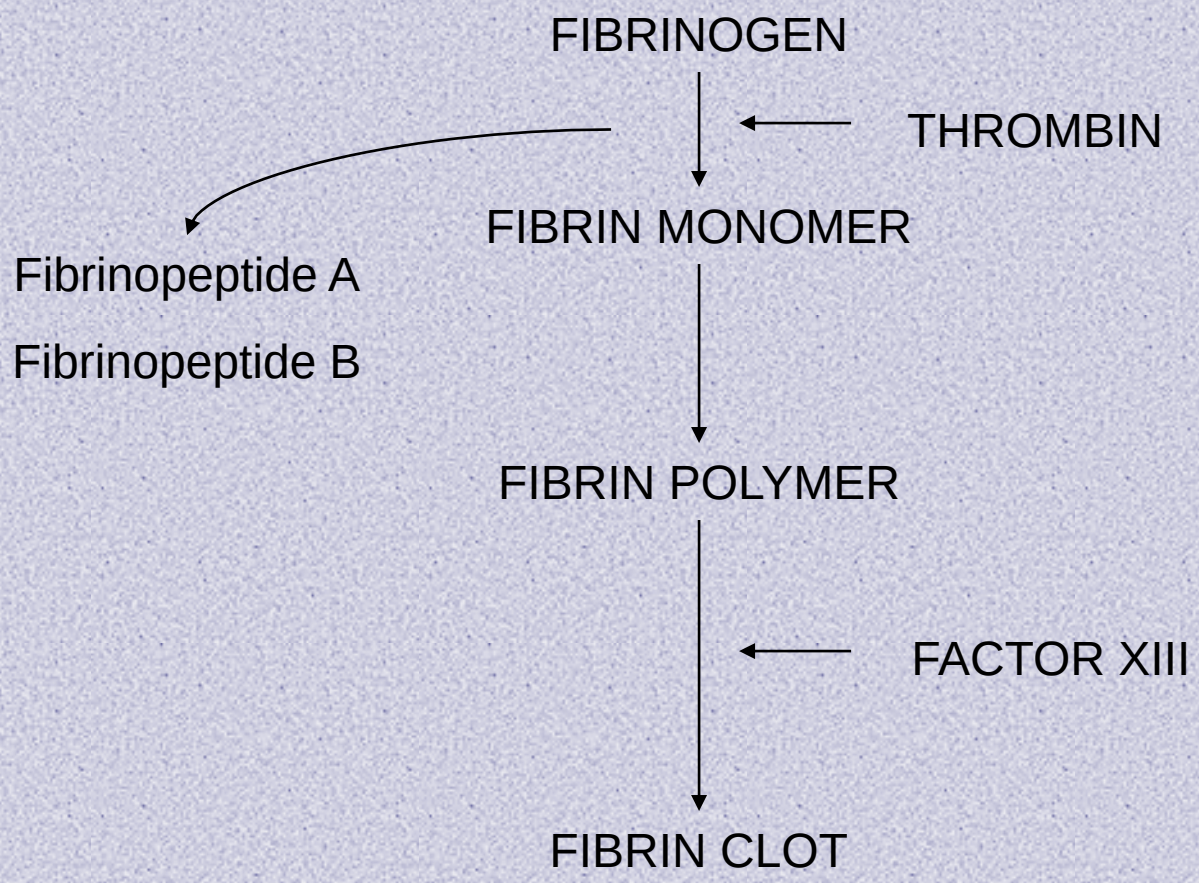
"Coagulation Factory"

- *Enzyme* binds a *cofactor* which is bonded by *calcium* to a *surface*
  - Enzyme – VIIa, IXa, Xa, IIa, protein C
  - Cofactor – V, VIII, tissue factor, protein S
    - Speeds up reactions by orders of magnitude
  - Calcium – binds protein to surfaces
  - Phospholipid surface
    - Negative charge
    - Brings proteins closer together

The "New" Model



Formation of Fibrin Clot



What about the other players?

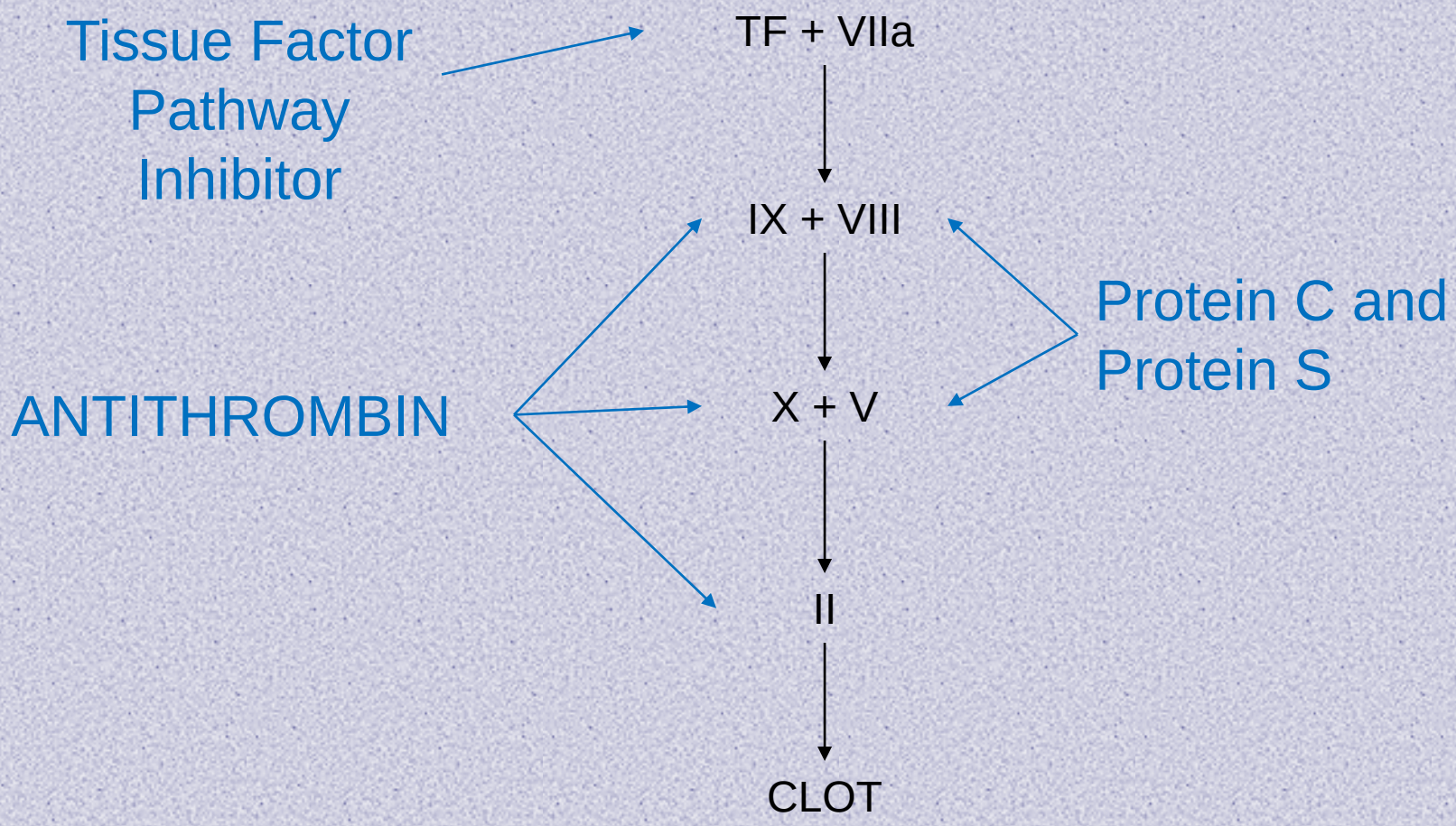
- Contact system
  - XII, kallikrein (also HK, PK)
  - Plays a role in inflammation
  - Deficiencies do not cause bleeding

- XI
  - Deficiencies cause bleeding, especially after surgery
  - Role is still emerging….

Thrombin (IIa)

- Multifunctional molecule
  - Cleaves **fibrinogen** into fibrin
  - Activates **Factors V and VIII**
  - Activates **Factor XIII**
  - Activates **Factor XI**
  - Activates **platelets**
  - Activates **thrombin activatable fibrinolysis inhibitor (TAFI)**
  - Activates **Fibrinolysis**
  - Activates **protein C**

Natural Anticoagulants

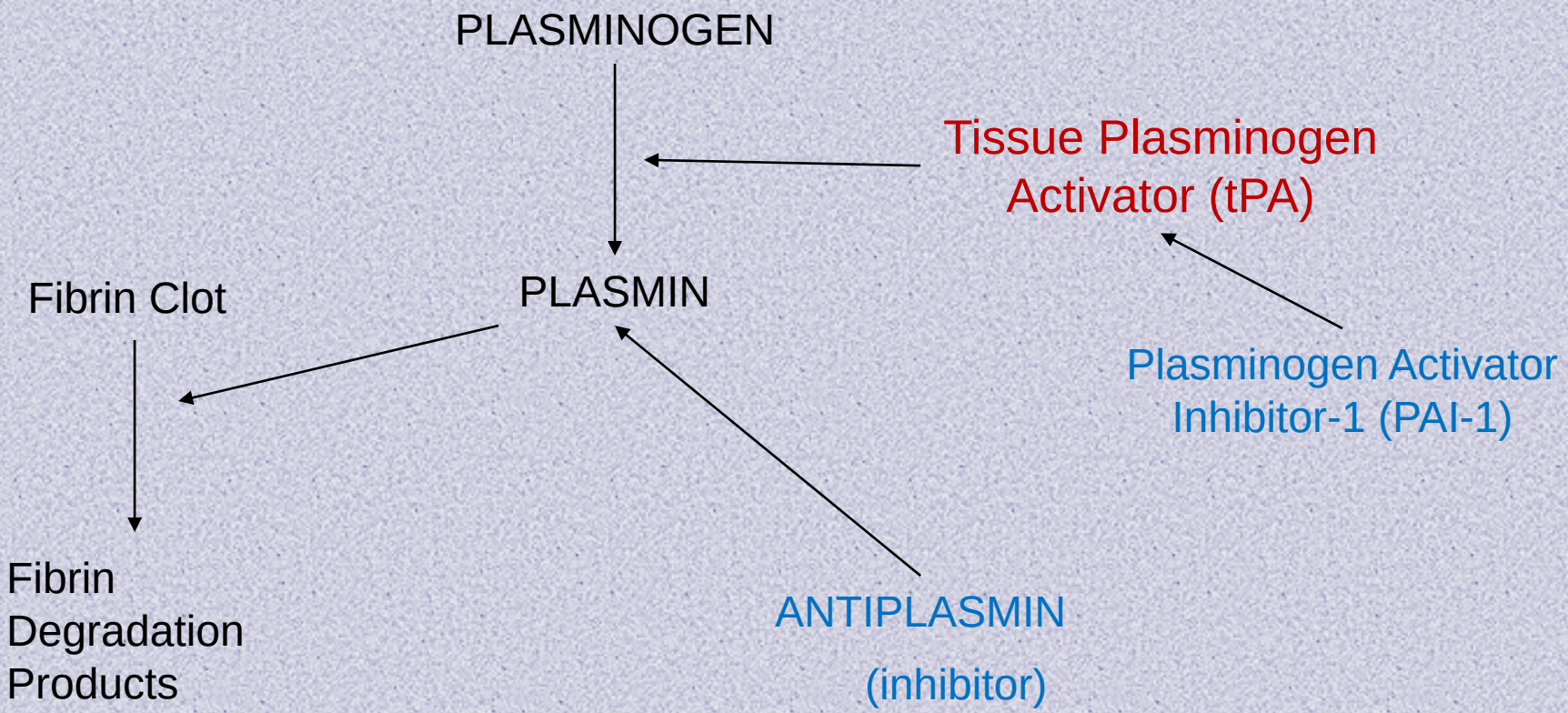


Fibrinolysis

- Breakdown of formed blood clots
  - Keeps thrombi from getting too large
  - Aids in wound healing
  - Prevents thrombosis in undesirable places

