I. Antibody Screen
-Only 0.3 - 2% of people tested will have unexpected antibodies in their serum.

A. Purpose - To detect irregular or unexpected antibodies in the serum/plasma of an individual. The goal, in most cases, is to maximize detection of clinically significant antibodies, which are antibodies that can cause decreased red cell survival, while minimizing the detection of clinically insignificant antibodies.

1. Red blood cells (RBCs) may be destroyed intravascularly, through the activation of complement.

2. There may be extravascular destruction of RBCs (coated with immunoglobulins or complement) by the macrophages of the RES system.

B. Population groups requiring an antibody screen:

1. Prenatal patients- to identify IgG antibodies which can cross the placenta and attach to fetal RBCs, causing destruction of fetal RBCs (Hemolytic Disease of the Fetus and Newborn – HDFN)

2. Pre-transfusion candidates - to evaluate the need for providing antigen negative blood. Clinically significant antibodies may cause a hemolytic transfusion reaction if the patient is transfused with RBCs carrying the corresponding antigen. The antibody screen is superior to the crossmatch for detecting unexpected antibodies for the following reasons:

   a. Reagent RBCs express most clinically significant antigens

   b. Reagent RBCs have homozygous expression of significant antigens

   c. Strength of antigens decrease on storage; reagent cells in preservative

3. Donor blood

   a. Units containing alloantibodies cause a passive transfer of antibodies and should be made into components having a minimal amount of plasma

   b. Donor could be evaluated for reagent preparation and inclusion in the rare donor file
C. Testing

1. Patient’s serum or plasma (unknown antibody) is tested against reagent RBCs (known antigens). Typically, sets of 2 to 4 cell suspensions are tested. Method is an application of the indirect antiglobulin test.

2. Characteristics of Screening Cells:
   a. Suspension of Group O RBCs
   b. Both Rh Positive and Rh Negative cells in the screen cell set. When using a three cell set, the Rh genotypes are: R1R1, R2R2, rr
   c. Homozygous expression of antigens in major blood group systems when possible (e.g., Kidd, Duffy, MNSs). Antigens from other systems are also present. A 2 cell screen will not have the same level of variety/distribution of antigens as the 3 or 4 cell screen.
   d. Each set of cells comes with an antigram – an antigen profile or matrix that lists the antigens present on each reagent red cell. The antigram is lot specific!
      - + indicates the antigen is present on the RBC.
      - 0 indicates antigen is not present.
   e. When screening donors, may use 2 screening cells that have been pooled into 1 vial. These cells may show mixed-field agglutination when one cell in the pool possesses the antigen and the other does not.

3. May include an immediate spin phase.
   a. Detects cold reacting IgM antibodies.
   b. This step is often omitted, to limit detection of insignificant antibodies.

4. Must include a 37°C phase.
   a. Allows for sensitization of RBCs with antibody.
   b. Often a potentiator (enhancement reagent) is added prior to incubation.
   c. Strong IgG antibodies may be detected as well as strong cold antibodies (ones with wide thermal amplitude).
   d. Antibodies that activate complement may demonstrate hemolysis at this phase.
e. Depending on the test method employed, reactions may not be evaluated at this phase.

5. Must include an Anti-Human Globulin (AHG) phase.
   
   a. Allows for “bridging” between sensitized RBCs (agglutination).
   
   b. Detects clinically significant (IgG) antibodies. May also detect insignificant antibodies that activate complement, if using polyspecific AHG reagent.

6. Interpretation of reaction results is “all or nothing”, not an interpretation of individual screen cells.
   
   a. If all screen cells tested yield negative reactions during all phases of testing, the screen is interpreted as negative.
   
   b. A positive reaction with one or more screen cells during any phase is interpreted as a positive antibody screen.
   
   c. All cells should be tested through all phases, even if a positive reaction has been obtained. The persistence and strength of the reaction may provide clues as to the identity of the antibody.
   
   d. An antibody identification panel should be performed to determine the specificity of the antibody(-ies) detected by the antibody screen.

