Blood Bank I

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The Fun Just Never Ends…

A. Blood Bank I
- Blood Groups

B. Blood Bank II
- Blood Donation and Autologous Blood
- Pretransfusion Testing

C. Blood Bank III
- Component Therapy

D. Blood Bank IV
- Transfusion Complications
  * Noninfectious (Transfusion Reactions)
  * Infectious (Transfusion-transmitted Diseases)

E. Blood Bank V (not discussed today but available at www.bbguy.org)
- Hematopoietic Progenitor Cell Transplantation

F. Blood Bank Practical
- Management of specific clinical situations
- Calculations, Antibody ID and no-pressure sample questions

Blood Bank I
Blood Groups

I. Basic Antigen-Antibody Testing

A. Basic Red Cell-Antibody Interactions

1. Agglutination
   a. Clumping of red cells due to antibody coating
   b. Main reaction we look for in Blood Banking
   c. Two stages:
      1) Coating of cells ("sensitization")
         a) Affected by antibody specificity, electrostatic RBC charge, temperature, amounts of antigen and antibody
         b) Low Ionic Strength Saline (LISS) decreases repulsive charges between RBCs; tends to enhance cold antibodies and autoantibodies
         c) Polyethylene glycol (PEG) excludes H_2O, tends to enhance warm antibodies and autoantibodies.
      2) Formation of bridges
         a) Lattice structure formed by antibodies and RBCs
         b) IgG isn't good at this; one antibody arm must attach to one cell and other arm to the other cell.
         c) IgM is better because of its pentameric structure.

2. Hemolysis
   a. Direct lysis of a red cell due to antibody coating
   b. Uncommon, but equal to agglutination.
      1) Requires complement fixation
      2) IgM antibodies do this better than IgG.

B. Tube testing

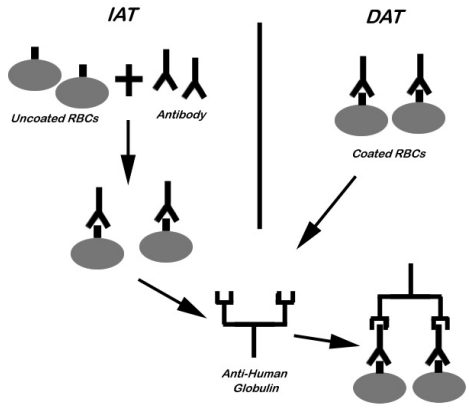
1. **Immediate spin phase**
   a. Mix serum, 2-5% RBC suspension; spin 15-30 sec.
      1) Most common: 2 drops serum, 1 drop RBCs.
   b. Antibodies reacting here are usually IgM
2. **37 C phase**
   a. Add potentiator (+/-), incubate at 37 C, spin.
   b. Potentiators and incubation times:
      1) 10-15 minutes for LISS
      2) 15-30 minutes for albumin or PEG
      3) 30-60 minutes for no potentiation
3. **Indirect antiglobulin** ("antihuman globulin") **phase**
   a. Wash above to remove unbound globulins.
   b. Add antihuman globulin, spin.

C. Alternatives to tube testing

1. **Column agglutination technology (Gel testing)**
   a. Add RBCs and plasma to top of tube, incubate, spin.
   b. Microtubes are filled with gel particles and anti-IgG
      1) Anti-IgG grabs onto IgG-coated RBCs and inhibits their migration through gel *immunologically*
      2) Gel particles separate RBC clusters *physically* (inhibit agglutinates from migrating through gel).
   c. Results:
      1) Negative: RBCs form button at bottom of microtube.
      2) Positive: RBCs stopped in areas through the microtube (more positive = higher position in tube)
   d. Can be automated (ProVue machine)
   e. Similar sensitivity to PEG tube testing
2. **Solid-phase Red Cell Adherence Testing**
   a. Antibody binds to lysed or intact RBC antigens that are bound by manufacturer to the sides of microwells
   b. Add patient serum, incubate, wash: If positive, antibody binds to test RBCs.
   c. Indicator RBCs (coated with monoclonal anti-IgG) attach to antibody on test RBCs.
   d. Spin and interpret
      1) Negative: RBCs in a button at bottom of microwell, (indicator cells didn't bind to the test RBCs).
      2) Positive: RBCs spread in a "carpet" all along the microwell (indicator cells did bind to test RBCs).
   e. Can be automated (Galileo, Galileo Echo, NEO)
   f. Similar sensitivity to PEG tube testing

D. The Antiglobulin Test ("Coombs Test")

1. Indirect: see above; demonstrates *in-vitro* RBC coating with antibody and/or complement.
2. Direct: red cells from patient washed, then mixed with antihuman globulin; demonstrates *in-vivo* RBC coating with antibody and/or complement.



