Pretransfusion Testing (Basic Immunohematology Part 2)

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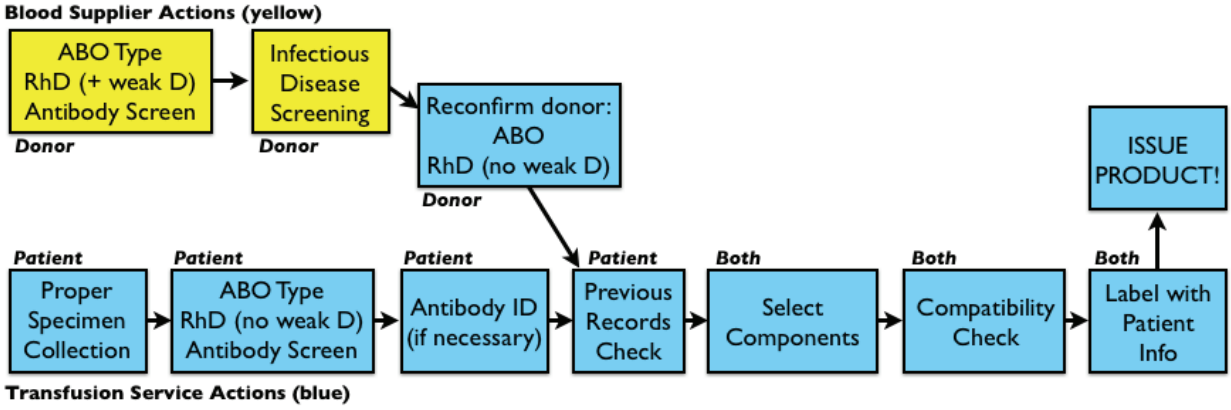
I. Outline:
- A. Overview of pretransfusion testing
- B. Testing methods
- C. Required components of the process
- D. Nomenclature of tests used

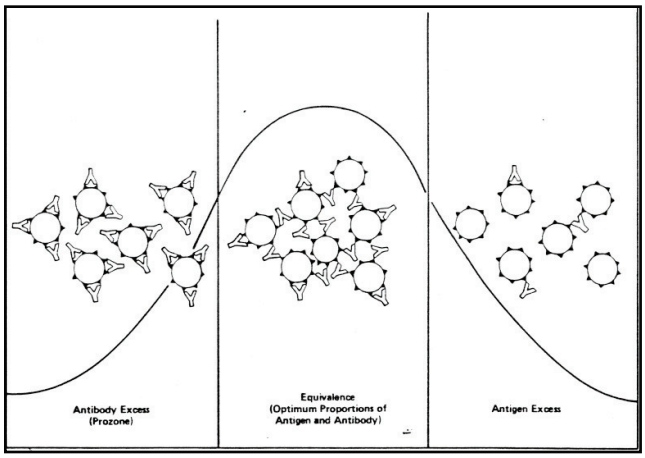
II. Overview
- A. Purpose
  1. Provide a blood component to a patient that will provide maximum benefit while minimizing potential for harm
     - a) Choose the most appropriate blood product from a safe, compatible donor and infuse it into a properly identified and thoroughly tested recipient
  2. This requires a systematic process that is fairly rigid
  3. Regulated in the US by:
     - a) CLIA (Clinical Laboratory Improvement Amendments, 1988)
       - (1) Determines the minimum standards for lab operations; inspections regulated by Centers for Medicare and Medicaid Services (CMS)
       - (2) Does not often inspect blood banks due to AABB and CAP deemed status
       - (3) Personnel, training, quality control, and proficiency testing requirements
       - (4) Regulates ABO and D typing, antibody detection, and crossmatching
     - b) AABB (formerly "American Association of Blood Banks")
       - (1) *Standards for Blood Banks and Transfusion Services* (currently 27th ed)
       - (2) Sets US standard of care for pretransfusion testing
       - (3) CLIA deemed status (AABB inspection may be used to satisfy CLIA inspection requirements in eyes of CMS)
     - c) College of American Pathologists (CAP)
       - (1) Biennial (every 2 yrs) inspection for compliance to laboratory guidelines
       - (2) Major provider of proficiency testing samples used in nearly all blood banks
       - (3) CLIA deemed status, like AABB
     - d) FDA
       - (1) More involved in component collection and manufacturing
       - (2) Specifies which antigens must be present in antibody detection tests
- B. Map of the process:



- C. Note that this discussion will be mostly limited to transfusion service actions

