

Blood Bank Practical

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Outline

1. Specific Situations
2. Calculations
3. Antibody ID

I. Specific Situations in Transfusion Medicine

A. Emergency transfusion/acute hemorrhage

1. Situations of acute blood loss where there may not be time for full pretransfusion workups
 - a. Need is more urgent for volume than for oxygen carrying capacity; fluids more critical in early stages
 - 1) Historically, crystalloid was used for volume
 - 2) Excessive crystalloid can be harmful; has led to re-emphasis on colloids instead
 - b. Most common in trauma settings, and commonly leads to massive transfusion (see below)
 - c. Loss of over 30% of blood volume leads to significant clinical consequences and increased mortality
2. Priority: Don't harm anyone by doing something stupid!
 - a. High stress situations
 - b. Staff must train and drill for these events; it's not the time to be learning!
 - c. Standardize blood choices based on maximum safety
3. Possible strategies (these are only estimates).

Blood needed in:	Give:
Less than 10 minutes ("NOW!")	Uncrossmatched Group O neg (may give O+ if male or older female)
10-30 minutes	Uncrossmatched ABO group and Rh type specific
Over 30 minutes	Crossmatched ABO group and Rh type specific

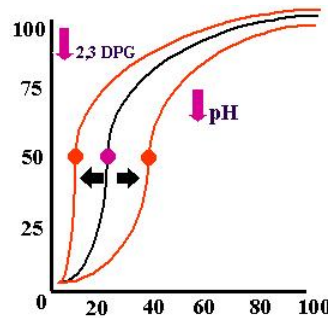
4. Documentation
 - a. Eventually need signed physician statement on chart (not a "waiver" of Blood Bank responsibility)
 - b. Don't delay therapy to get signature!
5. Rh issues
 - a. All transfusion services should have a policy for "standard" Rh types of blood to use in emergencies
 - b. For males and older females, it is acceptable to BEGIN by using O-positive blood in emergencies

- c. Use all possible means to give D-negative blood to childbearing age and younger females
- d. When D-neg blood is used, be prepared to switch to D-pos if massive use depletes inventory (see below)

B. Massive transfusion

1. Several definitions
 - a. Transfusion of an amount of blood equal to the patient's blood volume in 24 hours
 - b. Transfusion of 10+ units of blood in 24 hours
 - c. Transfusion of 50% of blood volume in 3 hours
 - d. Transfusion to replace blood loss of over 150 mL/min
2. Often follows emergency transfusion after trauma (see above), but also seen in:
 - a. Gastrointestinal hemorrhage
 - b. Cardiac bypass or other cardiovascular surgery
 - c. Post-partum or other obstetrical hemorrhage
3. Complications largely from "lethal triad" that greatly increases mortality in massive transfusion settings
 - a. Coagulopathy
 - 1) Thrombocytopenia due to platelet-poor transfusions
 - 2) Coagulopathy from dilution, factor-poor transfusions; decreased body temp and pH also affect coagulation
 - 3) Many trauma patients have acute coagulopathy before arriving at hospital
 - 4) See discussion of plasma transfusion in trauma in BBIII
 - 5) If coagulopathic, usually have increased bleeding and have increased risk of shock and death
 - b. Acidosis
 - 1) pH declines in stored blood
 - 2) Citrate anticoagulant may also contribute to acidosis
 - 3) Tissue injury and poor perfusion leads to lactic acidosis
 - c. Hypothermia
 - 1) Receiving large amounts of cold blood products may decrease body temperature
4. Summary of "storage lesion" of blood
 - a. What goes down:
 - 1) 2,3-DPG; see discussion below
 - 2) pH; see discussion below
 - 3) ATP; 50-60% levels at storage end
 - 4) Nitric oxide (NO); decreases oxygen delivery by decreasing ability to vasodilate
 - 5) RBC membrane flexibility; makes it more difficult to get into small capillaries
 - b. What goes up:
 - 1) Potassium; from hemolysis
 - 2) Free hemoglobin; from hemolysis

- 3) Biologic response modifiers; these have potential to be the main cause of increased mortality from “old” blood transfusions reported recently
5. Hemoglobin changes in stored blood (figure below)



- a. “p50” = partial pressure of oxygen where hemoglobin is 50% saturated.
 - b. “Shift to the left” = **decreased** p50 = **increased** oxygen affinity
 - 1) Decreasing 2,3-diphosphoglycerate (2,3-DPG); essentially zero after 14 days
 - c. “Shift to the right” = **increased** p50 = **decreased** oxygen affinity
 - 1) Decreasing pH in blood from glycolysis
 - d. End result: 2,3-DPG decline outweighs the pH decline, so curve is left-shifted in transfused blood.
6. Rh issues
 - a. Switching Rh types in D-negative patients
 - 1) Should be done only with medical director approval or according to pre-existing switching protocol
 - 2) Remember that if you use all D-negative blood for one patient, it is not available for anyone else
 - 3) Consider inventory levels and overall use when making policy
 - 4) Examples: Switch to O-pos when O-neg inventory gets to predetermined level (10 units, for example), or switch when D-neg person receives a predetermined number of D-neg units
 - 5) May also elect to switch ABO type before Rh type
 - b. What about RhIG?
 - 1) Risk of anti-D formation only approximately 20%
 - 2) In males, I do not recommend RhIG
 - 2) Consider in childbearing age and younger females, and has been reported to work
 - a) Should be IV form (“WinRho”)
 - b) Potential for hemolytic reaction
 7. ABO switching
 - a. Giving group O to a group A or B person may be necessary to conserve inventory
 - 1) This may lead to problems from incompatible plasma transfusion (eg, anti-A in a group A person)