3. IAT variations
   a. Unknown antibody check: Use RBCs with a known antigen profile, as in an antibody screen
   b. Unknown RBC antigen check: Use serum with known antibody specificity, as in RBC antigen testing
   c. Can be used to check for an unknown antigen *OR* unknown antibody, as in the crossmatch procedure
4. Specificity possibilities for the antiglobulin
   a. Anti-IgG, -C3d ("polyspecific"); most common to start
      1) Detect red cells coated with either of the above
      2) May also detect other immunoglobulins (because the anti-IgG detects light chains, too)
   b. Anti-IgG and anti-IgG (heavy chain-specific)
      1) Both detect IgG-coated red cells
      2) Anti-IgG used for PEG, gel, and solid phase tests
   c. Anti-C3b, -C3d
      1) Detects either of the above complement components
      2) Most useful in evaluating IgM-related hemolysis, cold agglutinin disease
5. IgG-sensitized RBCs ("Coomb's control", "check cells")
   a. Use after *negative* DAT or IAT tube test (not gel or solid-phase) to ensure functioning of AHG reagent
   b. Add IgG-coated cells to AHG-cell mixture
   c. Negative = bad AHG or no AHG added
   d. Other errors (e.g., omitting test serum) missed.

E. Dosage

1. Some antibodies react more strongly with RBC antigens that have homozygous gene expression.
2. For example, imagine a hypothetical anti-Z
   a. Patient 1 genotype: ZZ (Homozygous for Z)
   b. Patient 2 genotype: ZY (Heterozygous for Z)

c. If anti-Z shows dosage, it will react stronger with patient 1's RBCs (see below).

RBC Genotype	Reaction with anti-Z
ZZ	3+
ZY	1+

3. Most common in Kidd, Duffy, Rh and MNS systems

F. Enzymes

1. Proteolytic enzymes (e.g., ficin, papain) cleave RBC surface glycoproteins and can strengthen reactions by enhancing antigen expression or allowing antibodies to bind better to previously shielded antigens
2. Enzymes may also directly destroy other antigens
3. Useful in antibody identification to confirm or refute a particular antigen as target of an antibody (see table)
4. **The "Enzyme Classification"**

Enhanced	Decreased	Unaffected
ABO-related	**MNS System**	**Kell System**
ABO, H Systems	**Duffy System**	**Diego System**
Lewis System	**Lutheran System**	**Colton System**
I System		
P System		
Rh System		
Kidd System		

G. Neutralization

1. Certain substances, when mixed with a red cell antibody, inhibit the activity of that antibody against test red cells.
2. Some of these are pretty weird! (See table below)

Neutralization of Antibodies	
ABO	Saliva (secretor)
Lewis	Saliva (secretor for Le^b)
P1	Hydatid cyst fluid Pigeon egg whites
Sd^a	Human urine
Chido, Rodgers	Serum

H. Lectins

1. Seed/plant extracts react with certain RBC antigens
2. Especially useful in polyagglutination (T, Tn, etc)
3. May be commercial or homemade

Lectin	Specificity
Dolichos biflorus	A_1
Ulex europaeus	H
Vicia graminea	N
Arachis hypogea	T
Glycine max	T, Tn
Salvia	Tn

II. Blood Groups

A. General characteristics

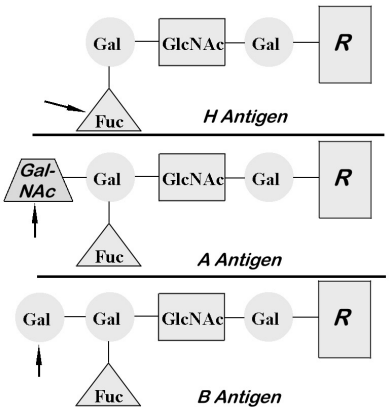
1. Definition
   a. Blood group antigen: Protein, glycoprotein, or glycolipid on RBCs, detected by an alloantibody
      1) NOTE: Antigens are not limited to RBCs
   b. Blood group system: Group of blood group antigens that are genetically linked (30 total systems per ISBT)
2. Significance
   a. "Significant" = antibody causes HTRs or HDFN
   b. Most significant antibodies are "warm reactive"; meaning they react best at IAT (37 C).
   c. Most insignificant antibodies are "cold reactive"; meaning they react best below 37 C.
   d. Warm antibodies most often IgG, colds usually IgM.
   e. IgM antibodies are usually "naturally occurring" (no transfusion or pregnancy required for their formation).
   f. ABO is the exception; see asterisks in table below

"WARM-REACTIVE"	"COLD-REACTIVE"
IgG	IgM
Require exposure	Naturally occurring
Cause HDN	No HDN*
Cause HTRs	No HTRs*
"Significant"	"Insignificant"*