<table>
<thead>
<tr>
<th>Phase</th>
<th>Antibodies Detected</th>
</tr>
</thead>
</table>
| IS    | Lewis, Lu^a, M, N, and P_1  
      | Cold auto antibodies (I, H, IH) |
| 37    | May see D, E, and K  
      | Hold over strong cold antibodies (e.g. Lewis) |
| AHG   | Rh, Kell, Kidd, Duffy, Ss, Lu^b, and Xg^a  
      | May see cold antibodies (if they activate complement), when using polyspecific AHG reagent. |
D. Methods

1. Tube Testing Procedure
   a. Two drops of patient’s serum (or plasma) is mixed with 1 drop of reagent screen cells. There will be a tube for each screen cell vial used (i.e. 2 cell screen = 2 tubes; 3 cell screen = 3 tubes). RBCs are in a 2-5% cell suspension with a preservative.
   b. Tubes are immediately centrifuged and read at room temperature (immediate spin) for hemolysis and agglutination. This phase is optional.
   c. Tubes are incubated at 37°C.
      1) If no enhancement is used, 45 min to 1 hour.
      2) If enhancement is used, 10-30 minutes.
   d. After incubation, the tubes may be centrifuged and read again (depending on enhancement used), looking for hemolysis and agglutination.
   e. The tubes are washed at least 3 times with saline. AHG reagent is added, and the tubes are centrifuged and read for agglutination. Reading may take place with the naked eye (macroscopic) and microscopically.
   f. Negative tests are controlled with Coombs Control Cells (check cells).

2. Gel Testing Procedure
   a. Patient’s serum is combined with the reagent RBCs in a reaction chamber. Below the chamber is a microtubule filled with dextran acrylamide gel.
      1) The reagent RBCs are suspended in LISS to form a 0.8% cell suspension.
      2) The reaction chamber/microtubule is mounted in a card. There are typically 6 reaction chamber/microtubules per card.
   b. The card is incubated at 37°C for 15 minutes, up to 1 hour.
   c. The card is centrifuged for 10 minutes, forcing the RBCs into the gel, which contains anti-IgG. Unbound immunoglobulins remain in the reaction chamber (no wash step is necessary).
   d. If an antigen/antibody reaction has occurred, the agglutinates become trapped in the gel, due to the interaction of the anti-IgG in the AHG layer and the IgG-coated RBCs. The screen would be considered positive.
   e. If no antigen/antibody reaction has occurred, the individual RBCs pass through
the gel, and form a pellet at the bottom of the microtubule.

3. Solid Phase Testing Procedure
   a. Patient’s serum is added to a set of microtiter wells, which have been coated with various red cell antigens (not intact RBCs).
   b. LISS is added as an enhancement.
   c. The microtiter plate is incubated at 37°C for 15-60 minutes.
   d. Each well in the plate is washed at least 2 times.
   e. IgG-coated indicator RBCs are added to each well.
   f. The microtiter plate is centrifuged for 1-3 minutes.
   g. If an antigen/antibody reaction has occurred, the IgG-coated red cells will be “trapped” in the matrix, forming a diffuse pattern throughout the well. The screen would be considered positive.
   h. If no reaction has occurred, the indicator cells will form a dense pellet at the bottom of the well.

E. Techniques to eliminate clinically insignificant antibodies:

   1. Pre-warming technique – avoid detection of cold agglutinins
      a. Warm cells and serum separately at 37°C before mixing together
      b. Incubate at 37°C up to 1 hour without an enhancement
      c. Wash with warm (37°C) saline
      d. Use monospecific AHG (anti-IgG)

   2. Adsorption - either warm or cold, to remove autoantibodies which may mask the presence of clinically significant antibodies.
      a. Cold - removal of cold reacting autoantibody (usually auto anti-I)
         1) Use patient’s cells if patient has not been transfused
         2) Use rabbit cells (RES) if patient has been transfused
3) Incubate patient’s serum with the absorbing cell at 4°C, to allow cold autoantibody to sensitize the absorbing cell (removes autoantibody from the serum).

4) Retest absorbed serum for unexpected antibodies that may have been masked.

b. Warm - removal of warm autoantibody

1) Use patient’s cells which have been treated to remove autoantibody coating, if patient has not been transfused

2) Use homologous cells if patient has been recently transfused
   - Helpful to know patient’s phenotype

3) Incubate patient’s serum and absorbing cell together at 37°C to allow warm autoantibody to sensitize the absorbing cell.

4) Retest absorbed serum for unexpected antibodies that may have been masked.

3. Saline replacement – removes false positive reactions caused by rouleaux, when using the tube method. Rouleaux is seen when there is excess/abnormal protein content in the serum.

   a. After centrifuging serum and cells, serum is removed using a pipette.

   b. Two drops of saline are added to the tube.

   c. The tube is centrifuged again and re-read.

   d.) Saline will disperse rouleaux; true agglutination will persist.

F. Limitations:

1. Will not detect ABO incompatibility

2. May not detect antibodies to low incident antigens (not present on reagent cells)

3. May not detect antibodies exhibiting dosage, if antigens are not expressed in a homozygous manner.

4. May not detect antibodies when titers are below clinically detectable levels. Studies have shown that 42% of antibodies decrease in titer to a point that the antibody is no
longer detectable 5 years later (unless patient is re-stimulated).