- b. Switching back
 - 1) Probably safest to wait until incompatible plasma has been cleared (anti-A in above example)
 - 2) Use fresh sample to crossmatch vs. donor blood
- 8. Massive transfusion protocols (MTPs)
 - a. Standardized, “cookbook” recipes for product use in massive transfusion
 - b. Most trauma centers have MTPs; technically required by trauma certifying organizations
 - c. MTPs are designed to be proactive rather than reactive
 - d. Most MTPs today try to use 1:1 red cell to plasma transfusion ratio, with platelets and cryo given variably depending on the protocol
 - e. Studies are early but appear promising (problems: patient selection, randomization, confounding factors)

C. Organ transplantation

- 1. 26,213 organs transplanted in US in 2010 (UNOS data)
 - a. 59% kidneys, 22% livers, 8% hearts
 - b. Data relatively stable since 2001 or so
- 2. Transfusion needs greatest in *heart* and *liver* transplants, minimal in kidney transplants
- 3. General principles:
 - a. ABO compatibility most important in organ transplants (above HLA); in contrast to stem cell transplants.
 - 1) ABO-mismatched organs reserved for emergent, dire circumstances; done more often now
 - 2) Plasma exchange and immunosuppression used to minimize incompatible recipient ABO antibodies
 - 3) A₂ donor organs are special; low A antigen levels on RBCs and endothelial cells may allow them to go to a group O or B recipient without a problem
 - b. Avoid HLA immunization before transplant if possible; especially in renal and cardiac transplant
 - c. Pre-renal transplant transfusion no longer required
 - d. CMV prevention is only an issue when a seronegative recipient gets an organ from a seronegative donor
 - 1) CMV seropositive donor is most likely source of infection in seronegative recipients; no real need to provide CMV safe products
 - 2) Most transplant centers are ok with leukocyte reduced products for CMV risk reduction
- 4. Modifications to “standard” transfusions
 - a. Leukocyte reduction: Standard (for HLA immunization prevention and CMV-safe)
 - b. Irradiation: Not standard (low risk of TA-GVHD), but many centers do it routinely anyway
 - c. ABO issues
 - 1) Group A and O patients, use ABO-identical RBCs.

- 2) Group AB and B may get A and O RBCs, respectively, to conserve more uncommon types.
- 3) In ABO-mismatched transplants, use type most compatible with donor and recipient for all transfusions
- 4) The AB patient may also be a challenge with FFP transfusion; group A FFP is probably OK during surgery
- d. Rh issues
 - 1) Avoid D immunization before surgery if possible
 - 2) D-negative childbearing age females should get D-negative blood unless supply can't support
 - 3) In males and older females, may either give all D-positive RBCs or use a staggered approach in hepatic and cardiac transplants (start with D-, switch to D+ in the middle, back to D- at the end)
5. Liver and heart transplants
 - a. Can be a HUGE stressor on transfusion services due to often massive transfusion and coagulation issues
 - 1) Recent reports of nearly bloodless hepatic transplantation surgeries have been seen
 - 2) Despite this, may severely strain supplies in many cases
 - b. Main issues are supply, communication and responsiveness.
 - 1) Because of above, large amounts of all products may be needed in a short time.
 - 2) Transplant team must notify Blood Bank ASAP when decision to transplant is made.
 - a) Transfusion services need this notice to increase supply, test recipient for antibodies, find antigen-negative units if necessary
6. Kidney transplants
 - a. In the past, used multiple transfusions, but erythropoietin therapy has decreased that need.
 - b. Historical interest due to the discovery of transfusion-associated immunosuppression in renal transplant patients transfused RBCs or WB before surgery
 - 1) Those transfused had less rejection.
 - c. Currently, main need is for CMV-safe products.
5. Other organs
 - a. Most are similar in management to major surgery of the individual organs involved.

D. Platelet refractoriness

1. Inability to respond to platelet transfusion with a significant qualitative increase in platelet count.
 - a. Big problem in multiply transfused patients
 - b. Nonimmune causes outweigh immune causes.