B. ABO and H Systems

1. Basic biochemistry (see figure below)
   a. Type 1 and 2 chains
      1) Type 1: Glycoproteins and glycolipids in secretions and plasma carrying free-floating antigens
      2) Type 2: Glycolipids and glycoproteins carrying bound antigens on RBCs.
   b. *Se* gene (FUT2; FUT = "fucosyltransferase")
      1) "Secretor" gene (chrom 19); Precursor to making A or B antigens in secretions
      2) FUT enzyme adds fucose to *type 1* chains at terminal galactose; **product is type 1 H antigen**
      3) 80% gene frequency
   c. *H* gene (FUT1)
      1) Closely linked to *Se* on chrom 19
      2) FUT enzyme adds fucose to *type 2* chains at terminal galactose; **product is type 2 H antigen.**
      3) Virtually 100% gene frequency (Bombay = *hh*).
   d. H antigen required before A and/or B can be made on RBCs (type 2 H) or in secretions (type 1 H).
      1) Single sugar added to a type 1 or 2 H antigen chain makes A or B antigens and eliminates H antigen.
         a) Group A sugar: **N-acetylgalactosamine**

b) Group B sugar: **Galactose**



2) As more A or B is made, less H remains.
   a) H amount: O > A_2 > B > A_2B > A_1 > A_1B

2. ABO antigens
   a. Genotype determined by three genes on long arm of chrom 9: ***A, B* and *O*** (*O* is nonfunctional).
   b. A and B genes code for transferase enzymes, not directly for an antigen (as above)
   c. ABO antigens begin to appear on fetal RBCs at 6 weeks gestation; reach adult levels by age 4.
      1) Also present on platelets, endothelium, kidney, heart, lung, bowel, pancreas tissue
3. ABO antibodies
   a. Antibodies clinically significant, naturally occurring
   b. Begin to appear at 4 months of age; reach adult levels by age 10 and may fade with advanced age
   f. Three antibodies: anti-A, anti-B and anti-A,B; differ by blood group
      1) Group A and B: Anti-A or –B is predominantly IgM, but each reacts strongly at body temperatures.
      2) Group O: Anti-A and –B are predominantly **IgG**, and react best at body temperatures
      3) Group O: Anti-A,B is IgG reacting against A and/or B cells (reactivity can't be separated into individual specificities).

Type	Whites	Blacks	Asians	Native Americans
O	45%	49%	40%	79%
A	40%	27%	28%	16%
B	11%	20%	27%	4%
AB	4%	4%	5%	<1%

4. ABO blood groups
   a. **Group O**
      1) The most common blood group across racial lines
      2) Genotype: *OO*
      3) Antigen: H
         a) *Ulex europaeus* lectin reacts with H antigen.

4) Antibodies: Anti-A, anti-B, anti-A,B (see above)
   a) Because of strong IgG component to all above antibodies, mild HDFN is common in O moms
   b) Why not severe? Weak fetal ABH expression, soluble ABH antigens (neutralize antibodies)

b. **Group A**
1) Possible genotypes: *AA, AO*
2) Antigens: A, H
3) Antibody: anti-B (primarily IgM).
4) A subgroups
   a) A_1 (80%) and A_2 (~20%) most important
   b) Monoclonal anti-A agglutinates both types well
   c) A_1 red cells carry about 5x more A on RBC surfaces than A_2 cells
   d) Qualitative differences also exist in the structure of the antigenic chains (type 3 and 4 for A_2).
   e) 1-8% of A_2 and 25% of A_2B form anti-A_1.
      • Usually clinically insignificant IgM
      • Common cause of ABO discrepancies.
      • If reactive at 37C, avoid A_1 RBC transfusion.
   f) *Dolichos biflorus* lectin agglutinates A_1 but not A_2 RBCs.

c. **Group B**
1) Genotypes: *BB, BO*
2) Antigens: B, H
3) Antibodies: Anti-A (primarily IgM).
4) B subgroups: Usually unimportant and less frequent

d. **Group AB**
1) Least frequent ABO blood type (about 4%)
2) Antigens: A and B (very little H)
   a) Can be further subdivided into A_1B or A_2B depending on the status of the A antigen
3) Antibodies: none

5. ABO testing

Cell		Serum		ABO Group
Anti-A	**Anti-B**	**A_1 cells**	**B cells**	
4+	0	0	**4+**	**A**
0	**4+**	**4+**	0	**B**
4+	**4+**	0	0	**AB**
0	0	**4+**	**4+**	**O**

a. Cell grouping ("forward grouping")
1) Patient red cells agglutinated by anti-A, anti-B.

b. Serum grouping ("reverse grouping", "back typing")
1) Patient serum (or plasma) against A_1 and B RBCs.

c. Note the opposite reactions!
1) If forward reactions are not opposite of reverse, an ABO discrepancy is present.

d. Both serum and cell grouping required unless testing babies < 4 months of age or reconfirming ABO testing done on donor blood (requires cell grouping only).