III. Testing principles (some covered in "Blood Groups" podcast and handout; December 2011)
  A. Agglutination
    1. Basic reaction in blood banking
    2. Two stages:
      a) Sensitization/coating
        (1) Binding of antibody to surface of RBCs
        (2) Dependent on multiple factors (see below)
      b) Bridge formation
        (1) Linkage of adjacent RBCs that are coated with antibody
        (2) IgM is far more capable of forming bridges between adjacent cells due to its pentameric structure (total of 10 possible bridging sites)
        (3) IgG has a harder time, since it only has two binding regions
          (a) Antigens that extend from the surface of the RBC, such as M, N and ABO, make it easier for IgG to directly bind and form bridges
          (b) Rh and other antigens that live closer to the surface of the cell are not usually directly agglutinated by IgG antibodies (see later)
    3. Sensitization is simply a chemical reaction
      a) Antigen + Antibody $\longleftrightarrow$ Antigen-Antibody
      b) K_0 = equilibrium constant of reaction
        (1) Larger K_0 means a push to the right side of the equation, with more stable and rapid reactions
      c) Affinity of RBC antigens and antibodies affected by multiple factors
        (1) Cold-reactive (usually IgM) vs. warm-reactive (usually IgG)
          (a) Must react in appropriate temperature for best antibody detection
            i) "Cold" antibodies are usually vs. carbohydrate antigens (ABO, Lewis, I/i, P, M, N)
            ii) "Warm" usually vs. protein antigens (Rh, Kell, Kidd, Duffy, etc.)
          (b) Warm antibodies most important (except ABO)
        (2) Size
          (a) An RBC is over 700 times bigger than an antibody!!!
            i) If an RBC were 100 yards long (length of a football field), an IgG molecule would be about 5 inches long (smaller than a football)
          (b) Overcoming this size difference in vitro requires manipulation of environment as well as forcing RBCs closer together (centrifugation)
        (3) Electrical repellence
          (a) RBC surfaces have a negative charge due to sialic acid at their surfaces
          (b) RBCs are naturally repelled by the negative charges ("zeta potential") and by the positively charged ionic cloud that forms around the cells
          (c) Reduce zeta potential with:
            i) Low ionic strength solutions (LISS) or albumin; fewer ions to surround RBCs
            ii) Water-exclusion (Polyethlyene glycol, "PEG")
          (d) Zeta potential is one reason that IgG molecules are challenged to directly agglutinate RBCs (limited reach of monomeric antibody)
            i) RBCs usually don't get closer than about 14 nm apart

(4) pH
(a) Optimal pH (7.0 in vitro) encourages an environment where:
i) RBC surfaces are negatively charged
ii) Antibodies are weakly positive
(b) Decreasing pH leads to dissociation of antibody from RBC surface
(5) Relative amounts of antibody and antigen
(a) Typical: 2 drops serum, 1 drop RBCs
(b) This gives mild antibody excess, to promote shift of equation to right
i) Too much antibody excess can give "prozone" effect (inhibiting agglutination), while too much antigen excess gives "postzone" effect (also inhibiting agglutination)



Source: http://faculty.matcmadison.edu/mljensen/BloodBank/lectures/Images/Zone_of_Equivalence.gif

4. Direct vs. indirect agglutination
a) Direct agglutination
(1) Antibody binds to multiple RBCs and causes agglutination without additional manipulation
(2) IgM is much better able to do this than IgG
(a) IgM maximum diameter is 30 nm (vs 14 nm for IgG), which is wide enough to more easily overcome RBC zeta potential
(b) Occasional IgG antibodies can do this, if antigens "stick up" fairly far from RBC surface (ABO-related & M and N antigens most commonly)
b) Indirect agglutination
(1) Antibody binds to, but does not form bridges with, RBCs
(a) Requires additional step to see agglutination (AHG phase described above; enzyme treatment of RBCs may also make an IgG capable of direct agglutination)
(2) Classically IgG rather than IgM, for reasons mentioned above
(3) Most significant antibodies cause this type of agglutination

B. Tube testing
1. Three main "phases"
a) Immediate spin (IS)
(1) 2 drops serum + 1 drop of 2-5% RBC solution, centrifuge 15-30 sec
(2) Detects IgM antibodies best
(3) Antibodies seen at this phase only are usually not clinically significant

b) 37 C

(1) Add potentiator, incubate at 37 C (see table), centrifuge

Potentiator	Incubation Time
None (saline)	30-60 min
LISS	10-15 min
PEG	15 min
Bovine albumin	15-30 min

(2) Occasional cold-reacting IgM antibodies and occasional warm-reacting IgG antibodies react at 37 C; in short, it's not that useful by itself

(3) Some do not perform a 37 C read, as it doesn't add much in most cases

(a) NOTE: Those using PEG potentiation should NOT do a 37 C read, as PEG can induce nonspecific positive reactions that are meaningless

c) Antihuman globulin (AHG)/Indirect antiglobulin test (IAT)

(1) AHG is antibody vs. human antibodies and/or complement

(2) Wash same tube as used for 37 C, add AHG, centrifuge

(a) Washing removes unbound globulins that "neutralize" (are bound by) AHG and cause false negative reactions

(3) Only required part of antibody detection, because it is best for detecting warm-reacting IgG antibodies

(4) Polyspecific or IgG-specific AHG may be used (lab preference)

d) Grading reactions

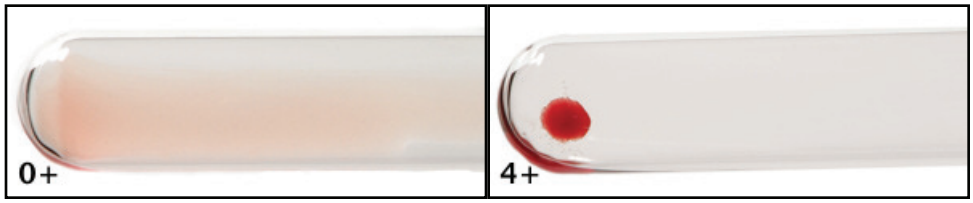
(1) Read after gentle resuspension of button (micro exam not necessary)