- Key proteins:
  - Plasminogen and Plasmin
  - Tissue Plasminogen Activator (tPA)
  - Urokinase (UK)
  - Inhibitors:
    - Plasminogen Activator Inhibitor (PAI-1)
    - $Alpha_2$ Antiplasmin

Fibrinolytic Pathway



Anticoagulants

Warfarin (Coumadin)

- Alters production of Vitamin K dependent factors
  - II, VII, IX, X
  - Protein C & Protein S
- Monitor with PT
  - Target INR 2.0-3.0
- Emergency reversal
  - Prothrombin Complex Concentrates (PCC)

Heparin

- Bind antithrombin to increase inhibitory effect
- Monitor with aPTT
  - 1.5-2.5x normal Therapeutic range
- Monitor with anti-Xa
  - 0.3-0.7 units/ml Therapeutic range
- Complication - HIT

Tests to Evaluate Hemostasis

Platelet Tests

- Quantitative test
  - Platelet count
- Function test
  - Platelet function (PFA)
  - Bleeding Time
  - Platelet Aggregation
- Antibody tests
  - Platelet Antibody Testing
  - Heparin Antibody Testing

Coagulation Tests

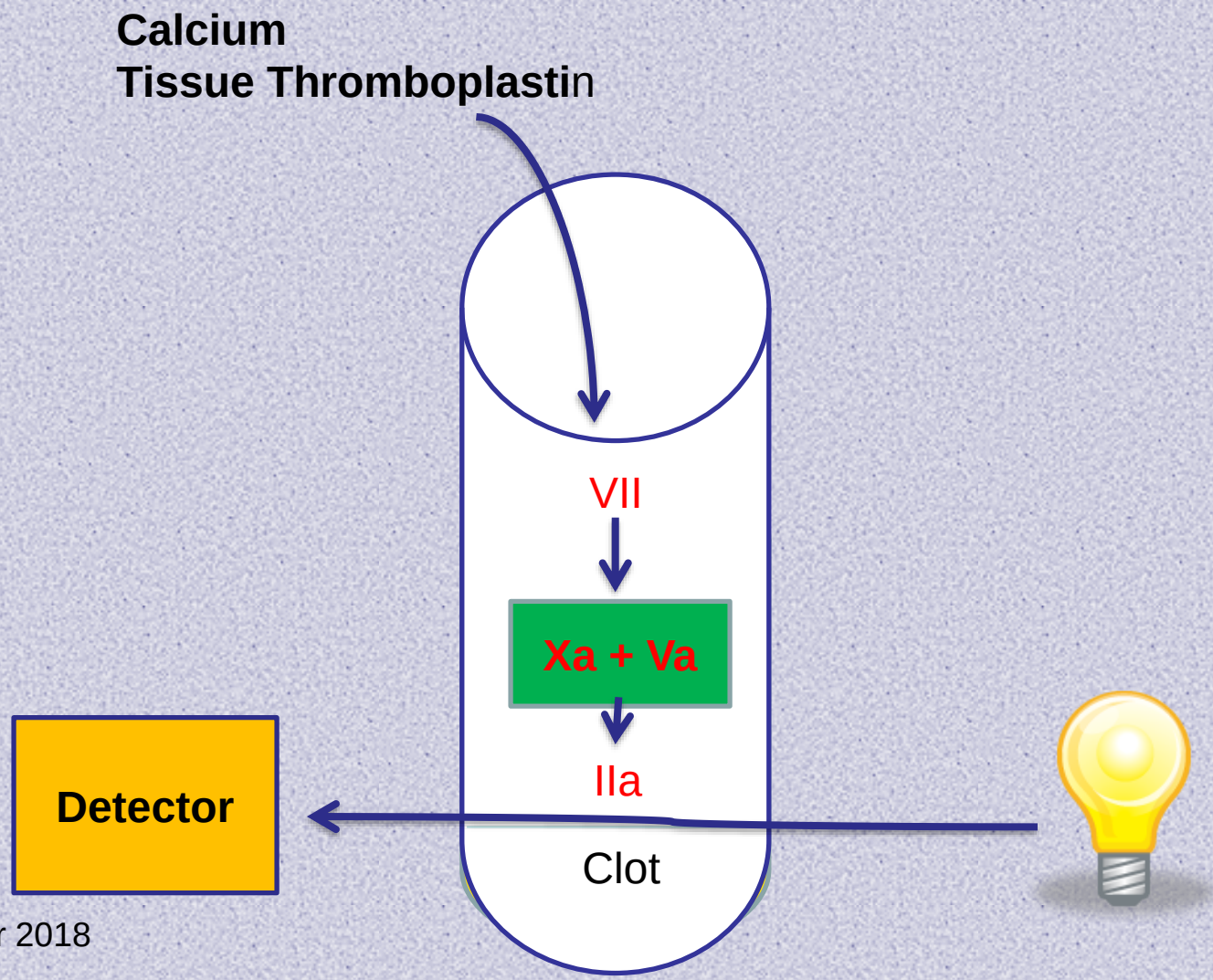
- Quantitative test
  - Fibrinogen
- Function test
  - PT & aPTT
    - Mixing studies
    - Factor assays
  - TT
  - vWF:RCo
  - TEG (platelet function too)
  - 5M Urea Lysis (FXIII)
- Fibrinolysis
  - FDP & D-dimer

Hillyer, C.D., Shaz, B.H., et. al. Transfusion Medicine and Hemostasis Clinical and Laboratory Aspects. Burlington, MA: Elsevier, 2009.

Prothrombin Time (PT)

- Measures time from formation of TF+VIIa complex to clot formation
  - Plasma + Calcium + Tissue Thromboplastin
- Major use is to monitor warfarin therapy
- Monitors Extrinsic pathway

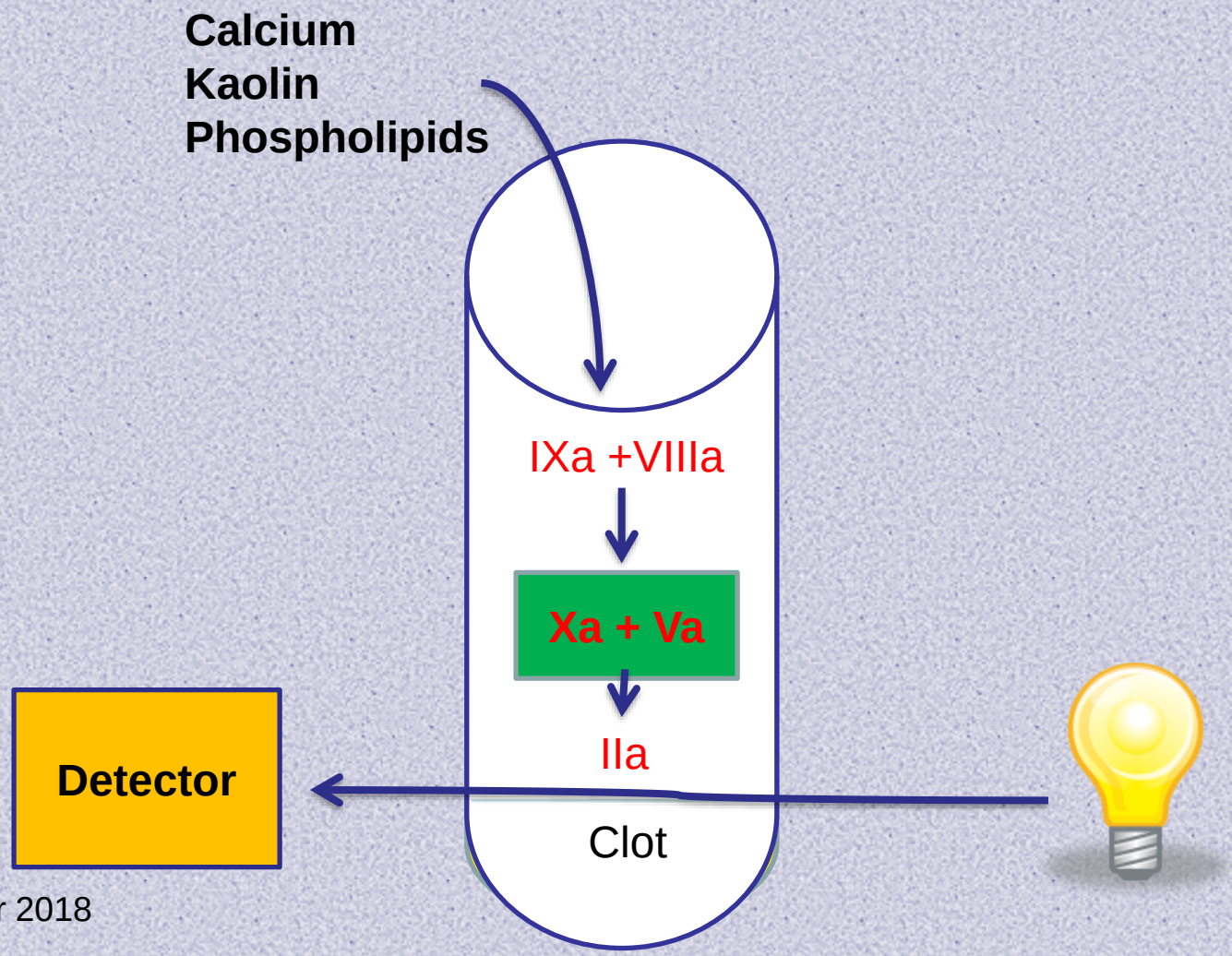
Prothrombin time (PT)



Activated Partial Thromboplastin Time (aPTT)

- Activator is added to plasma
  - Plasma + Calcium + Kaolin + Phospholipids
- Measures speed of contact pathway
  - (XII, kallikrein, XI) → IXa+VIIIa → Xa+Va → IIa → CLOT
- Monitors Intrinsic pathway

Activated Partial Thromboplastin Time (aPTT)



International Normalized Ratio (INR)

- Method of standardizing PT times obtained at different labs
- Derived by dividing PT time by control value and raising it to the International Sensitivity Index (ISI)
  - ISI is known for each PT reagent
- Use of INR results in better patient monitoring

Coagulation Disorders

- Primary Hemostasis
  - Vascular
  - Platelets
- Secondary Hemostasis
  - Coagulation factors

Primary: Vascular

- Marfan's Syndrome
- Hereditary Hemorrhagic Telangiectasia

- Characteristics
  - Easy bruising/bleeding
  - Painful

Primary: Platelets

- Acquired Causes of Thrombocytopenia
  - Ineffective Production
    - Bone Marrow Suppression
      - Chemotherapy/Irradiation
      - Drug induced thrombocytopenia
    - Infiltration of Bone Marrow
      - Myeloproliferative disorders
      - Lymphoproliferative disorders
      - Myeloma
      - Metastatic carcinoma
    - Aplastic Anemia
    - Treatment: Treat underlying disease & platelet transfusion
  - Abnormal Sequestration
    - Hypersplenism
    - Treatment: Platelet transfusion & splenectomy

Fung, M.K. et al. (Eds.). (2017). Technical Manual (19th ed.). Bethesda, MD: AABB Publications.