- 1) Nonimmune: fever, splenomegaly, DIC, bleeding (?), drugs (eg, Amphotericin)
 - 2) Immune: anti-HLA and/or anti-platelet antibodies
- c. Management
- 1) Correct nonimmune causes, if possible.
 - 2) Consider fresher, ABO identical products.
 - 3) Check for immune causes (platelet antibody screen) if nonimmune not apparent.
 - 4) Strategies for platelet transfusion
 - a) HLA matching
 - i) Traditional strategy, using platelets from a donor matching as many HLA class I (HLA-A and HLA-B) antigens as possible
 - ii) "HLA matched" usually means "as close as we could get."
 - iii) Match grades:
 - A: 4 antigen match
 - B1X: 1 antigen is cross-reactive
 - B1U: 1 antigen is unknown
 - B2UX: 1 unknown, 1 cross-reactive
 - C: 3 antigen match
 - D: 2 antigen match
 - iv) Irradiate platelets to prevent TA-GVHD.
 - b) Platelet crossmatching
 - Patient serum vs. platelets in inventory (or samples from potential donors); choose most compatible platelets
 - Uses solid-phase red cell adherence (SPRCA) technology (see BB1 section)
 - Some studies show more effectiveness compared to HLA matching
 - c) Matching for antibody specificities (antibody specificity method)
 - Determine specificity of antibodies, match for platelets lacking those antibodies
 - Analogous to giving someone with anti-K units that are K negative
 - Shown to be equal to HLA matching and platelet crossmatching in response
 - d. Prevention is the best strategy; thus, leukocyte reduction is used for patients likely to receive multiple transfusions (see BB3).

E. Warm autoantibodies

1. Causes:
 - a. Idiopathic (~ 50%)
 - b. Malignancies (CLL, NHL especially)
 - c. Drugs (alpha-methyl dopa)
 - d. Autoimmune disease (SLE)

2. Typically panagglutinins (against all cells in an antibody screen or panel), but may show Rh specificity.
3. Usually impossible to find completely compatible RBCs
4. Treatment: Corticosteroids, other immunosuppressives (including rituximab), treat underlying disease
5. Reflex thoughts:
 - a. Have I ruled out alloantibodies?
 - 1) Rh and Kell antibodies are very common.
 - 2) Autoadsorption +/- elution
 - b. Is clinician aware?
6. Use “least incompatible” red cells for transfusion.
7. Common questions from clinicians:
 - a. “Huh?”
 - 1) Smile, be nice, and explain it again
 - b. “Will my patient hemolyze this incompatible blood?”
 - 1) The best answer is a question: “Is your patient hemolyzing now?” If yes, chances are he’ll hemolyze this blood, too.
 - 2) Transfusion may strengthen autoantibodies.
 - c. “Is this transfusion more risky than regular ones?”
 - 1) Yes. Clinicians have to decide whether the increased risk (by definition, since the blood is not compatible) is justified by the clinical need.
 - 2) Use least amount of blood possible to achieve goal, and monitor patient closely during infusion.

F. Sickle cell disease

1. Common in African-Americans, with hemoglobin S mutation leading to RBC deformity and vasoocclusion
2. Acute or chronic transfusions or exchange transfusions in specific situations
 - a. Stroke (acute or prophylaxis)
 - b. Acute chest syndrome
 - c. Pre-surgery or other major procedure
3. Markedly increased alloantibody formation (reported as high as near 50%)
4. Hyperhemolysis: Hemolysis of both donor and recipient RBCs after transfusion
 - a. Poorly understood; worsened by continued transfusion
 - b. Perfect RBC matching may not prevent
 - c. Immunosuppression (steroids, IVIG) with erythropoietin may allow recovery
4. Specific transfusion needs:
 - a. Phenotypically matched blood
 - 1) Levels of “match”:
 - a) Common to match only Rh and K
 - b) Some add Duffy (Fya) and Kidd (Jkb) as well
 - c) Recent report of “full” matching leading to greatly decreased immunization

- 2) Some prefer to transfuse non-matched blood (or partially matched) and manage antibodies as they come up.
- b. Sickle-negative cells
 - 1) Many with sickle trait unaware of their condition.
 - 2) Some report ok results with sickle trait donors
 - 3) Best to ensure sickle-negative cells for transfusions.

G. Neonatal Transfusions

1. Red blood cells:
 - a. Generally higher RBC transfusion thresholds than in adults; common thresholds follow:
 - 1) No symptoms: 8 g/dL
 - 2) Cardiopulmonary disease, major surgery: 10 g/dL
 - 3) Severe cardiopulmonary disease: 12-13 g/dL
 - b. Choice of anticoagulant-preserved is controversial
 - 1) Most adult RBCs transfusions are with additive solutions
 - 2) Concern in neonates regarding mannitol and excess volume
 - 3) Despite this, most believe AS-RBCs are ok for small volume RBC transfusions
 - 4) For larger volumes, many use CPD or CPDA-1 units, or volume-reduce AS-RBCs before transfusion
2. Platelets
 - a. Also somewhat higher thresholds
 - 1) 100,000 for intracranial bleeds or ill premature neonates
 - 2) 50,000 for other bleeding
 - 3) 20,000 for prophylaxis
 - b. Special concern: ABO compatibility
 - 1) Minor ABO mismatch that works in adult PLT transfusions may be disastrous in neonates
 - 2) Remember incompatible plasma and smaller baby volumes; keep ABO compatible if at all possible
3. Plasma and Cryo
 - a. Similar indications to adults
 - b. FP24 ok for use in neonates
4. Granulocytes
 - a. Used often; may be more efficacious than in adults
 - b. Similar indications to adults, however
5. Modifications to baby blood:
 - a. Leukocyte reduction
 - 1) Pretty much standard of care in US
 - 2) CMV-safe blood required for low birth weight or very ill babies
 - 3) Same issue as adults: CMV-seronegative vs. leukoreduction