(2) Generally on a 0-4+ scale (some major labs use 0-12 scale instead)

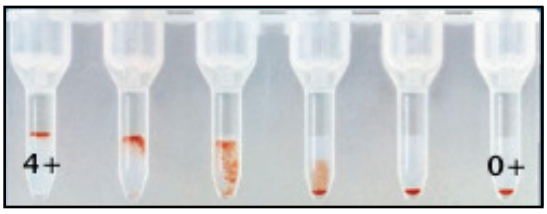
(3) Negative: Smooth, easily dispersed RBCs

(4) Strong positive (4+ or 12): Tight cell button, not easily dispersed

(5) Gradually increasing agglutinates from 1+ to 3+



C. Column agglutination ("gel") testing (see image below)

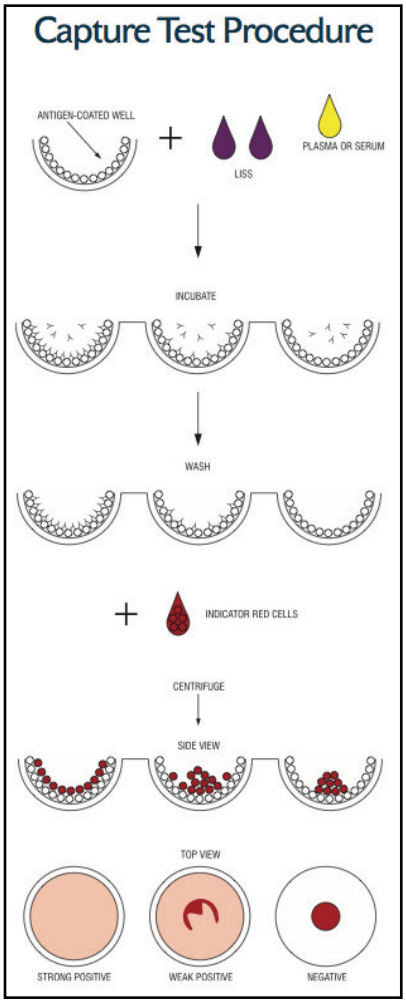


1. Essentially "skips" the IS phase, incorporates the 37 C incubation into the process, and gives an AHG result
2. Performed in microtubes with a chamber at the top for mixing plasma and RBCs and a column filled with gel and anti-IgG (see image at right)

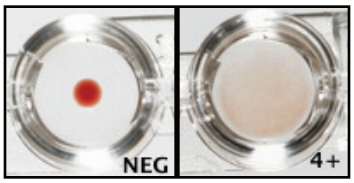
a) Add plasma and RBCs to chamber at top, incubate at 37 C, centrifuge

3. Gel separates agglutinates based on size and by binding to IgG-coated RBCs
   a) Strong positive gives agglutinates at the TOP of the gel (left side of above)
   b) Complete negative gives RBCs at the BOTTOM of the gel (right side)
4. Like PEG-enhanced tube testing, excellent for detecting warm antibodies (and has similar sensitivity)

D. Solid phase testing (see diagram at right; courtesy of Immucor)



1. RBC antigens are bound to microplate wells (either on intact or lysed RBC membranes), then plasma added, with incubation at 37 C
   a) If IgG against an RBC antigen(s) is present, antibody binds to antigen all over the bottom of the well
2. Wash away unbound antibodies, then add RBCs coated with anti-IgG, which bind to the previously attached IgG on the bottom of the well
3. Positive and negative are opposite of what you might think
   a) Negative: Solid "button" in the bottom of the well (indicating that there were no attached plasma antibodies with which the anti-IgG-coated indicators cells could bind)
   b) Strong positive (4+): Diffuse "carpet" of indicator RBCs spread all across the bottom of the well (indicating that the plasma antibody is attached to the well-bound RBC antigens)



4. Sensitivity basically equivalent to gel and PEG methods

E. Indirect antiglobulin test (IAT)
1. A test to detect *in-vitro* coating of RBCs with antibody and/or complement
2. Serves as the third phase of tube testing, and is the main part of the antibody screen
3. Procedure:

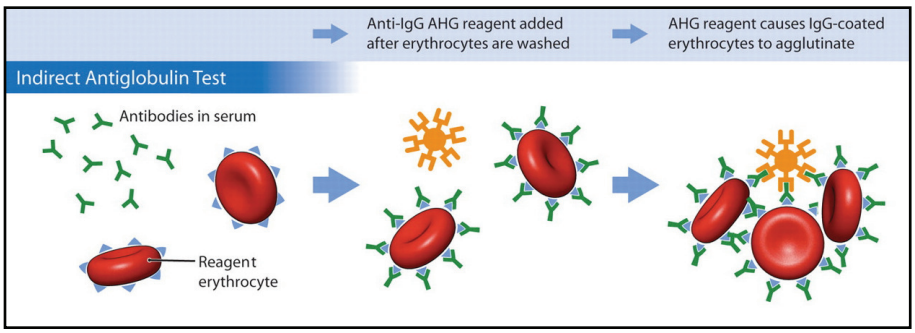


Image credit: Can Med Assoc Journal Jan 2006

a) In pretransfusion testing, patient serum added to solution of donor RBCs, to check for incompatibility between recipient antibodies and donor RBCs
b) Can be done with known serum and antibody and unknown RBCs, or with RBCs of a certain phenotype to check for serum antibody