Primary: Platelets

- Acquired Causes of Thrombocytopenia
  - Increased Destruction (Immune Mediated)
    - ITP (Idiopathic (or Immune) Thrombocytopenic Purpura)
      - Immune complexes
      - Acute: Children (infection) & Chronic: Adults
      - Treatment: Corticosteroids or IVIG
    - NAIT (Neonatal Alloimmune Thrombocytopenia)
      - Maternal antibody to platelet antigens
      - Treatment: IVIG & IUT with antigen negative platelets
    - PTP (post transfusion purpura)
      - See Transfusion Reaction section

Fung, M.K. et al. (Eds.). (2017). Technical Manual (19th ed.). Bethesda, MD: AABB Publications.

Primary: Platelets

- Acquired Causes of Thrombocytopenia
  - Increased Utilization
    - TTP (Thrombotic Thrombocytopenic Purpura)
      - Platelet/Fibrin microthrombi
      - Treatment: Plasma exchange
      - Contraindication: Platelet transfusion
    - HELLP (Hemolysis Elevated Liver Enzymes Low Platelets)
      - Obstetric patients
      - Treatment: Delivery
    - HIT (Heparing Induced Thromobocytopenia)
      - Antibody to heparin PF4 complex
      - Treatment: Discontinue heparin give alternative anticoagulant
    - Drug Induced Thrombocytopenia
    - DIC, HUS, Infection, ECMO

Primary: Platelets

- Inherited Causes of Thrombocytopenia
  - Glanzmann's Thrombasthenia
    - **GP IIb/IIIa abnormal**
    - Aggregation test abnormal with: epinephrine, collagen, ADP (**Normal with: ristocetin**)
  - Bernard-Soulier syndrome
    - **GP Ib abnormal**
    - Aggregation test normal with: epinephrine, collagen, ADP (**Abnormal with: ristocetin**)
- Avoid Antiplatelet Drugs

Platelet or HLA antibodies

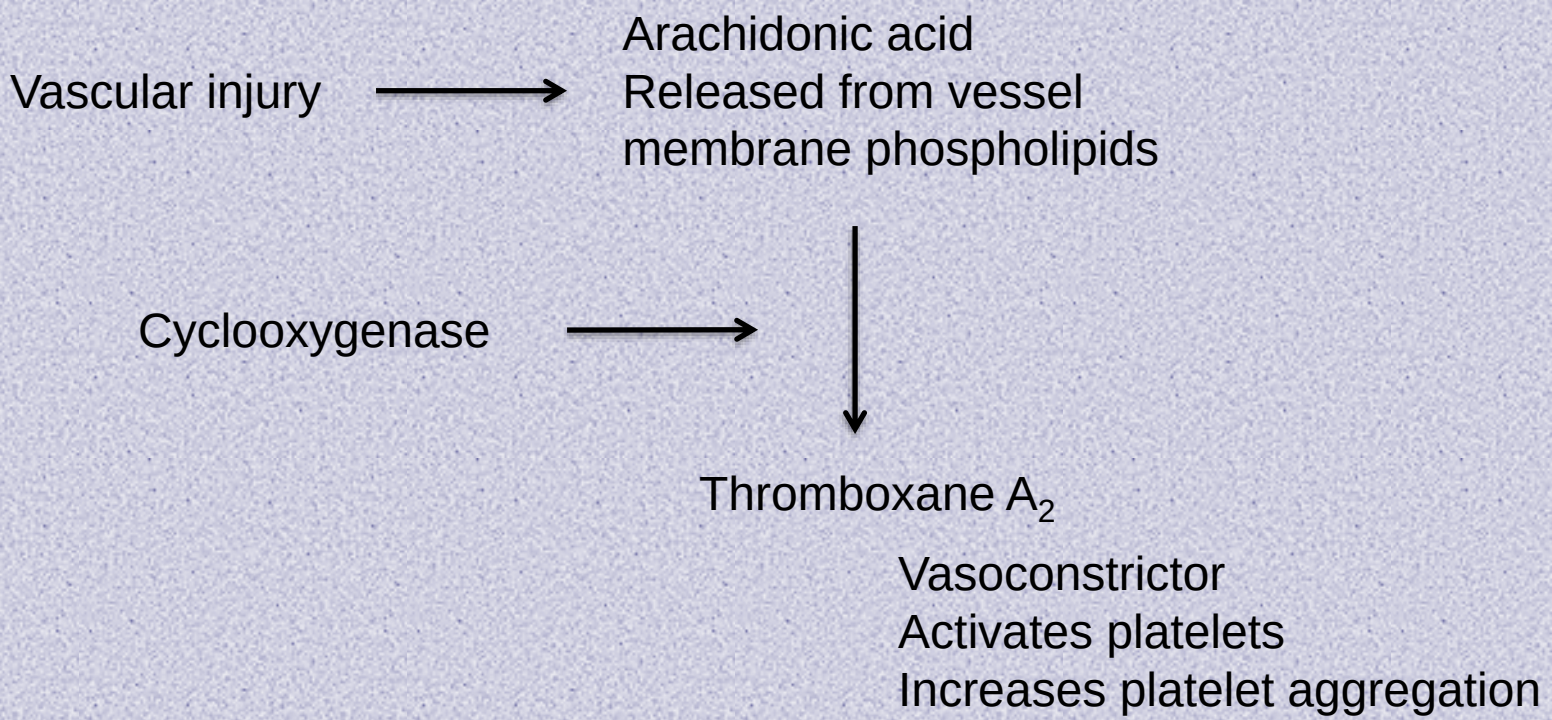
- Anti-HPA-1a
  - GP IIIa
  - If Transfusion needed,
    - Antigen negative Platelets

- Anti-HLA (Class I)
  - Treat platelets with Chloroquine diphosphate
    - Denatures HLA (Bg) antigens
  - If Transfusion needed,
    - HLA matched or Platelet Crossmatch

Fung, M.K. et al. (Eds.). (2017). Technical Manual (19th ed.). Bethesda, MD: AABB Publications.

Aspirin Effect

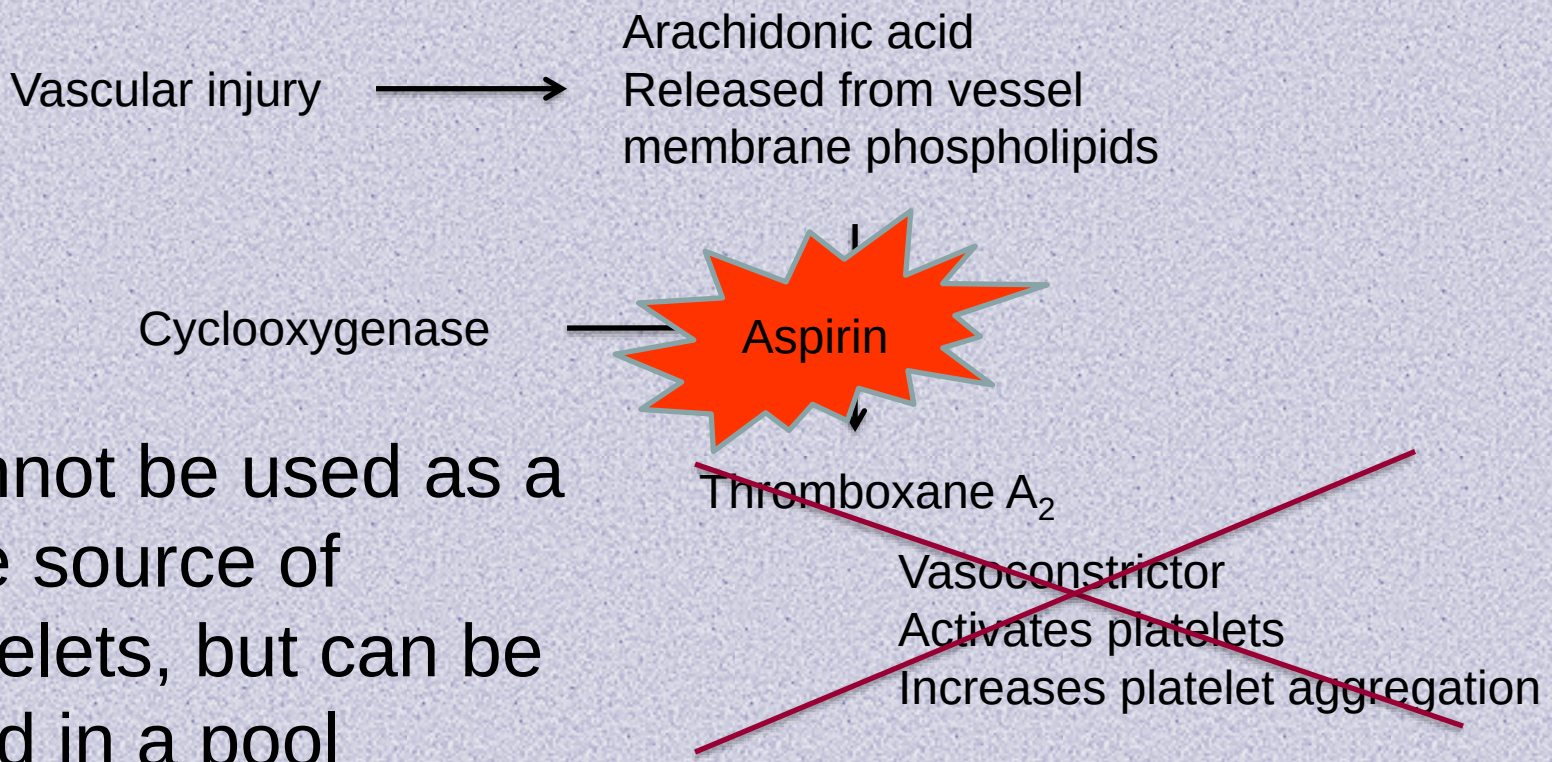
Normal Pathway



Fung, M.K. et al. (Eds.). (2017). Technical Manual (19th ed.). Bethesda, MD: AABB Publications.

Aspirin Effect

Add Aspirin



Cannot be used as a sole source of platelets, but can be used in a pool

Fung, M.K. et al. (Eds.). (2017). Technical Manual (19th ed.). Bethesda, MD: AABB Publications.