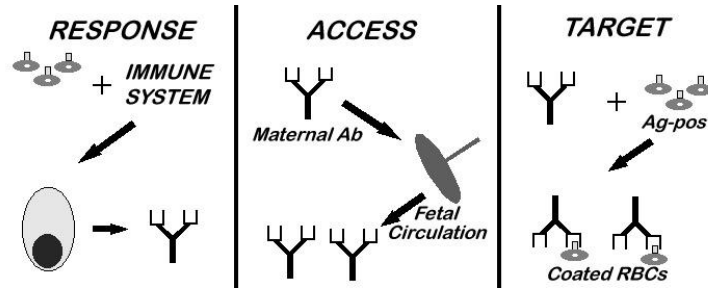
- b. Irradiation
 - 1) Some do routinely for all baby transfusions
 - 2) Probably only necessary in premature or those with immunodeficiencies

H. Therapeutic Apheresis

1. The use of apheresis technology to remove, replace, reduce, or modify a specific pathologic cellular or plasma component
2. Guidelines from American Society for Apheresis (ASFA), published in *Journal of Clinical Apheresis*
 - a. Class I: TA is accepted and proven for primary therapy
 - b. Class II: TA is accepted and useful for second-line therapy
 - c. Class III: TA is not proven but might be helpful
 - d. Class IV: TA is not helpful and may be harmful
3. A multitude of different types and methods available
 - a. Therapeutic plasma exchange (TPE)
 - 1) Removal of pathologic plasma component and replacement with colloid (plasma, albumin) and/or crystalloids
 - 2) Class I examples: TTP, Guillan-Barre, Goodpasture syndrome, myasthenia gravis, cryoglobulinemia, IgG/IgA paraproteinemic polyneuropathy, antibody-mediated renal transplant rejection
 - b. Cytapheresis
 - 1) Removal of pathologic levels of native red cells, platelets, or white cells (blasts)
 - 2) May include replenishment with “good” cells (red cell exchange)
 - 3) Class I examples: Acute stroke in sickle cell patients, severe babesiosis, leukostasis (blast crisis)
 - 4) Severe thrombocytosis is class II indication, as are acute chest syndrome and stroke prophylaxis in SCD
 - c. Extracorporeal photopheresis
 - 1) Modification of patient’s lymphocytes via removal, addition of psoralen, exposure to UV-A light (deactivates nucleated cells), reinfusion
 - i) Immune system responds to T-lymphocytes without general immunosuppression
 - 2) Class I in cardiac allograft rejection, cutaneous T-cell lymphoma
 - 3) Class II for skin GVHD treatment in stem cell transplant patients
 - d. Selective adsorption
 - 1) Removal of pathologic plasma components by various materials
 - 2) Class I: LDL pheresis; class II: Refractory ITP and RA

I. Hemolytic disease of the fetus/newborn

1. Requirements
 - a. The “RAT” model

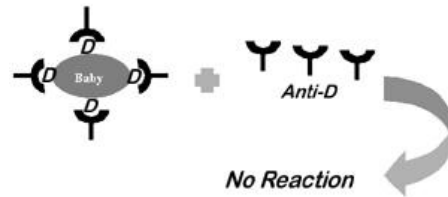


- 1) **R**esponse
 - a) Body responds to foreign antigen by making antibody.
 - 2) **A**ccess
 - a) Antibody formed must be able to cross placenta.
 - b) IgG1, IgG3, IgG4 cross, IgG2 and IgM do not.
 - 3) **T**arget
 - a) Target antigen must be present on fetal RBCs.
2. Manifestations
 - a. Severity depends on antibody involved.
 - 1) Most severe: anti-D
 - b. Lab findings
 - 1) Anemia
 - 2) Indirect hyperbilirubinemia/jaundice
 - 3) Positive DAT (unless all cells destroyed)
 - c. “Erythroblastosis fetalis”
 - 1) Abundant nucleated RBCs in circulation in response to accelerated destruction
 - d. Hydrops fetalis
 - 1) Most severe form of HDN, often fatal in utero
 - 2) EMH in liver may be so severe as to impair albumin synthesis, leading to hydrops.
 3. ABO HDN
 - a. **Most common** type of HDN
 - b. Generally mild or undiagnosed
 - c. First (or any) pregnancy
 - d. Group O moms, group A or B babies; See BB I
 - e. Lab testing
 - 1) DAT +/-
 - a) ABO antibodies are great at fixing complement, so coated cells might be destroyed, leaving nothing to be detected by the DAT!
 - 2) Antibody screen negative (all screen cells used are group O).
 - 3) Eluate from fetal cells reactive with A and/or B cells
 - f. Treatment

- 1) No intervention is usually necessary, but if severe, treat as outlined under Rh section below.