4. Types of anti-human globulin (AHG)
   a) "Polyspecific" (polyclonal anti-IgG + monoclonal anti-C3d)
      (1) Previously the most commonly used AHG, but less popular now
      (2) If positive, labs would then do tests with anti-IgG and anti-C3 individually
   b) Anti-IgG
      (1) Used for gel and solid-phase platforms exclusively
      (2) Many labs use anti-IgG only in tube tests, as well
      (3) Can get some cross-reactivity with other immunoglobulin types due to reaction with kappa and lambda light chains shared by immunoglobulins
   c) Anti-C3d
      (1) C3d is a nonreactive byproduct of complement fixation on RBCs
      (2) Anti-C3d is useful for evaluating IgM-related hemolysis and cold agglutinin disease, where antibodies are not usually detectable via anti-IgG
5. AHG control ("check cells")
   a) For all <u>negative</u> tube IAT or DATs, add reagent RBCs coated with antibody and/or complement; should see free AHG agglutinating the check cells
      (1) No agglutination means test or reagent problem
   b) Gel-negative IAT/DAT tests do NOT require an additional AHG control
   c) Solid-phase tests run a positive control in parallel, so no additional AHG control required

F. Direct antiglobulin test (DAT)
1. Detects whether antibody or complement coating of RBCs has occurred <u>*in vivo*</u>
2. Procedure:

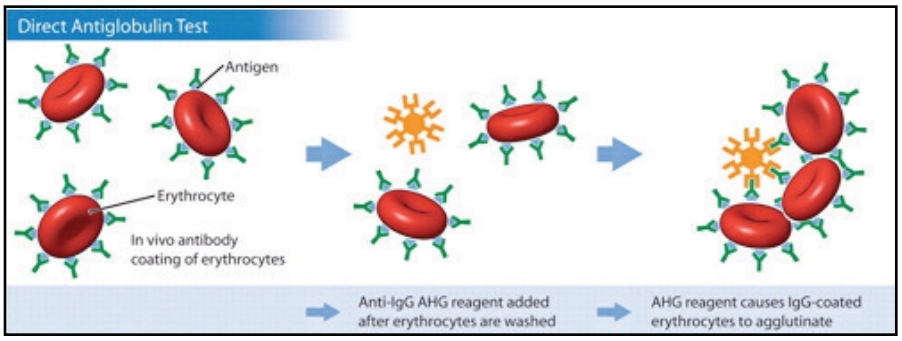


Image credit: Can Med Assoc Journal Jan 2006

   a) Essentially just the last part of performing an IAT.
3. DATs are useful in workup of:
   a) Transfusion reactions
   b) Autoantibodies and autoimmune hemolytic anemia
   c) Hemolytic disease of the fetus/newborn
   d) Drug-related hemolytic anemia
   e) Antibodies vs. recently transfused antigens
4. Positive DATs, however, are nonspecific, and are seen in up to 15% of hospitalized patients

G. A few other tools in the pretransfusion testing portfolio:
   1. Proteolytic enzymes
      a) Cleave proteins on RBC surface, may make underlying antigens more available
      b) Destroys some antigens (e.g., Duffy and MNS), enhances others (ABO, Rh, Jk)
      c) Not used routinely, but often used in complex or difficult cases
   2. Prewarming
      a) Performing pretransfusion testing with all reagents and samples incubated and kept at 37 C can help eliminate effects of cold auto- or alloantibodies
      b) NOT to be used as a way to get rid of reactivity of stuff you don't understand!
         (1) The procedure may weaken some significant antibodies
         (2) Should be used only as confirmation of the workup you have already done
   3. Adsorption
      a) Removal of specific antibodies from sample via incubation with antigen-positive RBCs
      b) Used to remove warm or cold <u>auto</u>antibodies ("autoadsorption") from sample in order to detect underlying <u>allo</u>antibodies
      c) May also be used to remove one or more <u>allo</u>antibodies ("alloadsorption") from sample in order to detect or confirm the presence of other alloantibodies
         (1) May be used with multiple antibodies to help clear a muddy picture
         (2) e.g., Sample has anti-K, anti-C, and anti-S but anti-S isn't visualized well. Use K+C+S– RBCs to adsorb the anti-K and anti-C and leave the anti-S in the "adsorbed serum" for clearer results.
   4. Elution
      a) Technique for removal of antibodies bound to RBC surface for analysis
      b) May be done with heat, cold, chemical (e.g., glycine) treatment
   5. Other RBC treatments
      a) Dithiothreitol (DTT) or 2-mercaptoethanol (2-ME)
         (1) Denatures surface RBC antigens of multiple groups (including Kell, Lutheran, Dombrock, Yt, LW)
         (2) Can also be used to remove IgM antibody activity from serum
      b) ZZAP
         (1) Combination of DTT and proteolytic enzyme
         (2) Acts on combination of enzyme sensitive and DTT-sensitive antigens
      c) Chloroquine
         (1) Removes IgG from coated (DAT-positive) RBCs to allow for accurate phenotyping (effective at least 80% of the time)
         (2) Also removes residual HLA antigens from RBCs (Bg antigens)