Primary: vWD

- von Willebrand's Disease (many types)
  - Type 1 vWD
    - Has decreased levels of vWF
    - Treatment: DDAVP (Desmopressin)
  - Type 2
    - Qualitative Defect in vWF
  - Type 3
    - Complete absence of vWF
  - Type 2 & 3 treated with Factor VIII that contains vWF (example: Humate-P)

Secondary: Coagulation Factors

	PT Normal	PT Abnormal
APTT Normal	Factor XIII deficiency	Factor VII deficiency
APTT Abnormal	Factor VIII, IX, XI, XII deficiency Factor VIII inhibitor	Factor I, II, V, X deficiency

Fung, M.K. et al. (Eds.). (2017). Technical Manual (19th ed.). Bethesda, MD: AABB Publications.

Hemophilia A

- Inherited deficiency or absence of FVIII
- FVIII levels
  - <1: Severe
  - 1-5: Moderate
  - >5: Mild

- Treatment: Factor VIII concentrates
  - Recombinant: Safest
  - Virus inactivated, plasma derived

Inhibitors to FVIII

- Bethesda units
  - <5 BU
    - Increased dose of FVIII
  - >5 BU
    - Factor VIIa
    - Activated Prothrombin Complex Concentrates (FEIBA)
    - Porcine FVIII

Hemophilia B

- Inhertied Factor IX deficiency
  - Recombinant Factor IX
  - Virus inactivated, Plasma derived Factor IX
- Patients with Inhibitors
  - Factor VIIa

Acquired Deficiency of Coagulation

- Vitamin K deficiency
  - II, VII, IX, X, Protein C and Protein S
  - Treatment: Vitamin K, Plasma or PCC

- Liver Disease
  - Decreased production of all coagulation factors
  - Treatment: Vitamin K, Plasma, DDAVP, PCC

DIC

- Acquired Deficiency due to increased utilization
- Disseminated Intravascular Coagulation
  - Increased: PT, PTT, TT, FDP's, D-dimers
  - Decreased: Platelets, Factor levels
- Treat underlying cause
- Transfusion Goal: Maintain hemostatic function
  - RBC's, Plasma, Cryoprecipitate
  - Platelets (except in cases of severe thrombosis)

Hypercoagulable States

- Associated with
  - Malignancy, post operative, pregnancy, oral contraceptives, nephrotic syndrome (DVT, PE)
- Lupus Anticoagulant
  - Acquired thrombophilia, Antiphospholipid antibody
  - Tests: Kaolin clotting time (KCT), Dilute Russell's Viper Venom time (DRVVT), Tissue thromboplastin inhibitor (TTI), modified aPTT
- Factor V Leiden
  - Thrombophilia, Mutation: FV decreased ability to inactivate Protein C
  - Tests: Activated Protein C resistance, Molecular testing for polymorphism

Coagulation Question 1

What coagulation factor may be deficient based on the following lab values:

Platelet count: 250 K
PT: 40 sec
aPTT: 30 sec

a. Factor V
b. Factor VII
c. Factor VIII
d. Factor XIII

Coagulation Question 1

What coagulation factor may be deficient based on the following lab values:

Platelet count: 250 K (150-450 K)
PT: 40 sec (10-14 sec)
aPTT: 30 sec (21-35 sec)

a. Factor V
b. Factor VII
c. Factor VIII
d. Factor XIII

Coagulation Question 1

	PT Normal	PT Abnormal
APTT Normal	Factor XIII deficiency	Factor VII deficiency
APTT Abnormal	Factor VIII, IX, XI, XII deficiency Factor VIII inhibitor	Factor I, II, V, X deficiency

Coagulation Question 1

What coagulation factor may be deficient based on the following lab values:

Platelet count: 250 K (150-450 K)
PT: 40 sec (10-14 sec)
aPTT: 30 sec (21-35 sec)

a. Factor V
b. Factor VII
c. Factor VIII
d. Factor XIII

Coagulation Question 2

- A 4 year old female arrives in the ER with a nosebleed that has persisted for three hours. Parents mention that patient has had some minor nosebleeds and bleeding of the gums in the past. What is your recommendation for treatment?
- Lab Values:

  Platelet count: 280 K

  PT: 12 sec

  aPTT: 28 sec

  PFA: 300 sec (epinephrine)

  vWF:Rco : 35 %

  RIPA: Decreased

a. Plasma
b. DDAVP
c. Factor VIII concentrate
d. Platelets

Coagulation Question 2

- A 4 year old female arrives in the ER with a nosebleed that has persisted for three hours. Parents mention that patient has had some minor nosebleeds and bleeding of the gums in the past. What is your recommendation for treatment?
- Lab Values:

  Platelet count: 280 K (150-450 K)

  PT: 12 sec (10-14 sec)

  aPTT: 28 sec (21-35 sec)

  PFA: 300 sec (epinephrine) (78-199 sec)

  vWF:Rco: 35 % (50-150 %)

  RIPA: Decreased (Normal)

Coagulation Question 2

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Rules out Thrombocytopenia

Coagulation Question 2

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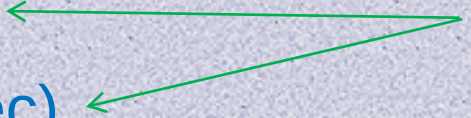

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  aPTT: 28 sec (21-35 sec)

  PFA: 300 sec (epinephrine) (78-199 sec)

  vWF:Rco : 35 % (50-150 %)

  RIPA: Decreased (Normal)

Abnormal Platelet Aggregation with Epinephrine & Ristocetin

Coagulation Question 2

- Bernard-Soulier Syndrome
  - Low platelet count
  - Decreased Ristocetin cofactor
- Glansmann Thrombasthenia
  - Normal platelet count
  - Normal Ristocetin Cofactor
- Factor VIII deficiency
  - Mostly affects males
  - Abnormal aPTT
  - Normal PT
- Factor XIII deficiency
  - Normal PT
  - Normal aPTT
  - Normal Platelet count
  - Normal PFA

Coagulati… 2

- Bernard-…drome
  - Low …
  - Dec… …s cof…
- Glans… Thron… …nia
  - Norm… …count
  - Norma… …Cofactor
- Fa… …eficiency
  - Mos… …males
  - Abnor…
  - Norma…
- …tor X… …ency
- …elet count
- …FA

Coagulation Question 2

- Von Willebrand's Disease
  - Normal Platelet count
  - Prolonged PFA
  - Decreased Ristocetin Cofactor
  - Decreased RIPA
  - Prolonged to Normal aPTT
- Treatment:
  - DDAVP (not Type 2b or platelet type)
  - Humate-P (Rco dosing), Alphanate, Koate HP
  - Cryoprecipitate (if others unavailable)

Coagulation Question 2

- A 4 year old female arrives in the ER with a nosebleed that has persisted for three hours. Parents mention that patient has had some minor nosebleeds and bleeding of the gums in the past. What is your recommendation for treatment?
- Lab Values:

  Platelet count: 280 K

  PT: 12 sec

  aPTT: 28 sec

  PFA: 300 sec (epinephrine)

  vWF:Rco : 35 %

  RIPA: Decreased

a. Plasma
b. DDAVP
c. Factor VIII concentrate
d. Platelets

Break

SBB/BB Exam Review

Blood Groups

Katrina Billingsley, MSTM, MT(ASCP) SBB^{CM}

LifeShare Blood Center

Shreveport, LA

Most Important

Read and know:

- Technical Manual
- Standards

General – Antigens

- Genetics
- Biochemistry
- Null phenotype
- Effect of chemicals
- Prevalence
- Racial variation
- Cord cell expression
- Soluble antigens

Reid, M.E., Lomas-Francis, C., & Olsson, M.L. (2012). *The Blood Group Antigen Facts Book* (3rd ed.). San Diego, CA: Elsevier, Academic Press

General – Antibodies

- Characteristic reactivity
- Techniques for detection/confirmation
- HTR
- HDFN

Reid, M.E., Lomas-Francis, C., & Olsson, M.L. (2012). *The Blood Group Antigen Facts Book* (3rd ed.). San Diego, CA: Elsevier, Academic Press

ABO

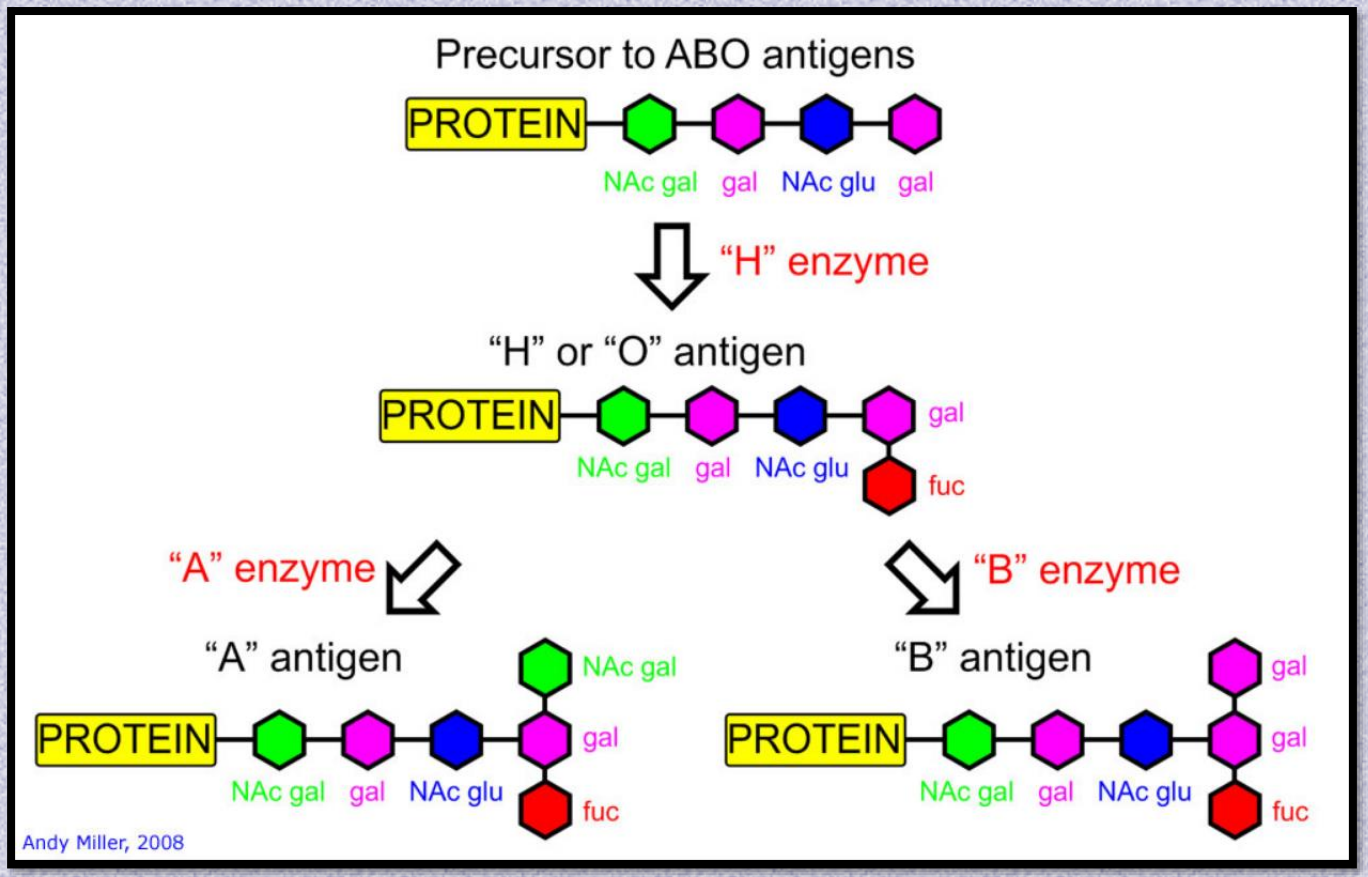
- Gene interaction - *A, B, H, Se*
- ABO gene produces glycosyltransferases
  - Adds sugar to paragloboside

Gene	Transferase	Sugar added
H	α-2-L- fucosyltransferase	L-fucose (to Type 2 chains)
Se	α-2-L- fucosyltransferase	L-fucose (to Type 1 chains)
A	α-3-N-acetyl-galactosaminyltransferase	N-acetyl-D-galactosamine
B	α-3-D-galactosyltransferase	D-galactose

Reid, M.E., Lomas-Francis, C., & Olsson, M.L. (2012). *The Blood Group Antigen Facts Book* (3rd ed.). San Diego, CA: Elsevier, Academic Press

ABO

- Antigen structure



ABO

- Antigen expression
  - Soluble antigen: saliva (secretors), body fluids
    - Built on Type 1 precursor chains
  - RBC membrane antigen: platelets, lymphocytes, etc.
    - Built on Type 2 precursor chains
- Cord cell expression weaker than adults
    - Fully developed by 2-4 years of age
- Reaction with enzymes/chemicals

Harmening, D.M. (2012). *Modern Blood Banking and Transfusion Practices* (6th ed.). Philadelphia: F.A. Davis Company.