4. Rh HDN

- a. Prototypical form of HDN
- b. Not first pregnancy (unless mom was transfused)
- c. D- moms, D+ babies
- d. Lab testing
 - 1) Infant D+ (unless “blocked D”)
 - a) “Blocked D”= false negative D antigen test due to excessive coating of D+ cells with anti-D



- 2) DAT strongly positive
 - 3) Antibody screen: anti-D
 - 4) Eluate: anti-D
 - 5) Indirect bilirubin elevated
 - 6) Increased change in amniotic fluid optical density at 450 nm (Liley graph)
- e. Treatment
- 1) Keep bilirubin < 20
 - a) Lower threshold in premature babies
 - b) Indirect bilirubin can cross the blood/brain barrier and cause kernicterus (basal ganglia bilirubin deposition).
 - 2) Phototherapy
 - 3) Exchange transfusion with D- blood as necessary
- f. Prevention
- 1) **RhIG**: commercially prepared anti-D
 - a) “RhoGam”, “BayRho”: IM forms
 - b) “WinRho”: IV or IM form
 - 2) Prevents D antigen recognition in vulnerable mom
 - 3) Obstetric indications
 - a) D- female at about 28 weeks gestation
 - b) D- female within 72 hours of D+ infant’s birth
 - c) D- female with pregnancy complications or invasive procedures (amnio, PUBS)
 - 4) Contraindications
 - a) D- pregnant female who has already made her own anti-D
 - Be sure to think about prior RhIG, especially on exams.
 - RhIG has a 25-day half-life, but may be detectable for as long as six months.

- Usually distinguishable from maternal anti-D by weak reactions and low titer (almost always ≤ 4); don't bet the house on it, though!
 - b) D+ females
 - c) D- mom, D- baby (no postpartum dose)
- 5) Dosage
- a) **One full dose vial (300 μg or equivalent) per 30 ml of D+ whole blood (15 ml D+ RBCs)**
 - “Mini-dose” (50 μg) used after 1st trimester abortion for ≤ 5 ml whole blood
 - b) Fetal blood screen (Rosette test)
 - Fetal D+ RBCs coated with anti-D then surrounded by D+ indicator RBCs
 - Qualitative (Yes/No)
 - c) **Kleihauer-Betke**
 - Quantitative
 - Acid-resistant Hgb F RBCs stain brightly
 - Flow cytometry being used in some centers samples many more cells and has potential to be far more accurate.
 - d) Using KB results
 - i) $\text{KB}\% \times \text{blood volume} = \text{volume of baby blood}$
 - Use 5000 ml if no maternal weight given.
 - If weight given, multiply weight $\times 70$ ml/Kg.
 - ii) $\text{Baby blood volume}/30 = \text{vials of RhIg needed}$: remember, one vial per 30 ml D+ blood.
 - iii) Rounding rules
 - If number after decimal is < 5 , round up.
 - If ≥ 5 , round up twice
 - So, “3.4” would mean give 4 vials, while “3.5” would mean to give 5 vials.
 - iv) Example: D- mom with D+ baby. $\text{KB}=2\%$
 - $0.02 \times 5000 = 100$ ml D+ blood
 - $100/30 = 3.33$
 - Round up once; give 4 vials
 - e) An easier way!
 - i) $\text{KB}\% \times 5/3 = \text{number of vials}$
 - ii) In above example, $(2 \times 5)/3 = 3.33$
- g. Compatibility testing
- 1) Mom's serum may be used to crossmatch.
 - a) Other choice: eluate from neonatal RBCs.
5. K HDN
- a. Relatively common; particularly severe
 - b. Anti-K attacks early RBC precursors and causes severe fetal anemia

II. Blood Bank Calculations

A. Chance of finding compatible units

1. Why bother?
 - a. In real life: to allow Blood Bank techs to estimate the difficulty in finding blood for patients with multiple alloantibodies.
 - b. On exams: to torment you and make you feel generally worthless and ignorant (don't let them succeed!).
2. Calculation
 - a. Find percentage of donors compatible:
 - 1) Likelihood of negativity = $1 - \text{Ag frequency}$
 - 2) Take likelihood of negativity for each antigen and multiply
 - 3) Example:
 - a) Patient with anti-Jk^a and anti-K (assume primarily Caucasian population)
 - b) Likelihood of Jk^a negativity = $1 - 0.77 = 0.23$
 - c) Likelihood of K negativity = $1 - 0.09 = 0.91$
 - d) Likelihood of Jk^a and K negativity = $0.23 \times 0.91 = 0.2093$, or 21% chance of this combination given this donor pool
 - b. Estimate number of units you would have to test in order to find x number of compatible units:
 - 1) Take number of units you need and divide by the likelihood of finding antigen-negative blood.
 - 2) Example:
 - a) From above, looking for Jk^a and K negative units, chance is 0.21.
 - b) For two units, divide 2 by 0.21 = 9.5 units that you would probably have to test to find two that were compatible (round to 10).
3. Pitfall
 - a. Can't use for paired alleles (like Jk^a and Jk^b)
4. Chart for antigen frequencies (for reference; compiled from *AABB Technical Manual*); see below