II. Antibody Identification

A. Panel cells:

1. Used to identify the specificity of antibodies directed against red cell antigens

2. Group O red cells selected to possess the major blood group antigens

3. Usually consist of eight to twenty vial sets

4. Supplied with a lot-specific antigen profile sheet showing the phenotype of each donor

5. Preservative maintains cell stability for 2-4 weeks at 4°C

B. Testing: Generally, the method used to detect the antibody in the screen is used in testing the panel cells.

1. May include an auto control (patient’s serum/plasma tested against patient’s cells)
   a. Detects autoantibodies
   b. Detects newly forming alloantibodies in the recently transfused (patient’s cell sample is actually a mixture of patient’s RBCs and donor RBCs)
   c. If auto control is positive, perform a DAT
   d. If DAT is positive with anti-IgG AHG, perform an elution and identify the antibody coating the cells

2. Additional techniques to enhance sensitivity of weakly reacting antibodies include:
   a. Increase serum to cell ratio (only if no enhancement is used)
   b. Increase incubation time
   c. Alteration of pH, e.g., lowering pH to 6.5 will enhance some anti-M
   d. Switch enhancement media, e.g., PEG, enzymes

III. Antibody Interpretation
A. Patient’s history:

1. Previous records of unexpected antibodies

2. Transfusion, transplantation and pregnancy history

3. Medication history

4. Diagnosis (e.g., AIHA)

5. Ethnicity (e.g., Black population has higher incidence of anti-Js<sup>h</sup>, -U, -Le<sup>a</sup>)

B. Evaluation of results with panel cells:

1. Exclusion by “cross out” technique
   - Using cells that did not react with the patient’s serum, eliminate antigen specificities that are present on the cells
     a. Rule out specificity if cell has homozygous antigen expression
     b. The following specificities can be excluded when the antigen is present in a heterozygous manner: K, Kp<sup>a</sup>, Js<sup>a</sup>, and Lu<sup>a</sup>
     c. When anti-D is present in the patient’s serum, it may be difficult to find a cell that is D - and C + c - (r’r’) in order to exclude C. In this situation only, it is acceptable to exclude C using a cell with heterozygous antigen expression (D- C+ c+…r’r). This exception is true for the E antigen also (rule out using r’r cell).

2. Inclusion pattern
   - compare the pattern of reactivity obtained with the test serum with that of the remaining antigen specificities. Consider the following:
     a. Phases where reactions occurred
     b. Reaction strength
     c. Dosage
     d. Reactivity of auto control

3. “Three positive; three negative” rule
   - Testing the patient’s serum with at least three antigen-positive and three antigen
negative cells will result in a probability ($P$) value of .05. (A $P$ value is a statistical measure of the probability that a certain set of events will happen by random chance.) The interpretation of the antibody identification will be correct 95% of the time.

4. Antigen type the patient to confirm the ability to produce the antibody.
   a. **Remember Landsteiner’s Law!** The patient’s cells should lack the antigen in order for the immune system to see the antigen as foreign and produce an antibody to it.
   b. Use a pre-transfusion sample, or reticulocytes.
   c. Test a cell that lacks the antigen and a cell that possesses the antigen and its allele (heterozygous) as controls for the anti-sera.
   d. A positive DAT or recent transfusion may invalidate the typing results.

5. If after the exclusion/inclusion process has been completed the antibody specificity is unclear, additional investigative techniques may be employed to eliminate or confirm specificities (see section that follows)

C. Variation in reaction strength:
   1. Dosage: reactions stronger with cells having homozygous antigen expression (e.g., MN, Lu, Rh, Kidd, Duffy)
   2. Variability: expression of antigens differ from person to person, (e.g., P, I, Lu)
   3. Multiple antibodies: a stronger reaction when more than one antigen is present on the same cell. However, dosage effect may also occur on same cell.

D. Phases of reactions: Consider which antibodies are detected at various phases and temperatures.
IV. Additional Investigative Tools

A. Selected cell panels

1. Additional panel cells that may be tested in order to eliminate additional antibody possibilities.

2. In patients with known antibody, selected cells may be tested to prove no additional antibodies have formed.

B. Cord cells: aid in identifying auto anti-I

C. A1, A2, B cells: for determining presence of unexpected anti-A, -B, -A,B, or -H

D. Antigen type the patient’s cells to see what antibodies could be produced

E. Reference center - rare donor cells

F. Enzyme enhancement
   - used when increased sensitivity is needed for detection or separation of antibody mixtures

1. Sources:
   a. Ficin - figs (most commonly used)
   b. Bromelin – pineapple
   c. Papain – papaya
   d. Trypsin - hog stomach