ABO

- Subgroup characteristics
  - Subgroups of A (A_1, A_2, A_3, A_m, A_x, A_{el})
  - Subgroups of B (B_3, B_m, B_x, Acquired B)
  - Characteristic reactions with antiserum
    - A3 and B3 – mixed field
    - Ax stronger with anti-A,B than anti-A
  - Reaction of serum with reagent RBCs
  - Saliva of secretors
- Bombay, parabombay

ABO

- Acquired B antigen
  - Found in group A1 individuals
  - Due to deacetylation of A antigen
  - Associated with colorectal carcinoma and bowel obstructions
- Important lectins
  - *Dolichos biflorous*
    - Appropriately diluted, reacts with A1 cells
    - ~80% of A and AB persons are A1+.

Harmening, D.M. (2012). *Modern Blood Banking and Transfusion Practices* (6th ed.). Philadelphia: F.A. Davis Company.

ABO

- Antibody Characteristics
  - Naturally occurring
  - Antibodies detected in an infant are maternal
  - Anti-A and anti-B production begins after the first few months of life
  - Production peaks at 5-10 years of age

Harmening, D.M. (2012). *Modern Blood Banking and Transfusion Practices* (6th ed.). Philadelphia: F.A. Davis Company.

ABO

- Causes of ABO discrepancies
  - Technical errors
    - Specimen mix-up
    - Inappropriate cell suspension
    - Incorrect interpretation
  - Specimen issues: RBCs
    - Variant A or B, transfusion, transplant, spontaneous agglutination due to cold agglutinates, aby to dyes
  - Specimen issues: serum
    - Weak subgroup, BMT, immunodeficient patients, detection of other alloantibodies

Harmening, D.M. (2012). *Modern Blood Banking and Transfusion Practices* (6th ed.). Philadelphia: F.A. Davis Company.

ABO

- Resolution of ABO discrepancies
  - Repeat test (same and new sample)
  - Wash pt and reagent RBCs
  - Incubate at RT
  - Treat pt cells with enzymes
  - Ads/elu studies
  - Saliva studies

Harmening, D.M. (2012). *Modern Blood Banking and Transfusion Practices* (6th ed.). Philadelphia: F.A. Davis Company.

MNS

- Glycophorin A – M/N
  - 5 terminal amino acids for M and N specificity
    - M: serine-serine-threonine-threonine-glycine
    - N: leucine-serine-threonine-threonine-glutamic acid
- Glycophorin B – S/s
  - Amino acid residue at position 48 (prev 29)
    - S – methionine
    - s – threonine

Reid, M.E., Lomas-Francis, C., & Olsson, M.L. (2012). *The Blood Group Antigen Facts Book* (3rd ed.). San Diego, CA: Elsevier, Academic Press

MNS

- Antigen
  - Prevalence in White and Black populations
  - Enzyme and chemical treatment
  - Hybrid SGPs
- Antibody
  - Immunoglobulin class
  - Optimal technique
  - Clinical significance

Reid, M.E., Lomas-Francis, C., & Olsson, M.L. (2012). *The Blood Group Antigen Facts Book* (3rd ed.). San Diego, CA: Elsevier, Academic Press

P and Globoside

- Antigen Structure
- Soluble antigen
  - Pigeon egg white, hydatid cyst fluid (P1, P^k)
- Autoanti-P and PCH
  - Donath-Landsteiner test, biphasic hemolysin
- Anti-PP_1P^k and spontaneous abortion
- P antigen receptor for Parvovirus B19

Reid, M.E., Lomas-Francis, C., & Olsson, M.L. (2012). *The Blood Group Antigen Facts Book* (3rd ed.). San Diego, CA: Elsevier, Academic Press

Rh

- Rh complex
  - Rh Associated glycoprotein (RhAG)
  - LW glycoprotein
  - CD47
  - Integrin associated protein
  - Glycophorin B
  - Fy5

Reid, M.E., Lomas-Francis, C., & Olsson, M.L. (2012). *The Blood Group Antigen Facts Book* (3rd ed.). San Diego, CA: Elsevier, Academic Press

Rh

- Types of Weak D
  - Quantitative: inherited gene, encodes less than normal number of D antigen sites
  - Position effect: weakening of D antigen by a C gene in *trans* to D gene
    - Dce/Ce (Ro/r')
  - Partial: lack part of the D antigen complex
- Standards for D typing
  - Donor vs. patient

Reid, M.E., Lomas-Francis, C., & Olsson, M.L. (2012). *The Blood Group Antigen Facts Book* (3rd ed.). San Diego, CA: Elsevier, Academic Press

Rh

- Rh_{null} types
  - Lack LW and Fy5
  - Anti-Rh29 (total Rh)
- Prevalence of 5 major antigens
- Antibody clinical significance
  - HTR
  - HDFN

Reid, M.E., Lomas-Francis, C., & Olsson, M.L. (2012). *The Blood Group Antigen Facts Book* (3rd ed.). San Diego, CA: Elsevier, Academic Press

Rh

- Antigens associated with D variants
  - Go^a associated with DIVa
  - D^w associated with Dva
- Compound antigens/antibodies
- Anti-G adsorption/elution
  - Prenatal cases

Reid, M.E., Lomas-Francis, C., & Olsson, M.L. (2012). *The Blood Group Antigen Facts Book* (3rd ed.). San Diego, CA: Elsevier, Academic Press

Kell

- Gene interaction – *XK1* gene
- Racial differences
- Chemical treatment
- KEL3 (Kp^a) in *cis* position
- McLeod phenotype
  - No Kx or Km antigens
  - Depressed Kell antigens
  - CGD association

Reid, M.E., Lomas-Francis, C., & Olsson, M.L. (2012). *The Blood Group Antigen Facts Book* (3rd ed.). San Diego, CA: Elsevier, Academic Press

Lewis

- Soluble antigen
- Gene interaction - *Le, Se*
- α-4-L-fucosyltransferase adds L-fucose to type 1 precursor chains
- Antigen structure

Reid, M.E., Lomas-Francis, C., & Olsson, M.L. (2012). *The Blood Group Antigen Facts Book* (3rd ed.). San Diego, CA: Elsevier, Academic Press

Duffy

- Racial differences
- Chemical treatment
- Anti-Fy3 vs Anti-Fy5
  - Rh null cells (Fy3+, Fy5-)
- Association with Malarial resistance

Reid, M.E., Lomas-Francis, C., & Olsson, M.L. (2012). *The Blood Group Antigen Facts Book* (3rd ed.). San Diego, CA: Elsevier, Academic Press

Kidd

- Inheritance for Jk(a-b-)
- Jk(a-b-) resistant to lysis in 2M urea
- Jk(a-b-) population
- Antibodies
  - May show dosage
  - May activate complement

Reid, M.E., Lomas-Francis, C., & Olsson, M.L. (2012). *The Blood Group Antigen Facts Book* (3rd ed.). San Diego, CA: Elsevier, Academic Press

Lutheran

- Lu(a-b-) inheritance
  - Recessive: *LuLu*
  - Inhibitor: *In(Lu)*
  - X-borne
- *Lu* linkage to *Se*
  - First example of autosomal linkage in man
- Association with ALG

Reid, M.E., Lomas-Francis, C., & Olsson, M.L. (2012). *The Blood Group Antigen Facts Book* (3rd ed.). San Diego, CA: Elsevier, Academic Press

LW

- Association with D
- Cord cell expression
- Distinction from anti-D
- Absent on Rh_{null} cells

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I System/Collection

- Soluble antigen
- Adult and cord cell expression
- Disease associations

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Others

- Diego
- Cartwright
- Scianna
- Dombrock
- Colton
- Indian
- Xg

Reid, M.E., Lomas-Francis, C., & Olsson, M.L. (2012). *The Blood Group Antigen Facts Book* (3rd ed.). San Diego, CA: Elsevier, Academic Press

Ab to High Incidence Antigens

Problem solving

- Phenotype clues
- Chemical treatment
- Ethnicity of antibody maker
- Source of units for transfusion
- HDFN

Harmening, D.M. (2012). *Modern Blood Banking and Transfusion Practices* (6th ed.). Philadelphia: F.A. Davis Company.

Chido/Rogers

- Chemical treatment
- Soluble antigen
- C4 coated cells

Knops

- Located on CR1
- Ethnicity of antibody maker
- Soluble antigen

Reid, M.E., Lomas-Francis, C., & Olsson, M.L. (2012). *The Blood Group Antigen Facts Book* (3rd ed.). San Diego, CA: Elsevier, Academic Press

SBB/BB Exam Review

Methods

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General

- Read Technical Manual Methods Section
- Principle of Method
- Interpretation of Method
- Applications of Method
- Limitations of Method

Antigen/Antibody Reactions

- Agglutination – crosslinking of antibody coated RBCs resulting in visible clumping
  - Stages
    - Stage I: sensitization, assoc. of ag with aby
    - Stage II: formation of bridges, lattice formation
  - Factors affecting agglutination
    - Stage I: Temp, pH, incubation, ag/aby ratio, etc.
    - Stage II: # Aby binding sites, # ag sites, zeta potential, centrifugation, etc.