ANTIGEN	CAUCASIANS	AFRICAN AMERICANS
D	85	92
C	68	27
c	80	96
E	29	22
e	98	98
K	9	2
k	99.8	99.9
Jk^a	77	91
Jk^b	72	43
Fy^a	66	10
Fy^b	83	23

M	78	70
N	72	74
S	55	31
s	89	97
Le^a	22	23
Le^b	72	55

B. Corrected count increment (CCI)

1. Why bother?
 - a. Honestly, many don't bother, but the idea is to objectively determine the response to platelet transfusion.
2. Calculation (most common formula)

$$\frac{\text{BSA} (\text{Platelet count}_{\text{post}} - \text{Platelet count}_{\text{pre}}) \times 10^{11}}{\text{Number of platelets transfused}}$$

BSA = body surface area in m²

3. Interpretation
 - a. 7500 or above means adequate response
 - b. Two consecutive inadequate CCIs define refractoriness.
4. Pitfalls
 - a. Precount should be as near as possible to the time of transfusion.
 - b. Post count is standardized for this equation at 1 hour post transfusion.
 - c. Use the 10¹¹ number behind the number of platelets transfused (this differs from previous versions)

C. Post-transfusion platelet recovery (PPR)

1. Why bother?
 - a. Another way (like CCI) to objectively determine the patient's response to platelet transfusion
2. Calculation

$$\frac{\text{Total blood volume} \times (\text{Platelet count}_{\text{post}} - \text{Platelet count}_{\text{pre}}) \times 10^3}{\text{Number of platelets transfused}}$$

3. Interpretation
 - a. 20% or above considered adequate
4. Pitfalls
 - a. Blood volume calculation:
 - 1) BV = bodyweight in Kg x 70 ml/Kg
 - b. Use the 10¹¹ on the bottom, as above.

D. Cryo dosage for hypofibrinogenemia

1. Why bother?
 - a. Again, often not done in real life (guesstimate)
 - b. Calculates bags to raise level desired amount
2. Critical data needed
 - a. Bodyweight in Kg, hematocrit and recent fibrinogen level

3. Calculation

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| <ol style="list-style-type: none">1. <u>Calculate Blood Volume</u>
Body weight x 70 ml/Kg2. <u>Calculate Plasma Volume (PV)</u>
Blood volume x (1 - hematocrit)3. <u>Calculate mg fibrinogen needed</u>
Plasma volume x concentration change desired
*Subtract desired level from current
(ie, 150 mg/dl – 50 mg/dl)
*Multiply level change by PV
(ie, 100 mg/dl x 3600 ml)
*Divide the answer by 100 to correct for
difference in units (dl to ml)4. <u>Calculate bags of cryo needed</u>
Fibrinogen needed / 250 mg per bag |
|--|

4. Pitfalls

- a. There must be at least 150 mg of fibrinogen in a bag of cryo, so it's tempting to use that number.
 - 1) 250 mg is used instead because most bags have closer to 250 than 150.
 - 2) Personally, I think this is inconsistent (see Factor VIII calculations below), but formula is standard
- b. It's easy to forget to divide by 100 in step 3 in the calculation above.
 - 1) This error would make you need about 686 bags of cryo to raise the fibrinogen of a 70 Kg person (HCT=30%) from 50 to 100 mg/dl. That would be just a teeny-weenie overdose!

E. Factor VIII calculations

1. Why bother?
 - a. Valuable and used often
 - b. Enables an accurate order to whoever stocks factor VIII in your institution
 - c. On tests, allows evil question writers to force you to commit malpractice and give cryo to a hemophiliac
2. Critical data
 - a. Bodyweight in Kg, hematocrit, factor VIII level, an idea of where you want to go
 - 1) General rules
 - a) For hemarthrosis, shoot for at least a 50% factor VIII level.
 - b) For major surgery or hemorrhage, go for 100% levels

3. Calculation

1. **Calculate Blood Volume**
Weight x 70 ml/Kg
2. **Calculate Plasma Volume**
Blood Volume x (1 - Hematocrit)
3. **Calculate F VIII units needed**
Plasma volume x % increase desired
(i.e., if you want to go from 4% to 50%,
multiply PV x 0.46)
STOP HERE IN REAL LIFE!!!
4. **Calculate bags of cryo needed**
F VIII units needed / 80 units per bag

4. Pitfalls

- a. On exams, the target level is often not specified, so you have to know the situations you would go for 50% or 100%, as outlined above.

F. Factor IX calculations

1. Easy! Same calculation as for factor VIII, just double the amount at the end.
 - a. Much of transfused factor IX is redistributed and lost very quickly after infusion.
2. Hey! You can't use Cryo for factor IX deficiency!

G. RhIG calculations

1. Discussed previously in HDN section

III. Antibody Identification

A. The process

1. Goal: determine the target antigen of the antibody found on the antibody detection screen.
2. Before you start:
 - a. Get history (pregnancy, transfusions, medications, race)
 - b. Understand your technology (tube vs. non-tube, enhancement vs. non-enhancement, etc)
3. Steps recommended:
 - a. **Check autocontrol**
 - 1) Positive autocontrols may change the entire meaning of the panel.
 - a) Autoantibodies, alloantibodies to recently transfused cells, drug-related antibodies
 - 2) Special techniques may be needed to understand the panel (Autoadsorption, elution, etc.
 - b. **Look at general pattern for guidance**
 - 1) Uniform reaction strength almost always means a single antibody
 - 2) Variable reactions at immediate spin (which is not required) vs. IAT: Consider multiple antibodies
 - 3) Take a glance at common antigens as target

- 4) Caution: This is just a preview, and it is still a good idea to complete the rest of the steps
- c. **Rule out antibodies against antigens present in nonreactive cells** (see below).