2. Techniques – used to remove antibody from cells sensitized in vivo or promote in vitro sensitization of cells for identification

   a. One step procedure - enzymes added directly to serum/ red cells in place of routine enhancement reagent.

   b. Two step procedure - red cells pre-treated with enzymes before addition of test serum
3. Mechanism of action
   a. Cleave glycoproteins (sialic acid) residues from the cell reducing red cell’s negative charge
   b. Destroys or depresses red cell antigens: Fy\textsuperscript{a}, Fy\textsuperscript{b}, M, N, S, and Xg\textsuperscript{a}
   c. Exposes or enhances expression of Kidd, Lewis, Rh, Ii, P\textsubscript{1} and ABO antigens.

G. Neutralization:
   1. Soluble substances (antigens) may bind with antibody, making the antibody unavailable to react with antigens on the reagent red cells. With the antibody neutralized, other antibodies may be detected, if present.
   2. Different fluids contain antigens as soluble substance:
      a. Saliva - Lewis
      b. Hydatid cyst fluid - P\textsubscript{1}
      c. Urine - Sd\textsuperscript{a}
      d. Serum - Chido and Rodgers
      e. Human milk – I

H. Summary of the typical sequence of antibody detection and identification steps.
   1. Antibody screen
   2. Panel
   3. Selected Cells (or other investigative tools) - if necessary
   4. “Three pos; three neg” rule
   5. Antigen type patient - if possible

V. Clinical Characteristics of Alloantibody vs. Autoantibody

A. Warm (IgG) alloantibodies - clinically significant
   1. Negative auto control - unless patient has positive DAT
   2. Most common antibodies identified at AHG phase (may be at 37°C)
      a. Rh - largest number of alloantibodies are made against Rh antigens
      b. Kell - account for 2/3 of non-Rh antibodies
3. If screen/panel cells are negative but an antiglobulin crossmatch (AGXM) is incompatible consider:
   a. Antibody to low frequency antigen
   b. Positive DAT on donor unit
   c. Bacterial contamination of unit

4. If all cells are positive consider:
   a. Multiple antibodies- may exhibit variation in reaction strength or phase
   b. Antibody to a high incident antigen – often react at the same phase/ reaction strength (e.g. HTLA)

B. Warm autoantibodies

1. Positive auto control

2. Autoantibodies may show specificity to Rh system - most common is autoanti-e

3. If all panel cells are positive consider:
   a. Broad Rh specificity
   b. A cold antibody binding complement also may also be present (detected using polyspecific AHG)
   c. The need to remove autoantibody to determine the presence of an alloantibody -38% of patients with a warm autoantibody will also have alloantibodies.

C. Cold (IgM) alloantibodies:

1. Negative auto control

2. Most common antibodies in I.S. phase of testing which may activate complement and continue to be reactive through the AHG phase are: Anti-Le, -P, -P₁, -M and N

D. Cold autoantibodies

1. Autoanti-I - assumes pathologic significance in cold agglutinin disease or mixed autoimmune hemolytic anemia and Mycoplasm pneumonia.
a. All panel cells positive

b. Auto control strongly positive

c. Cord cells negative or weakly positive

2. Anti-i - relatively weak cold agglutinin; also seen as transient potent antibody in infectious mononucleosis

3. Autoanti-IH

a. All panel cells positive

b. Cord cells negative or weakly positive

c. Auto control negative or weakly positive

4. Autoanti-H - seen in A_1 and A_1B individuals (not the alloanti-H of an O_h individual which reacts over a wide thermal range with all red cells except those of other O_h individuals)

a. All panel cells positive

b. Cord cells strongly positive

c. Auto control negative or weakly positive

   - H substance strongest on group: O > A_2 > B > A_2B > A_1 > A_1B

5. Anti-P - potent IgG hemolysin with wide thermal range (biphasic hemolysin). Seen in PCH

6. Cold agglutination of undetermined specificity

a. All panel cells positive

b. Cord cells and auto control strongly positive
VI. Additional Considerations:

A. Albumin agglutination:
   1. Rare phenomenon caused by antibody to sodium caprylate, a stabilizer in many commercial albumin preparations
   2. Most reactive at I.S. and 37°C; a few at AHG phase
   3. Auto control positive; DAT negative

B. Preservative antibodies - antibody produced to preservative solution

C. Panagglutination - an antibody capable of agglutinating all red blood cells including patient’s own cells

D. Polyagglutination - a state in which an individual’s red cells are agglutinated by all sera regardless of blood type

E. Rouleaux - coin-like stacking of red blood cells in the presence of plasma expanders or abnormal plasma proteins