Antigen/Antibody Reactions

- Hemolysis
  - Rupture of RBCs with release of hg
  - Does not happen in plasma (EDTA binds Ca)
- Precipitation
  - Applications
  - Ouchterlony double diffusion

Antigen/Antibody Reactions

- Complement Fixation
- ELISA
  - Indirect
  - Sandwich
  - Competitive

Flow Cytometry

- Principle
- Applications
  - Define cell markers
  - Detect minor cell populations
  - Antigen zygosity

Red Cell Survival

- Monocyte Monolayer Assay
  - Monocytes incubated with antibody coated RBCs
  - Phagocytosis predicts in vivo RBC survival
- In vivo crossmatch
  - Cr^{51}-labeled RBCs transfused
  - Radioactivity in recipient measured to predict RBC survival
- Applications

Adsorption

- Types
- Variables
  - Temperature, incubation time, etc.
- Applications
  - Remove autoantibody
  - Separate multiple antibodies
  - Confirm antigen or antibody specificity

Elution

- Principle
- Types
  - Optimal recovery
  - Limitations
- Applications
  - Investigate positive DAT
  - Remove antibody for phenotyping

Titration

- Be able to interpret a titration scheme
- Be careful about phenotypes of cells used
- Know how to score
- Applications
  - Prenatal studies
  - Antibody identification
  - Separate antibody specificities

Neutralizations

- Principle
- Look for dilutional control
- Sources and specificity of soluble substances
  - ABH
  - Lewis
  - P_1
  - Sd^a
  - Ch/Rg

Cell Separations

- Applications
- Microhematocrit
  - Principle
  - Limitations
- Hypotonic Wash
  - Principle
  - Sickle cell disease

Chloroquine Diphosphate

- Applications
  - Dissociate antibody from red cells
  - Denature Bg and HLA-related antigens
- Limitations
  - Complement not removed
  - Some antigens weakened with prolonged exposure

Enzymes

- One stage
  - Serum, cells and enzyme in one rxn mixture
- Two stage
  - Red cells pre-treated then add serum
- Standardization procedure
  - Method
  - Interpretation
- Effect on various antigens

Sulfhydryl Reagents

- AET, DTT, 2ME
- Principle
- Applications
  - Antigen
  - Antibody - know how to interpret serum treatment (look for dilutional control)

Enhancement Techniques

- Strengths and weakness of each
- LISS
- PEG
- Polybrene
- Bovine Albumin

Other Techniques for Antibody Detection/Identification

- Column Agglutination Technology
  - Principle
  - Unique components of system
- Solid Phase Red Cell Adherence
  - Principle
  - Unique components of system

Other

- Donath-Landsteiner Test
  - Diagnosis of PCH
  - Method and interpretation
- Saline Replacement
  - Rouleaux formation
- Tests for PNH
  - Sucrose lysis
  - Ham's test
- Tests for HLA
  - Serologic method
  - Molecular method

Molecular Methods

Polymerase Chain Reaction (PCR) & Transcription-Mediated Amplification (TMA)

- Principle
- Procedure
- Applications
  - PCR used for DNA amplification
  - TMA used for RNA amplification

DAT and Hemolytic Anemia Investigations

- Ab removal for Phenotyping
  - IgM removal: warm wash, heat, sulfhydryl
  - IgG removal: chloroquine, ZZAP, certain elution methods
- Ab removal by Adsorption
  - Cold: autologous, allogeneic, RESt
  - Warm: autologous, allogeneic known phenotype, allogeneic unknown phenotype

Drug Associated Hemolytic Anemias

- Drug dependent
  - React with drug coated cells (penicillin, most cephalosporins)
  - React in the presence of drug (piperacillin and some 2nd & 3rd generation cephalosporins, quinidine, many other drugs)
- Drug Independent
  - Serum/eluate reacts with all cells (methyldopa, fludarabine)
  - Cannot be distinguished serologically as different from idiopathic warm autoimmune hemolytic anemia
  - Difficult to prove drug-induced
- Non-immunologic protein adsorption
  - Positive DAT/IAT (eg, cephalothin, oxaliplatin, tazobactam)

Fung, M.K. et al. (Eds.). (2017). Technical Manual (19th ed.). Bethesda, MD: AABB Publications.

SBB/BB Exam Review

Blood Donors and Component Preparation

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Overview of the Donation Process

- Donor identification, qualification, consent and educational materials provided
- Donor History Questionnaire (DHQ)
- Physical examination (mini physical)
- Phlebotomy
- Care of the donor afterwards

Whole Blood Donor Qualification

Allogenic Donation

- Hemoglobin (Hgb) /Hematocrit (Hct)
  - ≥ 12.5 g/dl, Hct ≥ 38%
- Temperature
  - ≤ 99.5˚F or 37.5˚C
- Blood pressure – per FDA only
  - Systolic ≤180 mm Hg
  - Diastolic ≤100 mm Hg

Autologous Donation

- Physicians order
- Hgb ≥11 g/dl or Hct ≥33%
- Collected > 72 hours before surgery or transfusion
- Deferred if there is a risk of bacteremia

Must be

- **At least 17 years (16 in some states with parent permission), Iron acceptable**
- **Minimum 110lbs healthy person**

Donation Frequency

- Blood (whole blood) Every 56 days
- Platelets
  - Every 7 days, up to 24 times / year
- Plasma
  - Every 28 days, up to 13 times / year
- Double Red Cells
  - Every 112 days, up to 3 times / year

	Allogeneic Male	Allogeneic Female
Weight	≥130 lb	≥150 lb
Height	at least 5'1"	at least 5'5"
HCT	at least 40%	at least 40%
HGB	at least 13.3 g/dl	at least 13.3 g/dl

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Donor History Questionnaire

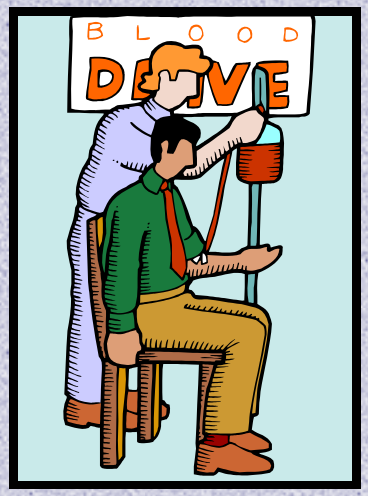
- The DHQ was developed by an interorganizational task force in 2006.
- Questions are based on regulations and guidance from the FDA and on AABB BBTS Standards
  - Medical and Drug History
  - Infectious Disease History or Risks
  - Immunizations
  - Travel

Phlebotomy and Collection of Samples

- Phlebotomist asks donor to confirm their identity.
- Phlebotomist insures all information is correct on the DHQ.
- Phlebotomist inspects bag for any defects and discoloration. Inspects anticoagulant and additive solution for particulate contamination.
- Phlebotomist insures a unique number is placed on DHQ, donor blood container and all attached bags and all sample tubes.

Fung, M.K. et al. (Eds.). (2017). Technical Manual (19th ed.). Bethesda, MD: AABB Publications.

Post -Phlebotomy Care



- Apply firm pressure
- Remain reclined until released
- Give the following instructions:
  - Eat and drink before leaving and wait to be released
  - Drink a lot of fluid over the next few days
  - Avoid alcohol until after a good meal
  - Avoid smoking for 30 minutes
  - If phlebotomy site begins to bleed, raise arm and apply pressure
  - Lie or sit down if feel faint or dizzy
  - Report any symptoms that persist
  - Remove bandage after a few hours

Fung, M.K. et al. (Eds.). (2017). Technical Manual (19th ed.). Bethesda, MD: AABB Publications.

SBB/BB Exam Review

Blood Components - Preparation and Storage and Transfusion Practice

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Anticoagulants

- Prevent clotting and maintain cell viability and function during storage
  - Dextrose: supports ATP generation
  - Adenine: provides substrate for ATP synthesis
  - Sodium biphosphate: controls the pH
  - Citrate: prevents coagulation
- 21 day storage = CPD, CP2D
- 35 day storage = CPDA-1
- 42 day storage
  - AS-1 (Adsol), AS-3 (Nutricel), AS-5 (Optisol)

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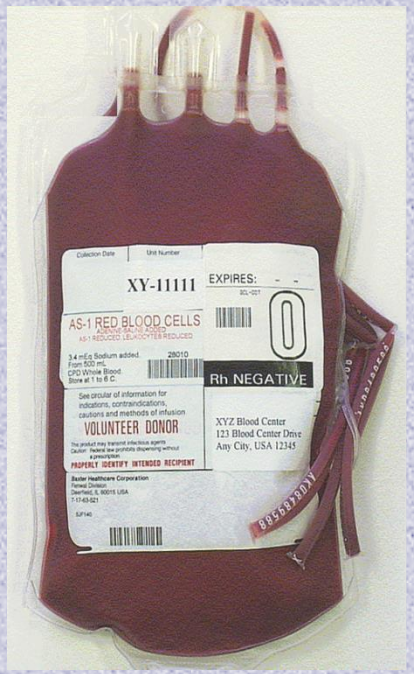
Red Cell Components



- Whole blood (1-6C)
- Red Blood Cells (1-6C)
- Frozen Red blood Cells (-65C or colder)
- Deglycerolized RBCs
  (1-6C, Open System 24 hours or Closed system 14 days)
- Washed RBCs (1-6C up to 24 hours)
- Leukoreduced RBCs (1-6C)
- RBCs Irradiated (1-6C, Exp. date or 28 days from irradiation)
- Apheresis RBCs (1-6C)
- Rejuvenated RBCs (1-6C)

Indications for Whole Blood

- Used infrequently
- Increases both oxygen carrying capacity and plasma volume

Indications for RBCs

- Used for increasing oxygen carrying capacity
  - Leukocyte-reduced
  - Washed
  - Frozen, deglycerolized

Effects of Storage on Red Cell Components

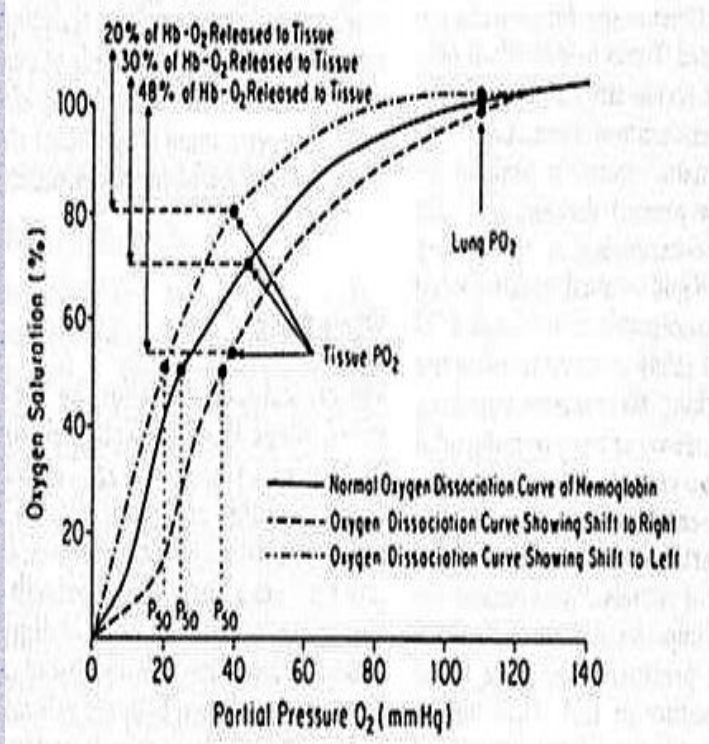
- ↓ 2,3 DPG levels
- ↓ATP
- ↑K+

Days of Storage	0	21	0	0	35	35	42	42	42
pH (measured at 37C)	7.2	6.84	7.6	7.55	6.98	6.71	6.6	6.5	6.5
ATP (% of initial value)	100	86	100	100	56(±16)	45 (±12)	60	59	68.5
2,3 DPG (% of initial value)	100	44	100	100	<20	<10	<5	<10	<5
Plasma K+ (mmol/L)	3.9	21	4.2	5.1	27.3	78.5	50	46	45.6
Plasma hgb (mg/L)	17	191	82	78	461	658	NA	386	NA