Fy ^a	Fy ^b	IS	37C	IAT
+	0	0	0	0
+	+	0	0	0
0	+	0	1+	2+

- 1) Exclude antibodies when the antigen is present but no reaction is seen
- 2) Start with cells that have totally negative reactions.
 - a) First two rows in above figure have no reaction.
 - b) On line 1, since Fy^a antigen is present, negative reaction suggests that it is not antibody target.
 - c) Indicate this by making a diagonal slash (cross-out) through the “Fy^a” at the top of the column.
- 3) Only cross out “homozygous” cells (except K)
 - a) Negatives may be nonpredictive when the antigen is “heterozygous” (dosage)
 - b) So, on line 2, do not cross off either Fy^a or Fy^b.
- 3) Horizontal vs vertical cross-outs
 - a) Common method: Do cross-outs all across a row, move down to the next negative row, do cross-outs all the way across that row, etc.
 - b) Alternative: Do cross-outs down a column, blood group by blood group (often goes faster).
- d. **Use reactive cells to try to fit a single antibody.**
 - 1) If available, evaluate reactions as “warm” or “cold.”
 - a) If all cold, look at Le^a, Le^b, M, N and P.
 - 2) If a mix of warm and cold, skip to step e.
- e. **Try to fit two or more antibodies in the same phase.**
 - 1) “Phase” only applicable in liquid tests
 - 2) If one antibody doesn’t explain all reactions, try two, then three, etc.
- f. **Try to fit one warm and one cold antibody.**
 - 1) Only applicable if immediate spin results present
 - 2) If necessary, do cross-outs based on only warm, then only cold reactions.
- g. **Consider special techniques**
 - 1) **Patient antigen typing**
 - a) Alloantibodies are vs. non-self antigens.
 - b) Confirms suspected specificity
 - c) Shows *possible* in complicated workups
 - 2) **Enzymes**
 - a) Weaken or strengthen certain antigens
 - b) Use to confirm preliminary conclusions

3) **Adsorption**

- a) Specific RBCs to remove antibody from serum.
- b) Patient's own RBCs (autoadsorption) or RBCs of known phenotype (alloadsorption).
- c) Remaining serum ("absorbed") may be tested to determine antibody specificity.

4) **Elution**

- a) Often used together with adsorption.
- b) Antibody removed ("eluted") from RBC surface by heat, cold, acid, solvent, or other treatment.
- c) Antibody ("eluate") may then be identified.

g. **Final rule ins/rule outs**

- 1) Two positives when antigen is present and two negatives when antigen is absent required by AABB
- 2) Labs may choose to require more or less

h. **Run screaming from the room**

- 1) We try to avoid getting to this stage!

B. Practical tips

- 1. Time to burn?
 - a. Expect somewhere between 1 and 5 antibody panels on standardized exams (occasionally, you'll get lucky and get none).
 - b. Despite how long you spend on each one, each counts for exactly one point!
 - c. Moral: Don't beat yourself up over a hard panel! Guess and Go!
- 2. Anti-D
 - a. Every time an anti-D shows up, your first question in the exam world should be: "Has the patient had RhIG?"
 - b. RhIG can hang around and be detected for as long as six months (half-life is about 25 days, though).
- 3. Autoantibodies (see next section)
- 4. Look for instant recognition (see next section)

C. Liquid panels patterns to instantly recognize

	A	B	C	D	E										
	IS	37	IAT	IS	37	IAT	IS	37	IAT	IS	37	IAT	IS	37	IAT
1	0	0	2+	1	2+	0	0	0	1	0	0	1+	1	1+	0
2	0	0	2+	2	2+	0	0	0	2	0	0	1+	2	1+	0
3	0	0	2+	3	2+	0	0	0	3	0	0	1+	3	1+	0
4	0	0	2+	4	2+	0	0	0	4	0	0	1+	4	1+	0
5	0	0	2+	5	2+	0	0	2+	5	0	0	1+	5	1+	0
6	0	0	2+	6	2+	0	0	0	6	0	0	1+	6	1+	0
7	0	0	2+	7	2+	0	0	0	7	0	0	1+	7	1+	0
8	0	0	2+	8	2+	0	0	0	8	0	0	1+	8	1+	0
9	0	0	2+	9	2+	0	0	2+	9	0	0	1+	9	1+	0
10	0	0	2+	10	2+	0	0	0	10	0	0	1+	10	1+	0
P _C	0	0	2+	P _C	2+	0	0	0	P _C	0	0	0	P _C	1+	0

(mf)