IV. Required components of pretransfusion testing:

Donor Evaluation	Patient Evaluation	Donor/Patient Evaluation
Test donor sample	Obtain patient sample	Select components
	Test patient sample	Check for compatibility
	Check previous records	Label/final records check

V. Donor Evaluation

A. Test donor sample

1. Blood supplier responsibilities:

a) ABO grouping

(1) Red cell (forward) and serum (reverse) grouping

(2) Often done on automated platforms

b) RhD type

(1) If donor tests D-negative, weak D test is required

(2) Weak D

(a) Variant of D antigen where truly D-positive individuals test as D-negative or very weakly positive on initial direct anti-D testing

(b) There is a theoretical risk that these donors, if called D-negative, could expose D-negative recipients sufficiently for anti-D to be formed

(c) Weak D test is simply an indirect antiglobulin test (see image above)

i) After addition of anti-D, centrifugation, and immediate reading, wash away unbound anti-D, add anti-human globulin

c) Antibody detection

(1) All donated units get a screen for unexpected antibodies

(2) If present:

(a) RBCs: Can be transfused, but must be labeled with the antibody name

i) Functionally, few hospitals will accept these units, so they are often discarded

(b) Plasma, platelets: Are not transfused due to large amounts of plasma

d) Infectious disease screen

(1) See "Transfusion-transmitted Disease I" podcast for details

2. Transfusion service responsibilities:

a) ABO group confirmation

(1) Forward grouping only

(2) Any discrepancy must be resolved before transfusing the product

b) RhD type

(1) Only direct agglutination testing required; not required to confirm D-negative labeling with weak D testing

c) NO requirement to repeat infectious disease screening or phenotyping results when special antigen negative units are ordered

VI. Patient Evaluation

A. Obtain patient sample

1. Procedure for collection

a) Identification and labeling process is critical

b) Training and attention to detail essential to avoid errors in collection

(1) "Wrong Blood in Tube" (WBIT): Blood in tube is not from the person on the label; risk estimated as 1 in 2000 samples!!

(2) WBIT is potentially catastrophic because everything could be done perfectly in the blood bank and disaster could happen anyway

c) Steps for accurate specimen collection:

(1) Generate request (electronic or hand-written)

(2) Identify patient by evaluating wristband; if possible, ASK the patient to identify himself (spell name, recite date of birth).
  (a) If wristband has been removed, staff must apply a new one
  (b) In operative settings, wristbands are sometimes removed and placed on chart or taped to IV pole or OR table
    i) This is suboptimal, but if facility does this in a uniform manner, can be acceptable
    ii) Requires extra vigilance both at time of specimen collection and transfusion
(3) Compare wristband and stated identifying information to each other and to the requisition
  (a) Note that this may be done in some systems using technology such as barcode readers and radio frequency identification (RFID)
(4) Draw required sample into unlabeled tubes
  (a) Sample can be either serum (red top) or plasma (lavender top)
  (b) Facility can specify one or the other, depending on technology used (gel/solid phase platform users prefer plasma, tube users like serum)
(5) Label tubes at the patient's side
  (a) Do's:
    i) Do label the tube with at least two identifiers (name, unique hospital ID number, DOB) and date of collection
      (1) This technique will vary by facility
      (2) Some use all printed labels, some use printed labels and separate hand-written blood bank labels
      (3) Whatever technique is used, make sure everything matches (down to the last i dotted and t crossed!)
    ii) Do ensure that the phlebotomist is identified on the tube (or on the requisition or in the computer system, as applicable)
    iii) Do compare the information with that on the requisition
  (b) Don'ts:
    i) Don't prelabel the tubes before collection
    ii) Don't label the tubes back at the nursing station
    iii) Don't try to correct errors in writing out information; start over with a new label!

2. Transfusion service evaluation of sample
  a) Compare identifying information on tube to that on requisition
    (1) Strict requirement for re-collection of mismatched specimen-requisition is recommended
    (2) This will NOT be popular, especially if it is a change
    (3) Do NOT allow correction of errors (even typos!)
      (a) One study showed a 40 times greater risk of blood group discrepancy between sample and donor when labeling errors were present (ref 8)
      (b) This must be proceduralized and part of the culture of the hospital
  b) Ensure that requisition has required information
    (1) What is needed and when needed

(2) Ordering provider
(3) Modifications to product (leukocyte reduction, irradiation, washing, etc.)

c) Timing and sample expiration
(1) If patient transfused or pregnant within last 3 months (or if history unknown)
(a) In these patients, a blood sample is considered "predictive" for a limited time (patients may be in process of developing new or stronger antibodies against potentially transfused RBC antigens)
(b) New sample for compatibility testing is required every three days
(c) This is often calculated in a weird way (see image below)

Sun	Mon	Tues	Wed	Thurs	Fri	Sat
Sample drawn @ 1 pm	Sample used	Sample used	Sample expires @ midnight	New sample drawn @ 6 am	New sample used	New sample used
Day 0	Day 1	Day 2	Day 3	Day 0	Day 1	Day 2

i) Date of sample collection is day 0 (Sunday in above example), then sample expires at midnight of day 3 (Wednesday above)
ii) After midnight, day 3, new sample needed for testing