Affect of Anemia on the Patient

- Hemoglobin-Oxygen Affinity (shift in the curve)
  - Increase in 2,3 DPG
  - Decrease in pH
- Cardiac
  - Increased heart rate and stroke volume
- Blood Flow
  - Increase in blood flow, vasodilatation and shunting
- Oxygen Extraction
  - Increase in Oxygen extraction from 25 to 75%

http://www.ncbi.nlm.nih.gov/pmc/articles/PMC2918661/

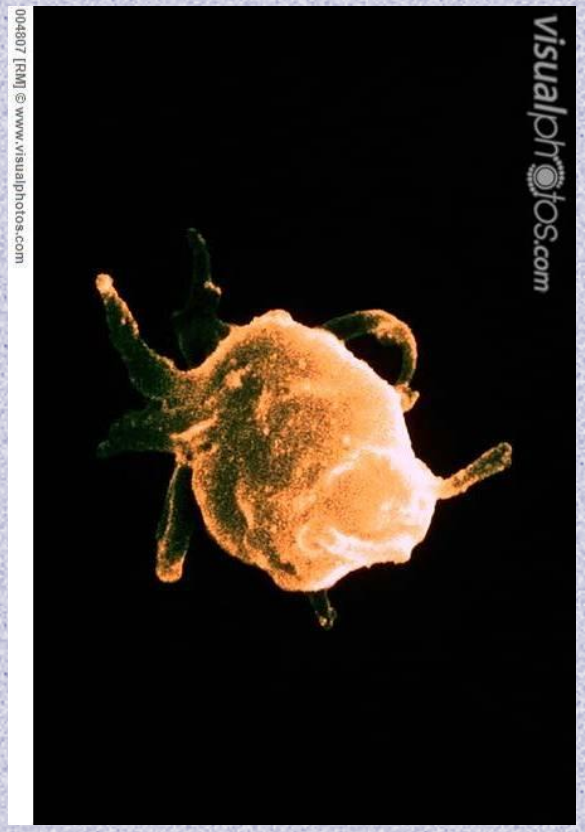


Platelets

- Platelet Concentrates
- Platelet - Apheresis
- Modifications of platelets
  - Irradiated
  - Leukoreduced
  - Volume-reduced
  - Aliquots
  - Washed
  - Frozen

Indications for Platelets

- Improve hemostasis
- Given
  - Bleeding
  - Low platelet count
  - Platelets are not working properly
- Do not give for TTP or ITP unless absolutely necessary

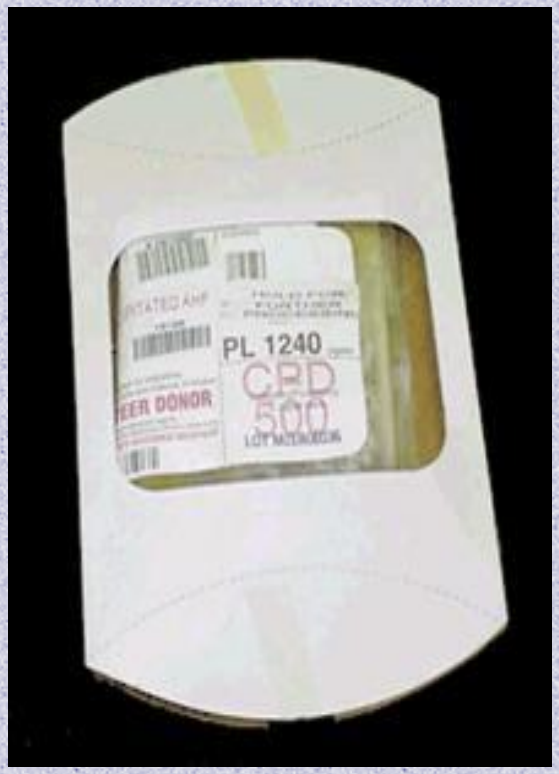


Activated Platelet

www.aabb.org/tm/coi/Documents/coi1017.pdf

Plasma Components

- Fresh Frozen Plasma
- Thawed Plasma
- Plasma Frozen Within 24 hours of collection
- Plasma and Liquid Plasma
- Plasma Cryoprecipitate Reduced
- Recovered Plasma
- Cryoprecipitate

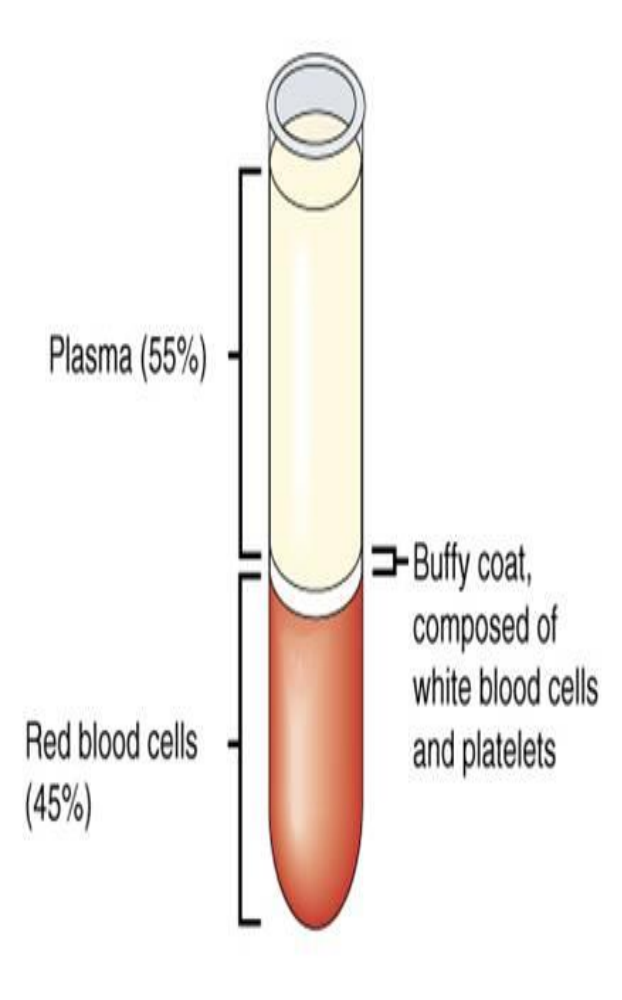


Indications for Plasma

- FFP
  - Used to treat coagulation factor deficiency
- Cryo-reduced plasma
  - Primarily used to treat TTP patients
- Cryoprecipitate
  - Source of Fibrinogen for “Fibrin Glue”
  - Used to replace Factor VIII, vWF

Granulocytes

- Usually collected by apheresis
- Buffy coat harvest
- Yield must be a minimum of 1.0×10^{10} granulocytes
- Transfuse as soon as possible after collection
  - Indications
    - Used to fight infection in neutropenic patients
    - Should be irradiated



Component Modifications

- CMV-negative
  - Reduces the risk of CMV transmission
  - Leukoreduced may be an alternative
- Irradiated
  - Prevents T lymphocyte proliferation; the primary cause of GVHD

SBB/BB Exam Review

Donor Testing, Transfusion Transmitted Disease Testing & Re-entry

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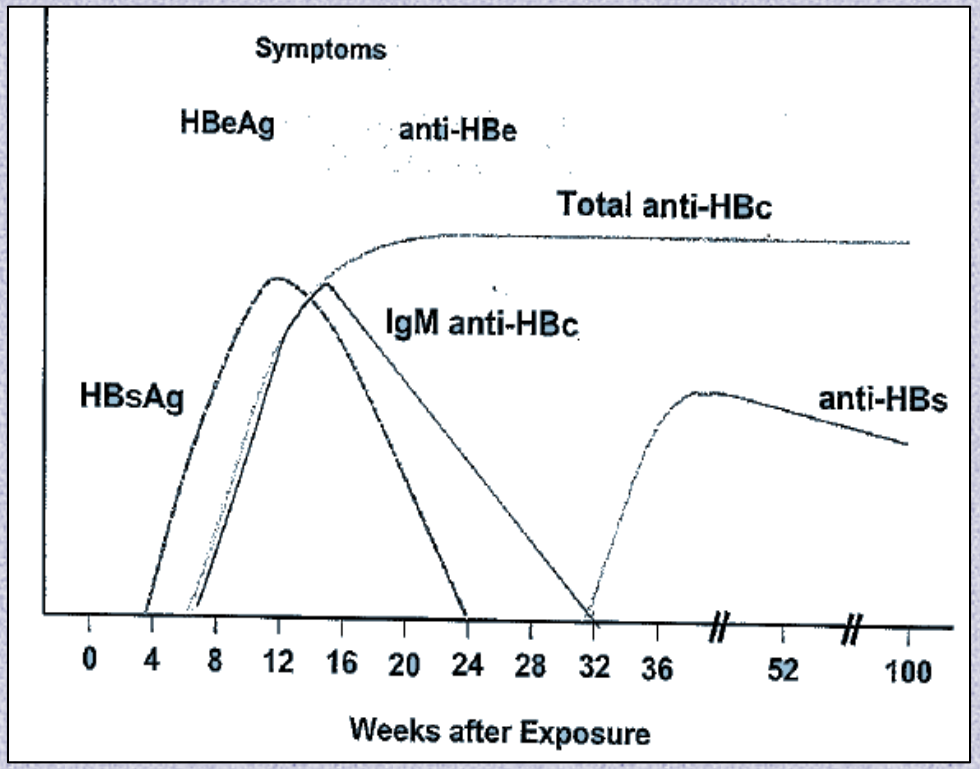
General Requirements

- ABO and Rh
- HBsAg
- Anti-HCV
- Anti-HIV-1/2
- Anti-HTLV-I/II
- Syphilis
- Antibodies to *Trypanosoma cruzi* (tested once)
- Bacterial Detection (platelets)
- Zika (newest requirement)

- Antibody Screen
- Anti-HBc
- HBV and HCV-RNA (NAT)
- HIV-1 RNA (NAT)
- WNV RNA

Physiology of Infection

- Routes of Infection
- Onset
- Incubation
- Window Period
- Chronic or Acute
- Mortality



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Supplemental and Confirmatory Tests

Test	Confirmatory Test
Anti-HTLV-I/II	None
Anti-HBc	None
HBsAg	Neutralization
Anti-HCV	• As of 2013, RIBA (Recombinant immunoblot assay, a supplemental test) is not currently on the market. or • HCV RNA
Anti-HIV-1/2	• HIV-1 WB (Western Blot) or IFA (Indirect Fluorescence Assay) • HIV-2 EIA • HIV-2 WB or IFA
Syphilis	Treponemal test •FTA-Abs •TPI (*Treponema pallidum* immobilization) •TPHA (*T. pallidum* hemagglutination)
Anti-*T. cruzi*	RIPA (Radioimmunoprecipitation Assay) or IFA

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Re-entry Algorithm and Loook Back