1. **Warm autoantibodies (panel A)**
 - a. Across-the-board positivity (at IAT +/- 37 C) with positive autocontrol (panel "A" in chart above)
 - b. Positive DAT
 - c. Antibody specificity: very broad, likely basic Rh component.
 - d. Strategy
 - 1) Rule out underlying ("masked") *allo*antibodies.
 - 2) Use autologous adsorption.
 - 3) Once alloAb ruled out, give least incompatible blood unless specificity is clear (usually not).
2. **Cold autoantibodies (panel B)**
 - a. Across-the-board positivity (at IS +/- 37 C) with positive autocontrol (panel "B" above)
 - b. Positive DAT (usually for complement components only)
 - c. Antibody specificity: usually I, sometimes i
 - d. Strategy
 - 1) Consider prewarmed crossmatches.
 - 2) Consider transfusion through a blood warmer.
3. **Antibodies vs recently transfused antigens (panel C)**
 - a. One or more antibodies in a panel with positive autocontrol and history of recent transfusion (panel "C" above)
 - 1) Classic autocontrol description: "mixed field"
 - a) Definition: two groups of RBCs, with/without agglutination.
 - 2) Of most clinical importance when antibody screen was negative *before* transfusion
 - b. Positive DAT (also "mixed field")
 - c. Famous with Kidd and Duffy antibodies
 - d. Strategy
 - 1) Ensure that the patient is stable clinically (rule out delayed hemolysis); support as necessary.
 - 2) Phenotype transfused unit, if possible.
 - 3) Give antigen negative blood in future.
4. **High-titer, low-avidity antibodies (HTLA) (panel D)**
 - a. Classically 1+ positivity at AHG only, with negative autocontrol (panel "D" above)
 - 1) Occasional HTLA's can give positive autocontrol and DATs; uncommon
 - b. Still positive after many dilutions ("high titer") but weakly reacting ("low avidity")
 - c. Chido, Rodgers most common antigens
 - 1) Complement components
 - 2) Neutralize with serum
 - d. Clinically insignificant (No HDN, no HTRs)

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- e. NOTE: The pattern of this panel could also be seen in other high-frequency antibodies that may be significant!
- 5. **Reagent-related antibodies (panel E)**
 - a. Antibodies against reagents used in testing (eg, preservatives in LISS)
 - b. Across-the-board positivity at IS/37 C, negative at IAT, positive autocontrol (panel “E” above)
 - c. DAT **negative** (due to washing step)
 - d. Run reactions without offending reagent.

Sample antibody ID problems begin on the next page.

Answers to Panels

1. **Anti-Le^b**

Single cold-reacting antibody. Anti-Le^b reacting at these temperatures is not clinically significant, so no specific interventions will be necessary. You might consider using prewarmed crossmatches to eliminate the antibody activity.

2. **Anti-D**

Note that this is likely a gel or solid-phase panel (though it could be a liquid panel only recording the IAT reactions). Single warm-reacting antibody. Fairly straightforward identification. Check the clinical situation (and don't forget to ask about recent RhIG injection or infusion!).

3. **Anti-K and anti-E**

After your cross-offs, no single antibody explains all of the reactions, so you should try to fit two antibodies (again, this panel only shows IAT results, so no concern about different "phases" here). Anti-K and anti-E is the best fit. Note the slightly weaker reactions in cell 6 due to dosage.

4. **Anti-Fy^b and anti-Le^a**

Just looking at this panel should make it very clear to you that no single antibody will explain all of these results. No antibody that I know of will give you this wide disparity in reaction strengths and temperatures. Postulate a single warm and a single cold antibody, and you come up with a perfect fit with the above. However, if you also said that you cannot rule out anti-C, you are correct. In the real world, you would have more work to do, but for our purposes, you are done.

5. **Anti-Jk^a**

Did you notice the positive autocontrol? A positive autocontrol with a mixed field pattern suggests an antibody against recently transfused antigens. This is classic for Kidd antibodies. You should check the patient first to ensure that he's not in the midst of a delayed hemolytic reaction, and then check the prior hospital to see if they picked up the anti-Jk^a in their testing (it's entirely possible that it was not detectable).

Additional Resources

1. Books
 - a. *AABB Technical Manual* (17th ed. is new and current).
 - b. *AABB Standards* (27th ed. is current).
 - c. Transfusion Medicine and Hemostasis, Chris Hillyer, Beth Shaz, James Zimring, and Thomas Abshire, ed.,; 2009, Elsevier (WOW, what a great book! Everything you need in an easy-reading beauty of a spiral bound book! Under 40 bucks on Amazon. Go get it, right now!)
 - d. Transfusion Medicine, 2nd ed., Jeffrey McCullough, 2005, McGraw-Hill (my former favorite; truly excellent while still being manageable. A little dated, but still really good)
 - e. Practical Transfusion Medicine, 2nd ed., Murphy and Pamphilon, 2005, Blackwell (fairly concise and practical).
 - f. Transfusion Medicine: Self-Assessment and Review, Helekar, et al, AABB Press (Review questions based topically on Tech Manual).
2. Web
 - a. For Blood Banking, there are a few sites that are quite good. Check www.bbguy.org/links.htm for a listing of some of them.