(2) If patient NOT transfused or pregnant within last 3 months
(a) No upper limit for how long a specimen may be used (many use for as long as 30-45 days)
(b) Facilities may choose their own limit based on practical considerations of specimen storage, patient reliability, and package insert
(c) Many choose to use 3 days regardless, for ease and consistency
(3) Retain sample for 7 days after transfusion
(4) Unusual situations
(a) Pre-operative or outpatient surgery specimens
i) Variety of methods, including keep band on for days-weeks, new armband on surgery day, bring armband on surgery day for re-attachment
ii) No upper time limit defined if no recent pregnancy/transfusion
(b) Emergencies
i) Must set up a system in advance
ii) Temporary ID/ alias (usually alphanumeric)
iii) Define a mechanism to change to proper ID once established

d) Evaluate sample integrity
(1) Serum vs plasma
(a) Most labs doing tube testing prefer to use serum (clotted) samples
(b) Tubeless testing (gel, solid phase) prefer plasma due to particulate debris interference from incomplete clotting
(c) Plasma samples, however, can inhibit or weaken detection of certain antibodies that are complement-dependent (e.g., Kidd [Jk] antibodies) due to calcium inhibition of complement fixation

(2) Hemolysis
(a) Hemolysis in plasma makes it impossible to rule out an in vitro hemolytic antibody
(3) Lipemia
(a) Interferes with the detection of hemolysis in serum/plasma
(b) May interfere with automated testing platforms for tubeless testing

B. Test patient sample
1. Required tests
a) ABO grouping
(1) Forward and reverse required, with resolution of discrepancies
b) RhD typing
(1) Direct agglutination (immediate spin) test only done (usually with monoclonal/polyclonal anti-D)
(2) Avoid weak D test for those testing D-negative on direct test
(a) Current formulations will call most partial D patients D-negative, and most formerly weak D patients D positive
(b) This is what you WANT, as those who are partial D should receive D-negative blood, so they don't form anti-D
c) Antibody detection ("screen")
(1) Test patient serum vs. RBCs from two, three, or four fully phenotyped group O individuals
(2) MUST read at AHG (37 C and IS not required)
(3) Antigens required by FDA on reagent RBCs used in antibody screens:
(a) D, C, c, E, e, Fy^a, Fy^b, Jk^a, Jk^b, K, k, Le^a, Le^b, M, N, P1, S, s
(4) If screen is positive, must identify antibody
(5) If antibody is significant, must provide antigen-negative blood
(a) "Significant" is up to facility, but most are very similar
d) Antibody identification (next podcast)

C. Check previous records (AABB *Standard* 5.13.5)
1. Previous ABO/Rh type
a) Compare with previous records for at least the last 12 months
b) Document the comparison manually or electronically
c) What if no previous results?
(1) Some repeat on same sample
(2) Others require a second draw and full repeat testing
2. History of clinically significant RBC antibodies
a) Many antibodies disappear over time (1/3 after one year, 1/2 after 10 years)
b) ALWAYS honor a history of significant antibodies, regardless of whether it is present right now
3. History of difficult compatibility testing or blood grouping
4. History of transfusion reactions
5. History of previous special interventions (washing, irradiation, etc.)

VII. Donor and Patient Evaluation

A. Select components
1. Choose products that have the best chance of maximum benefit and minimum harm

2. Choose component based on provider order order, blood bank serologic testing, and check of historical records (for things like irradiation, etc; see above)
   a) For questions about historically requested modifications, clarify with provider
   b) If no clarification possible, provide the safest product possible based on information available
      (1) For example, blood bank might provide irradiated products until order is rescinded, even if current order does not specify to irradiate
3. ABO compatibility (Modified from *AABB Technical Manual*, 17th ed, 2011, Table 15-5, pg 447)

Component	Requirement
Whole blood	ABO identical to recipient
Red blood cells	ABO compatible with recipient plasma
Granulocytes	ABO compatible with recipient plasma
FFP/FP24	ABO compatible with recipient RBCs
Platelets	ABO identical preferred (avoid group O to non-O recipients when possible, esp. in young, small recipients)
Cryoprecipitate	All ABO types acceptable

4. RhD compatibility
   a) RBC products
      (1) D-negative premenopausal females should receive D-negative RBCs, granulocytes, or whole blood unless in dire circumstances
      (2) D-negative males and older females may receive D-positive RBCs when necessary (trauma, massive transfusion, transplant) unless have anti-D
         (a) In hospitalized patients, risk of anti-D formation in this setting is less than thought previously.
         (b) Previous studies: 80% formed anti-D
         (c) Current studies: Approximately **22%** of hospitalized patients receiving D+ RBC transfusion form anti-D (Ref 9)
   b) Platelet products
      (1) Same general rules as above, though risk is considerably less with RBCs
         (a) Older reports as high as 19% incidence of anti-D in D-negative patients receiving D-positive platelet products
         (b) Recent 10 year study of over 1000 patients showed **3.8%** incidence of anti-D without RhIG (Ref 10)
      (2) Reasonable strategy: Consider use of prophylactic RhIG to prevent immunization when giving D+ platelets to D- premenopausal females
   c) Plasma products (FFP/FP24/CRYO)
      (1) Not necessary to match for RhD