- See Guidance Document
  - Re-entry
    - Test and its associated confirmatory test
    - Re-entry possibilities based on the results
  - Lookback actions
    - Donor center
    - Transfusion Service

SBB/BB Exam Review

Lab Operations, Education and Quality

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Lab Operations

- Organization
  - Structure: chain of command
  - Mission: purpose of organization
  - Vision: long term organizational goals
- Leadership
  - Planning Decision Making
    - Problem solver, inspire, communicate
- Management
  - Dealing with things or people
    - Plan, organize, implement

Lab Operations

- Human Resources
  - Interviewing, Evaluation, Discipline
  - Laws & Regulations
    - Equal Employment Opportunity
    - Equal Pay Act
    - Fair Labor Standards Act of 1938
    - Family and Medical Leave Act of 1993
    - Uniformed Services Employment and Reemployment Rights Act
    - National Labor Relations Act

Lab Operations

- Financial Management
  - Budget, Reimbursement
  - Cost benefit analysis
- Operations
  - Compliance, Workflow, Staffing
- Compliance
  - CLIA 88
    - Waived Tests
    - Moderate & High Complexity Tests

Education

- Job Related
  - Orientation – Safety, Confidentiality, Policies
  - Training – to learn a new skill or task
  - Competency Assessment- Measure ability to perform skill or task
    - Interval:
      - Twice within the first year
      - Annually thereafter

Harmening, D.M. (2013). *Laboratory Management: Principles and Processes* (3rd ed.). St. Petersburg: D.H. Pub. & Consulting, Inc

Education

- Learning Objective Components
  - Condition (When? After what?)
  - Audience (Who?)
  - Action Verb (do not use Learn or Understand)
  - Standard (How do you determine if satisfactory)
- Example: At the completion of training, the new employee will be able to grade agglutination within one grade of the trainer.

Harmening, D.M. (2013). *Laboratory Management: Principles and Processes* (3rd ed.). St. Petersburg: D.H. Pub. & Consulting, Inc

Education

- Learning Domains
  - Cognitive (thinking)
  - Psychomotor (doing)
  - Affective (feeling)
- Bloom's Taxonomy Levels – cognitive domain
  1. Knowledge/recall – remembering
  2. Comprehension – understanding
  3. Application – utilization of learned material
  4. Analysis – break down material into parts
  5. Synthesis – generate new material
  6. Evaluation – judge the quality of new material

Harmening, D.M. (2013). *Laboratory Management: Principles and Processes* (3rd ed.). St. Petersburg: D.H. Pub. & Consulting, Inc

Quality

- Governing Bodies
  - Regulatory (FDA, CMS)
  - Accrediting (AABB, CAP, TJC)
  - State Agencies
- Quality Assurance
  - Quality Control
  - SOP management
  - Training/Competency
  - Validation
  - Audits
  - Event Management

The SBB and BB Exams

Resources
Preparation Guide
Testing Strategies

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Resources

- AABB Technical Manual: Cover to cover, each chapter, each method
- AABB Blood Bank and Transfusion Service Standards, especially chapter 5 and associated charts
- Blood Transfusion Therapy: A Physician's Handbook

Reference Lists

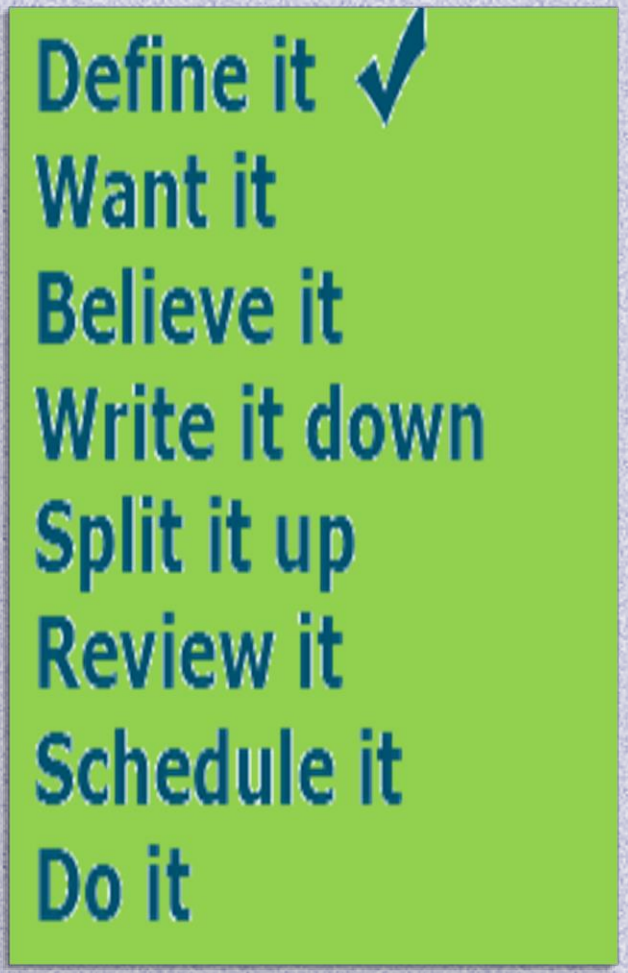
- Exam prep suggested reading ascp.org
  - Links to purchase references
- Blood Banking reference list aabb.org
  - Can search by keyword
  - Links to purchase references

List of CAAHEP SBB Programs

Correction: www.redcrossblood.org/socal/commun

Study Plan

- Consistent study and review time
  - Make an action plan and time line
  - Stick to your plan and study time!
  - Final review
    - According to category
    - Focus on weaker areas



Study Plan (Cont.)

- Compile all Blood Group information
  - Genetics
  - Biochemistry
  - Antigens & antibodies
  - Highlights, unique points
- Compile serological testing information
  - Procedures
  - Quality control
  - Appropriate use
  - Results

Study Plan (Cont.)

- Compile component information
  - Collection & preparation
  - Storage requirements
  - Expiration dates
  - Content
  - Quality control
  - Appropriate use

- Compile donor information
- Compile complement & coagulation pathways

Getting in the Door

- Admission letter
- 2 valid IDs
  - Name must match admission letter
- Palm vein image
- Say Cheese!
  - Picture
  - Audio / video
- Purse/bag, etc. will be locked in a locker

Inside the Testing Center

- Testing center will provide
  - White paper or white board
  - Master panel booklet for antibody ID
- You may use non-programmable calculator
- No cell phones allowed

Starting the Exam

- Do your “brain dump”
- 2.5 hours for 100 questions
  - Timing does not start until you click “start”
  - Keep an eye on the time throughout exam
- Questions are multiple choice, presented one at a time
- Click A, B, C, or D; enter or next

Starting the Exam (Cont.)

- Visual material such as graphs or photographs appear on the screen with the question
- Must give best guess before next question
- You can flag questions to review later
- 10 questions will not be graded
  - New questions to be evaluated

Computer Exam

- Exam Categories
  - Computer gives fixed number of questions from each category
  - Does not give more or less, regardless of performance
- Question Difficulty Rating
  - Taxonomy Level of questions will vary according to your ability to achieve the correct answer

Taxonomy Levels

Cognitive Skill	Purpose	Performance / Ability Required
Recall (Level 1)	Measure memory	Recall knowledge ranging from specific facts to complete theories
Application (Level 2)	Measure basic interpretation of data	Use recall to interpret or apply data
Analysis (Level 3)	Measure application of knowledge	Use recall and interpretive skills to resolve problems and/or make an appropriate decision

Computer Adaptive Testing

- Computer adapts exam to your performance
- Chooses each specific category question by difficulty
- Computer estimates your ability and selects questions with matching difficulty
- The weight value given to each question is determined by difficulty level (taxonomy level)

How Does the Computer do That?

- Student answers a few questions
- Computer makes rough estimate of ability based on those answers
- Computer gives student a question equal to that ability

Answers

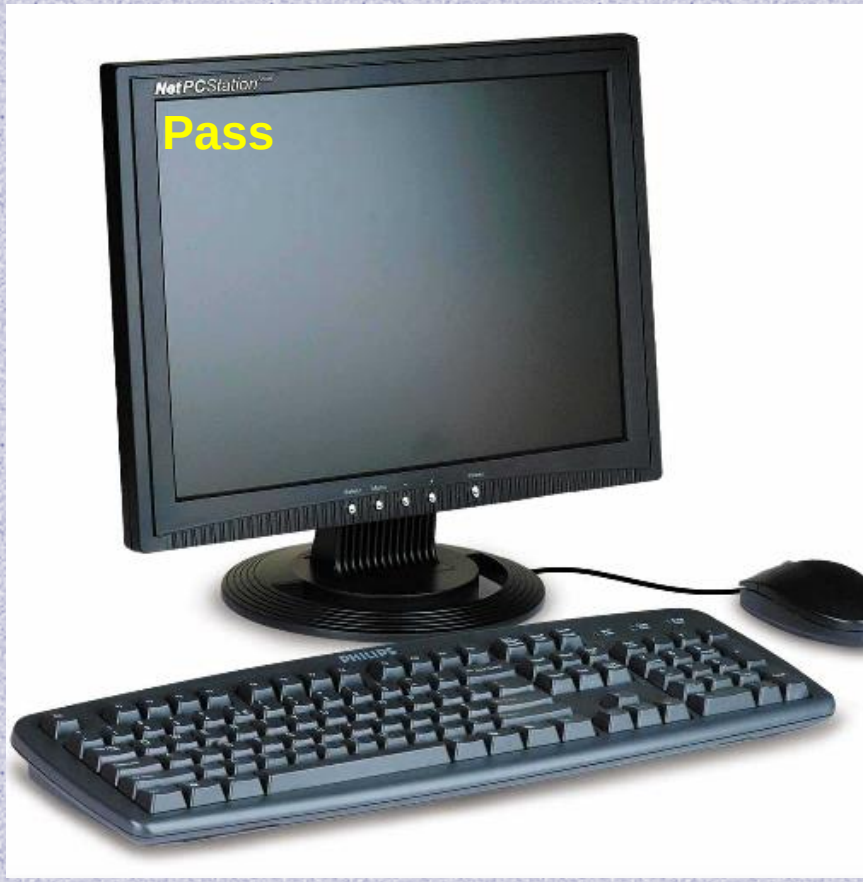
- Answer correctly, ability is boosted
  - Next question has a slightly higher difficulty level
  - Difficulty level continues to increase until a question is answered incorrectly
- Answer incorrectly, a slightly easier question is presented
- In this way the test is tailored to the individual's ability level

CAT Summary

- Computer makes rough estimate of ability
- Each question answered boosts or lowers estimated ability
- With each answered question, estimate of ability becomes more statistically correct
- Passing score range is 400-999

Exam Score

- When finished, preliminary Pass/Fail shows on screen
- ASCP will provide exam report to view score
  - Break down of scores for each subtest category



Certificate

- Certificate will be mailed within 4-6 weeks of exam date
  - valid for 3 years

- Certification Maintenance Program (CMP) information available
  - CMP is mandatory and is a way to document your continuing education/competency
  - Submit CMP with your renewal in 3 years

If You do NOT Pass - the First Time

- DO NOT GIVE UP!
- Make a study plan based on subtest scores
- Don’t delay!
  - Register for the next exam period
  - Readjust your study plan as needed
- You can take same exam by same eligibility route up to 5 times

Good Luck!!!

You can do it…