6. ABO discrepancies
   a. Disagreement between the interpretations of cell and serum grouping (e.g., forward = A, reverse = O); caused by antigen and/or antibody problems or technical errors.
   b. Antigen problems
      1) Missing antigens
         a) A or B subgroups
         b) Transfusion or transplantation
         c) Leukemia or other malignancies
      2) Unexpected antigens
         a) Transfusion/transplantation out-of-group
         b) Acquired B phenotype (more below)
         c) Recent marrow/stem cell transplant.
         d) Polyagglutination
   c. Antibody problems
      1) Missing antibodies
         a) Immunodeficiency
         b) Neonates, elderly, or immunocompromised
         c) Transplantation or transfusion
         d) ABO subgroups
      2) Unexpected antibodies
         a) Cold antibodies (auto- or allo-)
         b) **Anti-A_1**
         c) Rouleaux/plasma expanders (false positive)
         d) Transfusion or transplantation
         e) Reagent-related antibodies
   d. Technical errors
      1) Sample/reagent prep, mix-ups, or interpretation errors
7. Weird stuff about ABO
   a. **Acquired B phenotype**
      1) A_1 RBC contact with enteric gram negatives: Colon cancer, intestinal obstruction, gram-negative sepsis
      2) AB forward (with weak anti-B reactions), A reverse
      3) Bacterial enzymes deacetylate group A GalNAc; remaining galactosamine looks like B and reacts with forms of monoclonal anti-B (ES-4 clone).

Cell Typing			Serum Typing		
Anti-A	**Anti-B**	**Interp**	**A_1 cells**	**B cells**	**Interp**
4+	1-2+	**AB**	0	4+	**A**

      4) Use monoclonal anti-B that does NOT recognize acquired B, acidify serum (no reaction with anti-B)

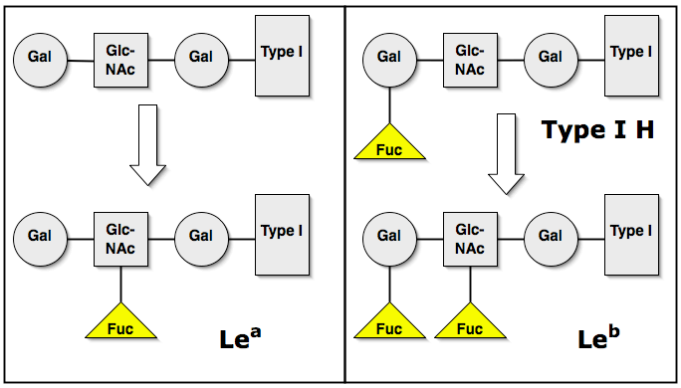
b. **B(A) phenotype**
   1) Opposite of acquired B (group B patients with weak A activity); this condition is inherited, not acquired
   2) Cross-reaction with a specific monoclonal anti-A; test using different anti-A shows the patient as B.

c. **Bombay (O_h) phenotype**
   1) Total lack of H, A and B antigens due to lack of *H* and *Se* genes (genotype: *hh*, *sese*)
   2) Naturally occurring strong anti-H, anti-A, anti-B
   3) Testing: O forward, O reverse, but antibody screen wildly positive and all units incompatible
   4) "Para-Bombay" phenotype
      a) Like Bombays, are *hh*, but unlike Bombays, have at least one *Se* gene
      b) Phenotypes: A_h, B_h, AB_h
      c) RBCs may be Bombay-like, but may also show free or RBC A or B antigens (unless group O).
      d) Allo-anti-H present in serum.
   5) Both Bombay and Para-Bombay need H-negative blood (from Bombay donors)

8. Consequences of ABO incompatibility
   a. Severe acute hemolytic transfusion reactions
      1) Among most common blood bank fatalities
      2) Clerical errors
   b. Most frequent HDFN; usually mild, however

C. Lewis System

1. Biochemistry (see figure below)
   a. Type 1 chains only
   b. One gene: *Le* (FUT3)
      1) Second gene, *le*, is nonfunctional
   c. FUT enzyme adds fucose to **subterminal GlcNAc** (left side of figure below).
      1) This makes **Le^a** (Lewis A) antigen.
      2) Le^a antigens **cannot** be modified to make Le^b.



   d. In secretors, *Se* product (FUT2) adds fucose, then *Le* product adds fucose; this makes **Le^b** (Lewis B).
      1) In secretors, Le^b formation occurs preferentially.

2) As a result, the vast majority of the chains of those who carry *Le* and *Se* are Le^b rather than Le^a.
3) In non-secretors, Le^a is only possible Lewis antigen.

e. Unlike ABO, antigens are not tightly bound (remember, they are made from *type 1 chains*); rather, they **adsorb** onto the surface of RBCs.
1) Le^b does this better than Le^a; another reason that most adults with both *Le* and *Se* will be Le(a-b+).
2) Le(a-b+) people still have Le^a, just in much smaller quantities that may not show up on RBCs.

f. Same chain can carry Le and ABO antigens (unlike the inverse relationship with ABO and H).