5. Antigen-negative red blood cell components
   a) Required when current or historical testing shows one or more significant RBC antibody
   b) Serologic methods
      (1) Units selected by testing with licensed specific antisera (e.g., anti-K, anti-C, anti-Fy^a, etc)
      (2) This may just be for confirmation, as most blood centers have already performed RBC phenotyping on many of their donors
      (3) May be more difficult if just pulling random units off the shelf in a hospital transfusion service
         (a) Calculation: Estimated units to screen to find particular antigen profile:
            i) QUESTION: A donor has anti-K and anti-Fya. How many units should a transfusion service expect to screen in order to find two compatible units?
            ii) Take percentages of antigen negative donors and multiply
               (1) Example: K-negative 91%, Fy^a negative 32%
                  (a) Note that these percentages assume a primarily caucasian donor base; adjust according to local situation
               (2) 0.91 x 0.32 = 0.29 (29% of donors would be expected to be compatible)
                  (a) This is only an estimate, of course
                  (b) ABO and RhD status will influence likelihood of finding compatible units
            iii) Divide the number of units needed by the percentage of compatible donors to find estimated units to screen
               (1) Example: 2 / 0.29 = 6.9 units screened to find 2 compatible
            iv) Likelihood of available product on transfusion service shelves
               (1) In this example, it's reasonably likely that units can be found
               (2) A HUGE number, however, suggests the need to call blood supplier and find uncommon/rare units
   c) Molecular methods
      (1) Genotyping technology available for screening via single nucleotide polymorphism for genes for an enormous number of antigens
      (2) Used in blood centers and transfusion services
      (3) Results must still be confirmed serologically when licensed antisera is available
      (4) Also useful for determining true genotype of recently transfused patients

B. <u>Check for compatibility</u>
   1. "Crossmatch" is used to determine compatibility between donor and patient
      a) When we say "crossmatch" we usually mean "Major" crossmatch, showing compatibility between recipient serum and donor RBCs
   2. In particular, the MAIN reason to do a crossmatch is to ensure <u>ABO</u> compatibility!
      a) Added benefit: May detect antibody vs. low-incidence antigen not present on screening cells but present on donor cells
      b) Also helps detect incompatibility when antibody screen performed incorrectly

3. Required before transfusion of any product that contains at least 2 mL of RBCs
   a) Functionally, this means crossmatches are needed for transfusion of:
      (1) Whole blood
      (2) Red blood cells
      (3) Granulocyte concentrate
   b) But are NOT needed for transfusion of:
      (1) Plasma (FFP or FP24)
      (2) Platelets (unless heavily contaminated with RBCs)
      (3) Cryoprecipitate
4. Three main types of major crossmatch:
   a) Serologic crossmatch
      (1) Full (AHG) crossmatch
         (a) Transfusion services may choose to perform AHG crossmatches on all samples, but such a strategy is overkill with no antibody on the screen
            i) AHG crossmatch is <u>required</u>, however, when patient has history of clinically significant RBC antibodies or has one or more currently
         (b) Most commonly uses washed donor cells in 2-5% suspension mixed with patient serum in a test tube with LISS enhancement (see earlier)
            i) Can use solid phase or gel technology for crossmatch, but it may require additional steps to prove ABO compatibility
            ii) LISS/gel > PEG/albumin/saline > solid phase
         (c) The only phase that MUST be read is AHG
            i) However, agglutination or hemolysis after 37 C incubation is also a positive reaction showing incompatibility
      (2) Immediate-spin (abbreviated) crossmatch
         (a) By definition, may ONLY be performed if antibody screen is negative and there is no history of significant RBC antibodies
         (b) Is simply a final ABO compatibility check
         (c) Procedure:
            i) Mix patient serum with donor 2-5% RBC solution (2 drops serum to 1 drop RBCs)
            ii) Centrifuge and observe for agglutination or hemolysis
         (d) Why do it?
            i) Saves time and reagents
            ii) Decreases workload for transfusion service workers
            iii) Demonstrated to be safe (<0.1% risk of acute hemolysis); this rate is actually very similar to that with an AHG crossmatch
   b) Electronic (“computer”) crossmatch (Image on pg 15 from Ref 11)
      (1) Like immediate-spin crossmatch, may <u>only</u> be used when current antibody screen is negative and there is no history of significant RBC antibodies
         (a) Chart below: ANY significant antibody triggers serologic crossmatch
      (2) Other requirements:
         (a) FDA-approved, locally validated computer system capable of making logic judgments about ABO compatibility between donor and patient

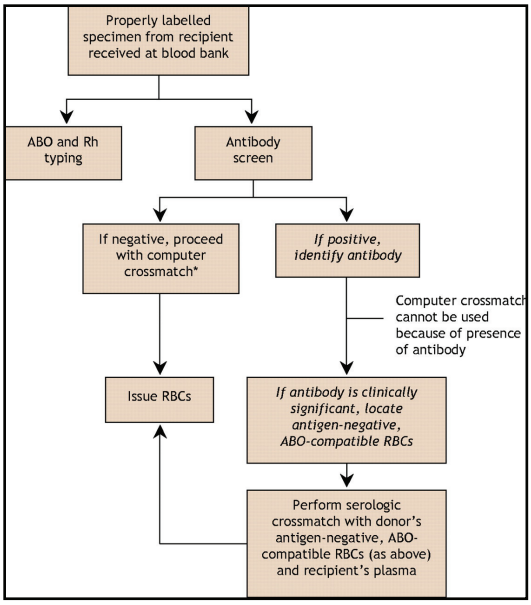
i) Part of the validation includes demonstrating that the computer will ALERT the transfusion service when it sees incompatibilities