2. Lewis phenotypes, antigens, and antibodies
a. Phenotypes: Le(a-b+), Le(a+b-), Le(a-b-)
b. **22% of blacks are Le(a-b-), vs. only 6% of whites**.
c. Antibodies are naturally occurring, cold-reacting IgM.
1) Primarily in Le(a-b-)
2) Neutralize with saliva from secretors.
3) Antibodies commonly also show ABH specificity (e.g., anti-Le^{bH} reacts best with O or A_2 RBCs)

3. Consequences of incompatibility
a. Antibodies are generally insignificant
b. Rare HTRs (more commonly with anti-Le^a)
c. No HDFN (antibody doesn't cross placenta and Le antigens are not present on fetal RBCs).

4. Weird stuff about Lewis
a. Lewis antigens decrease during pregnancy.
1) Pregnant patients may appear Le(a-b-) and have transient, insignificant Lewis antibodies.
2) Increased plasma volume dilutes the antigens and increased plasma lipoproteins strip the antigens
b. Le(a-b+) people don't make anti-Le^a.
1) Still have Le^a, just not visible on their RBCs.
c. Children's Lewis type may vary, as antigen chains are converted [more Le^a than Le^b initially, with a transient period of Le(a+b+)]; by age 2, are Le(a-b+)
d. Infection associations:
1) *H. pylori* attaches to gastric mucosa via Le^b antigen.
2) Norwalk virus also attaches via Le^b
3) Le(a-b-) are at risk for *Candida* and *E. coli* infection

D. I System

1. Antigens built on type 2 chains.
2. Expression is age-dependent.
a. Simple chains found on neonates make i antigen.
b. Branched chains in adults make I antigen.
c. "Big I in big people, little i in little people"
d. Occasional adults lack I; they are known as "i_{adult}"; more common in Asians

3. Antibodies (usually autoantibodies)
   a. Cold reacting IgM, with auto-anti-I seen commonly
   b. Naturally occurring, common, usually insignificant
   c. Like Lewis, antibodies commonly have H specificity as well (e.g., anti-IH reacts better against O and A_2)
4. Classic associations
   a. Auto-anti-I
      1) Cold agglutinin disease
      2) ***Mycoplasma pneumoniae*** **infection**
   b. Auto-anti-i
      1) Associated with **infectious mononucleosis**
      2) Less often a problem than auto-anti-I
   c. I_{adult} phenotype
      1) Cataracts
      2) HEMPAS

E. P System (the cool one)

1. Also built on ABO-related chains
2. Antigens
   a. P1 is the only antigen
      1) P, P^k not officially in P system anymore
      2) These three antigens define the overall P phenotype.
      3) Most common P phenotype: $\mathbf{P_1}$ ($P+P1+P^k-$).
   b. Very rare people lack all three and make anti-$PP1P^k$.
      1) Acute HTR and early spontaneous abortions
   c. P antigen is **parvovirus B19** receptor.
   d. P^k antigen is receptor for various bacteria and toxins
3. Antibodies (anti-P1)
   a. Cold reacting, naturally occurring, insignificant IgM; rare anti-P1 reactive at AHG is potentially significant
   b. Titers elevated in those with hydatid cyst disease (*Echinococcus*) and bird handlers
      1) Bird feces contains P1-like substance.
   c. Neutralized by hydatid cyst fluid, pigeon egg whites
4. Association with paroxysmal cold hemoglobinuria
   a. Biphasic IgG with anti-P (not P1) specificity
      1) Binds in cold temps, hemolyzes when warmed
      2) "Donath-Landsteiner biphasic hemolysin"
   b. Historically in syphilis, now after viral infx in children

F. Rh System

1. Second most important blood group (after ABO)
2. Old (incorrect) Rh antigen terminology systems
   a. **Fisher-Race (DCE or CDE)**
      1) Five major antigens: D, C, E, c, e
         a) "Rh positive" really means "D positive."
         b) Absence of D designated "d" (no d antigen)
         c) C/c and E/e are antithetical (e.g., can't have both C and c or E and e from same chromosome)
      2) Eight potential combinations based on presence of genes for above antigens (ie, "DCe", "dce", etc.)

b. **Wiener (Rh-Hr)**
   1) Different, archaic names for the five main antigens
   2) Believed that main Rh genes (for presence or absence of D, for C or c and for E or e) inherited as one genetically linked group, or "haplotype."
   3) Shorthand names to the haplotypes; nomenclature is still in use and is essential to know (though theory of how these are inherited has been disproven).

Wiener's "Haplotypes" (with DCE Equivalents)	
R_1: DCe	**r' : dCe**
R_2: DcE	**r": dcE**
R_0: Dce	**r : dce**
R_z: DCE	**r^y : dCE**

   a) Rules for converting Wiener's modified haplotypes into Fisher-Race terminology:
      - "R" = D, "r" = d
      - "1" or "prime" = C
      - "2" or "double prime" = E
      - "0" or "blank" = ce
      - Any sub- or superscript letter = CE
   4) Only four of the above combinations occur with significant frequency: **R_1, R_2, R_0 and r**. (~97% of blacks and whites use only these four).
      - R_0 most common in blacks, least common in whites.
      - r is always second in frequency.
      - R_1 always comes before R_2.