(b) Patient who has had two separate ABO determinations (including one for this transfusion episode)

i) Acceptable: Historical ABO type and current sample ABO type

ii) Acceptable: No historical ABO type, test current sample ABO type twice

iii) Acceptable: No historical ABO type, test current sample ABO type, require a second ABO type from a second phlebotomy



(c) Why do it?

i) Potential to save LOTS of time (even more than immediate spin)

ii) Decreased workload and reagent cost in the transfusion service

iii) No significant difference in safety compared to immediate spin or AHG crossmatch (same less than 0.1% risk of hemolysis)

5. Issues with positive crossmatch results

a) Positive crossmatch after negative antibody screen

(1) Positive immediate-spin crossmatch

(a) Donor RBCs are ABO incompatible with recipient antibodies

(b) Anti-A_1 in a group A_2 or other A subgroup patient

(c) Cold-reactive antibodies in recipient tested only for warm antibodies

(d) Polyagglutinable donor RBCs

(2) Positive AHG crossmatch

(a) Antibody vs. low-frequency antigen on donor RBCs

(b) False negative antibody screen

(c) Donor RBCs coated with antibody or complement (positive DAT)

b) Positive crossmatch after positive antibody screen

(1) Autocontrol <u>positive</u>

(a) Warm autoantibody

(b) Antibody vs. recently transfused RBC antigens

(c) Cold autoantibody

(d) Passive alloantibodies (IVIG, transfusion, transplantation, RhIG)

(2) Autocontrol <u>negative</u>

(a) Expected with antibody vs. high frequency antigen

(b) If unit selected as antigen-negative:

i) Incorrectly performed antigen testing

ii) Incorrectly identified antibody
iii) Antibody vs. low-frequency antigen on donor RBCs

C. Label components
1. Component must have a tag or label affixed that includes:
a) The recipient's two independent identifiers
b) The donor unit number
c) Results of compatibility testing (if performed)
2. Other information will already be on the standard label, including (to name a few):
a) Component name
b) ABO/RhD type
c) Expiration date and storage temperature
d) Collection facility
e) Approximate volume

D. Final clerical checks
1. At issue
a) Verification of patient records noted above as well as component characteristics; the request, component, and records all must match
b) The following are required by AABB *Standards* (27th ed):
(1) Recipient information:
(a) Two independent identifiers
(b) ABO group
(c) RhD type
(2) Donor/product information:
(a) Donor identification number
(b) ABO group
(c) RhD type (if required)
(d) Compatibility testing results (if applicable)
(e) Special requirements (irradiation, leukocyte reduction, washing, etc.)
(f) Expiration date/time
(g) Issue date/time
c) Check of all of the above is usually done with person checking the blood out of the transfusion service, and must be documented
2. At bedside
a) Usually out of transfusion service control, but is VITAL!
b) Pre-transfusion verification required by AABB Standards (27th ed.); NOTE that this is the same list as above except for checking issue date/time:
(1) Recipient information:
(a) Two independent identifiers
(b) ABO group
(c) RhD type
(2) Donor/product information:
(a) Donor identification number
(b) ABO group
(c) RhD type (if required)
(d) Compatibility testing results (if applicable)

(e) Special requirements (irradiation, leukocyte reduction, washing, etc.)
(f) Expiration date/time

c) This is really the "last defense" against mistransfusion, and transfusing staff must be thoroughly trained and aware of importance of this final check

VIII. Testing/ordering nomenclature

A. Hold clot
1. Uncommonly used
2. Clotted sample held in transfusion service but is not tested at all

B. Type and hold
1. Uncommonly used
2. ABO and RhD typing done, but no other testing (no antibody detection)

C. Type and screen
1. Should be most common pretransfusion order
2. Check of previous records for comparison with current results only
3. ABO, RhD typing done, antibody detection performed
   a) If antibody present, identification is performed
4. Very simple to convert from a type and screen to a type and cross, if necessary
   a) If antibody screen is negative, only an ABO check is required (accomplished via immediate spin or computer crossmatch)
   b) If antibody screen is positive, most transfusion services automatically identify antibody and convert test to "type and crossmatch" below, after selecting antigen-negative donor RBCs (if antibody is clinically significant)

D. Type and crossmatch ("type and cross")
1. Same as type and screen, but adds crossmatching (serologic or electronic) for a specified number of units of RBCs
2. RBC units are then designated (reversibly) for that patient
3. Effective strategy: Maximize T&S, minimize T&C whenever possible

E. MSBOS
1. "Maximum Surgical Blood Ordering Schedule"
2. Hospital-specific guide to appropriate routine ordering quantities for specific procedures
3. Generally, a list of surgical and other procedures followed by a recommended blood order for that procedure
   a) Order may be "None," "Type and Screen," or "Type and Crossmatch for (X) Units"
4. Helps conserve resources and promotes consistency
5. Must be formally approved (with maximum physician input) and promoted widely to be effective
6. Not required (except in certain states), not widely utilized effectively

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