"The Big Four"
Whites: $R_1 > r > R_2 > R_0$
Blacks: $R_0 > r > R_1 > R_2$

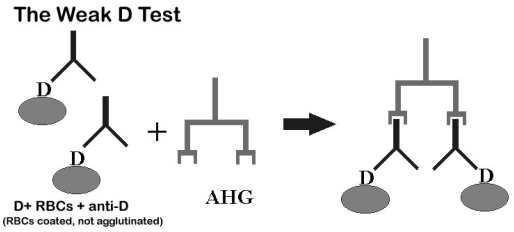
   5) Asians us. D+; their order is $R_1 > R_2 > r$ and R_0.

c. Current understanting of Rh genetics/structure
   1) Two genes, *RHD* and *RHCE* (chromosome 1) code for two main Rh proteins (RHD and RHCE)
   2) D type determined by presence/absence of RHD
   3) One protein gives both C/c and E/e antigens; combination determined by which alleles of *RHCE* are present (*CE*, *Ce*, *cE*, or *ce*)

3. Rh antibodies
   a. Exposure-requiring, warm-reacting IgG
   b. D induces the most antibodies, then c and E
      1) Traditional: 80-85% of D negatives make anti-D when exposed to one unit of D pos RBCs
      2) Recent data: 20-30% in hospital settings
   c HTRs with extravascular hemolysis

d Severe and prototypical HDFN with anti-D, severe HDFN with anti-c, mild HDFN with anti-C, -E, -e

4. Weird stuff about Rh
   a. **D-negative phenotype**
      1) Unusual because caused by mutations and deletions rather than by synthetic actions of a gene product
      2) Caucasians: D-negatives have deletion of *RHD* gene
      3) African-Americans: Point mutations in *RHD* gene ("pseudogene")
      4) Asians: Usually have inactive *RHD* gene
   b. **D Variants**
      1) **Weak D** (formerly "D^u")
         a) Usual D testing: Monoclonal IgM with polyclonal IgG read only at immediate spin
         b) Almost all D+ test as D+ with these reagents
         b) Some D+ individuals have decreased D expression and require IAT to detect D antigen.



         c) Possible reasons for weak D
            - Mutated form of *RHD*
              - Point mutation causing altered amino acids in membrane or inner part of RHD
              - Type 1 common in Caucasians
            - *RHCe* on opposite chromosome to *RHD* ("C in trans") inhibits D expression
         d) Testing requirements
            - Weak D test for all D-negative blood donors
            - Not required for D-negative blood recipients
              - Previously a concern, for fear of wasting D-neg units on D+ patients
              - Monoclonal antibodies mentioned above make this very unlikely
              - The only patients who definitely need weak D testing are apparently D-negative babies with D-negative moms.
         e) Weak D moms do not need RhIG prophylaxis
      2) **Partial D** ("D Category", "D mosaic")
         a) At one time considered a form of weak D
         b) Lack portions (epitopes) of D antigen.
         c) *RHD* gene mutations leading to alteration of exterior part of RHD antigen
         d) Antibodies form against absent parts of RHD; this antibody appears to be anti-D at first glance

e) Classic: Anti-D in a D-positive person
f) Most common: DVI (D "six") in whites
- Monoclonal anti-D usually types these as D-negative (prevents D exposure as recipients)

g) Note that partial C and partial e antigens exist, and can result in unusual antibodies
h) Partial D moms do need RhIG prophylaxis
i) *Partial D vs. weak D may be impossible without molecular testing; if in doubt for prenatal testing, consider patient D-negative*

3) **D_{el}** ("D-E-L")
a) Appear D-neg but have tiny amounts of D seen after elution of reagent anti-D from RBCs
b) Primarily seen in Asian populations (up to 1/3 of D-negative Asians)

c. These antibodies go together…
1) Anti-E formation commonly accompanied by anti-c (not necessarily vice-versa)
2) Think "Big 4"; R_2R_2 gives both E and c exposure

d. **Compound Rh antigens**
1) **G** = Antigen present when either C or D is present
- Anti-G reacts against (D+C-), (D-C+), or (D+C+) RBCs (rarely against D-C-G+)
- Common presentation: D-negative person forms anti-D when not obviously exposed to D
- Important because if D-neg mom has anti-G, she DOES still need RhIG to prevent anti-D
- Can cause HTRs (give D-C- blood)
- See bbguy.blogspot.com/2011/08/g-whiz.html

2) **f** = Present when *RHce* is inherited (r and R_0).
- Anti-f is often seen with anti-e or anti-c
- Can cause mild HDFN and HTR

G. Kidd System

1. Kidd antigens
a. Jk^a, Jk^b, Jk3 (very high frequency)
b. Jk^a slightly more common than Jk^b in African Americans but similar in whites and Asians
c. Antigens reside on a urea transport protein

2. Kidd antibodies
a. Exposure requiring, warm-reacting IgG (often with IgM component as well)
1) Can fix complement (with IgM component)
2) Severe acute HTRs possible

b. **Marked dosage effect**
1) Antibodies may not react at all against cells with heterozygous Kidd antigens

c. **Variable antibody expression**
1) Antibody often disappears with time/storage.

3. Weird stuff about Kidd
   a. **Delayed HTRs (most famous association)**
      1) Anamnestic response
      2) Intravascular and often severe
   b. **Mild HDFN at worst**
      1) Child can only be one antigen different from mom; remember dosage discussion above.

H. MNS System

1. Basic biochemistry
   a. Glycophorin A (GPA) carries M or N antigens.
   b. Glycophorin B (GPB) carries S or s, and U antigens.
2. MNS antigens
   a. M frequency roughly equals N (each ~75%)
   b. s (~90%) is more frequent than S (~50%W, ~30%B)
   c. If S-s- (as seen in 2% of African-Americans), may also be U-negative (U is extremely high frequency).
   d. *Vicea graminea* lectin reacts against N antigens
   e. **Mur**: Hybrid antigen seen in nearly 10% of Chinese
      1) Significant antibodies can form; more frequent in some areas than anything but anti-A or -B
3. MNS antibodies
   a. M and N antibodies are mostly opposite of S, s and U antibodies (see below)

Anti-M & anti-N	Anti-S, -s and -U
Naturally occurring	Require exposure
Cold IgM	Warm IgG
Dosage	Minimal dosage
Insignificant	Significant

   b. Anti-M and anti-N can usually be ignored unless reactive at 37C; not so with anti-S and anti-s
      1) Though anti-M is usually insignificant, it has been rarely associated with severe HDFN.
   c. Effect varies by enzyme, but enzymes generally decrease all MNS antigens except U
4. Weird stuff about MNS
   a. **N-like antigen ('N')**
      1) GPB always has terminal 5 amino acid sequence that matches GPA's terminal sequence when it is expressing N; this is known as 'N'.
         a) Not really true N antigen, but it's close enough to prevent most M+N- from making anti-N.
      2) Seen in all except those who lack glycophorin B.
         a) <1% of blacks lack S, s, and U; rare in whites
         b) Anti-N nearly exclusive to African-Americans
   b. Auto-a**nti-N induced by hemodialysis**
      1) Formaldehyde sterilization of machine

2) Modification of N leads to rare autoantibody

I. Duffy System

1. Duffy antigens and genes
   a. Fy^a from *Fy^a* gene; high frequency in Asians
   b. Fy^b from *Fy^b* gene; high frequency in caucasians
   c. Absence of both antigens, **Fy (a-b-), is most common Fy phenotype in African-Americans (68%, even higher in Africa).**
      1) Due to inheritance of two copies of *Fy* gene, which gives no functioning Duffy glycoprotein
      2) *Fy* is an *Fy^b* gene variant, and gives *Fy^b* antigen in non-RBC tissues
2. Duffy antibodies
   a. Anti-Fy^a more common and significant than anti-Fy^b
   b. Exposure requiring, warm-reactive IgG
   c. Marked dosage and variable expression like Kidd Abs
3. Consequences of incompatibility
   a. Severe HTRs, usually delayed and extravascular
   b. Often mild, occasionally severe HDFN
4. Weird stuff about Duffy
   a. **Fy(a-b-) and malarial resistance**
      1) Fy(a-b-) humans are resistant to *Plasmodium vivax* and *P. knowlesi* infection.

J. Kell System

1. Extremely important group clinically and serologically
2. Kell antigens
   a. Low frequency: **K, also known as "KEL1" (9% whites, 2% blacks)**, Js^a, Kp^a
   b. High frequency: k or "KEL2" (99.8%), Js^b, Kp^b
   c. Kx antigen: Bound to Kell glycoprotein on the red cell membrane; required for proper Kell antigen expression
      1) Actually a separate blood group (Kx system)
      2) When Kell antigens decrease, Kx increases (as in K_0, aka "Kell null")
      3) When Kx decreases (as in "McLeod syndrome", see later), Kell antigens decrease, too.
   d. Kell system antigens destroyed by thiol reagents (2-ME, DTT, ZZAP) but not by enzymes alone.
3. Kell antibodies
   a. Anti-K
      1) Most common non-ABO antibody after anti-D
      2) Exposure-requiring, warm reacting IgG1
      3) More common from <u>transfusion</u> than pregnancy
   b. Anti-k
      1) Very uncommon due to high antigen frequency
      2) Antibody is just like anti-K
4. Consequences of incompatibility
   a. Severe HTRs
      1) May be acute or delayed; usually extravascular.

b. Severe HDFN
   1) Less common than ABO or RHD HDFN
   2) Damages EARLY RBC <u>precursors</u>, so tends to be *suppressive* rather than hemolytic
      a) Lower bilirubin and reticulocytopenia than with anti-D HDFN

5. Weird stuff about Kell
   a. **Kell null phenotype ("K_0")**
      1) All Kell antigens decreased, Kx increased
      2) Significant anti-Ku ("universal") with exposure
   b. **McLeod phenotype**
      1) Kx absent, all Kell antigens markedly decreased
      2) No anti-Ku like K_0, but can form anti-Kx and anti-Km (Kell "McLeod"); only compatible with McLeod RBCs
      3) Phenotype is part of McLeod "syndrome"
         a) Hemolytic anemia with **acanthocytes**
         b) Myopathy, ataxia, peripheral neuropathy, cognitive impairment, cardiomyopathy
         c) Occasional association with X-linked chronic granulomatous disease
            • NADPH oxidase deficit
            • Organisms phagocytized but not killed
            • Catalase-positive organisms (*Staph*)

K. Diego System

1. Over 20 antigen system built on "band 3"
   a. Important RBC membrane structure
   b. Carries HCO_3^- anions out of RBCs (for CO_2 removal), and anchors membrane to cytoskeleton
2. Diego antigens
   a. Di^a and Di^b antithetical pair
      1) Di^a very <u>low</u> frequency except in some South Americans and Asians
      2) Di^b very <u>high</u> frequency in all populations
   b. Wr^a and Wr^b antithetical pair
      1) Wr = "Wright"
      2) Wr^a very <u>low</u> frequency, Wr^b very <u>high</u> frequency
3. Diego antibodies
   a. Di antibodies are IgG, while Wr antibodies may have IgM component
   b. Both anti-Di^a and –Di^b can cause HDFN that may be severe but generally not HTRs
   c. Anti-Di^b can show marked dosage effect
   d. Anti-Wr^a is common, naturally occurring, and may cause both HTRs and severe HDFN (IgG + IgM)
   e. Anti-Wr^b, on the other hand, is rarely seen as an alloantibody but may be an autoantibody in autoimmune hemolytic anemia (AIHA)

L. A few other systems and antigens (in brief)

1. Dombrock System
   a. Do^a/Do^b antigens; Do^b more frequent
      1) Either antibody may cause HTRs but generally don't cause HDFN
      2) Warm-reactive IgG
   b. High frequency antigens Jo^a, Gy^a, Hy
      1) Mild HTRs or HDFN possible, but antibodies are very rare
      2) Near 100% incidence for all of these
      3) Jo^a- and Hy negative exclusively in blacks
      4) Gy^a negative in Japanese and eastern Europeans
2. Colton (Co) System
   a. Antigens (Co^a and Co^b) located on water transport membrane protein (aquaporin 1)
   b. Co^a very high frequency (near 100%), Co^b about 10%
   c. Both antibodies may cause significant HDFN
3. Lutheran (Lu) System
   a. Lu^a (low frequency; 5-8%) and Lu^b (very high frequency; 99.8%) antigens
   b. Antibodies uncommon, may be naturally occurring (anti-Lu^a), and not usually significant
   c. Most enzymes decrease Lu antigen activity.
4. Xg System
   a. Gene carried on X chromosome ("X-linked")
      1) Seen in 66% of males and 90% of females
   b. Antibody insignificant
5. Yt System
   a. Formerly "Cartwright"
   b. Yt^a (very high frequency; 99.8%), Yt^b (8%)
   c. Antibodies are IgG but not usually significant (occasional anti-Yt^a can cause HTRs, however)
6. Vel Antigen
   a. Extremely high frequency antigen (>99% in all populations)
   b. Antibody is mix of IgG and IgM
      1) May cause severe HTRs and HDFN
      2) May interfere with ABO typing due to reaction at room temperatures
      3) May be allo- or autoantibody
7. Landsteiner-Wiener (LW) System
   a. LW^a antigen is more abundant on D-positive RBCs
   b. LW antigens were originally thought to be Rh antigens
   c. Antibodies are not generally significant
8. Sd^a ("Sid") antigen
   a. High frequency (96%)
   b. Refractile, small immune complexes with naturally occurring IgM

c. Lectin of *Dolichos biflorus* agglutinates Sd^a positive RBCs (like A^1)
d. Neutralize with guinea pig or human Sd^a+ urine!

9. Antibodies with "high titer, low avidity" (HTLA) features
   a. High frequency antigens that are generally clinically benign (no HTRs or HDN)
   b. **Chido, Rodgers** most frequent
      1) Complement components (C4)
   c. Multiple others known
      1) Knops (Kn^a), McCoy (McC^a), JMH
   d. Must be careful, because some antibodies with similar features may be significant (anti-Vel, anti-Yt^a)