Complex Antibody Identification and Special Techniques
Panel Interpretation

The following should be looked at when interpreting an antibody panel:

- Phase of reactivity
- Reaction strength
- Autocontrol
- “Ruling out”
- Matching the pattern
- Rule of three
- Phenotype the patient
Multiple Antibodies

- Serum with two or more alloantibodies may make interpretation of test results difficult.

- Some clues that indicate you may have multiple antibodies:
  - Observed pattern does not fit that of a single ab
    - See if pattern fits combined specificities
  - Reactivity is present at different test phases
    - Evaluate each phase separately.
Multiple Antibodies

– Unexpected reactions occur when trying to confirm the specificity of a suspected single ab
  • Test selected cells

– No discernable pattern
  • Perform exclusions to eliminate some specificities
  • Test cells with strong antigen expression or increase sensitivity of the test system
  • Phenotype the patient
  • Enzymes
Selected Cells

- Chosen from other panels to help rule in or rule out antibodies
- If using the same panel manufacturer for select cells, ensure the selected cell is not the same as one on the original panel
  - Most manufacturers give cells a donor number or code
- Remember to use your screening cell results – the screen may have the selected cell you need!
Enhancement Techniques:

- Weak reactivity with no specificity
- Antibody suspected but not demonstrated
- Autocontrol should be included
  - LISS, PEG
  - Temperature reduction
  - Increased serum-to-cell ratio
  - Increased incubation time
  - Alteration of pH
Special Serologic Techniques

- Enzyme techniques
- Elution
- Adsorption
- Neutralization
- Chemicals
- Titers (quantification of antibody)
Enzymes

- Eliminates and enhances antibody activity
- Works by removing sialic acid residues from the RBC membrane
  - Some antigens removed, some exposed
- Enzyme-treated cells are not tested with potentiator and are read only at AHG phase to avoid false positive reactions
- **DO NOT USE AS ONLY ID METHOD!!**
Enzyme Techniques:

- Treatment of reagent red cells with enzymes (ficin or papain) will enhance or destroy certain antibody specificities

<table>
<thead>
<tr>
<th>Enhanced</th>
<th>Inactivated</th>
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<tbody>
<tr>
<td>Rh</td>
<td>Duffy</td>
</tr>
<tr>
<td>Kidd</td>
<td>MNS</td>
</tr>
<tr>
<td>Lewis</td>
<td></td>
</tr>
<tr>
<td>P₁</td>
<td></td>
</tr>
<tr>
<td>I</td>
<td></td>
</tr>
</tbody>
</table>

- Enzyme-pretreated RBC panels
- Help to separate and Id multiple Ab’s
- Enzyme panel must be run with regular panel
Antibody to High-Incidence Antigen

- All reagent RBCs are reactive, AC negative
- Strength and test phase are uniform for all cells
- Resolution can be difficult
  - Knowing patient race can help in deciding testing course
  - System null cells can help
    - DTT-treatment makes Ko cells
    - Use of enzyme treated cells
- Refer sample to reference lab
HTLA Antibodies

“High-titer, low-avidity”
Usually directed to high-incidence antigen
Weak reactivity but diluted out to high titer despite weak reaction strength
React at AHG phase
Inconsistent, “nebulous” reactions
Usually not clinically significant, but can mask clinically significant alloabs
Antibodies to Low-Incidence Antigens

- Usually found in sera of multi-transfused or multiparous patients
- May occur alone
  - Suspect when screen is negative and crossmatch is positive
- Panel with one reactive cell suggests this type of antibody
- No need to identify
- Blood that is crossmatch compatible through AHG phase is acceptable to transfuse
Autoantibodies

- First clue to an autoantibody is serum that reacts with most or all cells tested, and a positive DAT or AC
- Patient diagnosis and medication history are helpful to determine what type of autoantibody may be present
- Must use techniques that detect any underlying clinically significant alloantibodies
Cold Autoantibodies

- Typical reactions are seen at IS phase, diminish through 37°C, and negative at AHG phase
  - Some pathologic cold autoabs can have high thermal amplitude (30°C or above)
- DAT is positive with anti-complement only
Resolution of Cold Autoabs

- Determining the specificity can be helpful to decide what additional techniques to use
  - “Mini-cold” panel
- Circumvent the cold autoab to allow detection of clinically significant alloabs
  - Skip IS or RT phases
  - Prewarmed technique
  - Use of anti-IgG rather than poly AHG
  - Cold autoadsorption
  - RESt adsorption
**Mini-Cold Panel**

<table>
<thead>
<tr>
<th></th>
<th>SC I</th>
<th>SC II</th>
<th>AC</th>
<th>Cord</th>
<th>Cord</th>
<th>A₁</th>
<th>A₂</th>
</tr>
</thead>
<tbody>
<tr>
<td>4ºC</td>
<td>3+</td>
<td>3+</td>
<td>3+</td>
<td>0</td>
<td>0</td>
<td>NT</td>
<td>NT</td>
</tr>
</tbody>
</table>

Group O patient with cold autoanti-I. Cord cells are negative. A cells not tested because of existing anti-A in patient.

<table>
<thead>
<tr>
<th></th>
<th>SC I</th>
<th>SC II</th>
<th>AC</th>
<th>Cord</th>
<th>Cord</th>
<th>A₁</th>
<th>A₂</th>
</tr>
</thead>
<tbody>
<tr>
<td>4ºC</td>
<td>3+</td>
<td>3+</td>
<td>3+</td>
<td>1+</td>
<td>1+</td>
<td>1+</td>
<td>2+</td>
</tr>
</tbody>
</table>

Group A patient with cold autoanti-IH.
Common Procedures to Avoid Cold Autoantibodies

- **Monospecific IgG**: cold antibodies avoided because membrane-bound complement not detected by IgG at AHG phase
- **Prewarm Procedure**: RBCs and serum to be tested are kept at 37°C before they are combined
- **Cold adsorption**: cold autoantibodies can be removed by adsorption with autologous or rabbit RBC’s
  - Rabbit RBC’s rich in I and H antigen
  - RESt
- **DTT**: denature IgM antibodies by breaking disulfide bonds
Warm Autoantibodies

- Usually IgG and react at body temp
- Uncommon but usually pathogenic
- **React with all cells including autocontrol**
- DAT positive
- Eluate reacts with all cells uniformly
- To rule out presence of alloantibody the autoantibody must be removed by adsorption
- Perform “warm autoadsorption” unless pt. transfused w/in last three months
- Once removed the adsorbed serum can be tested as usual for underlying alloantibodies
Warm Autoantibodies

- Serum reacts with virtually all cells tested
  - AC pos, DAT pos
- Goal is to detect underlying alloabs
- Warm autoab activity is enhanced with potentiatotors
- Specificity
  - Usually directed to Rh system
  - Can show anti-e specificity
DAT

- Usually positive due to IgG, but C3 may also be present
- Mixed-field reactivity
  - Implications in recently transfused patient
  - Patient could be forming new alloab
- Elution should be performed, especially in recently transfused patients
Elution

- Technique to dissociate IgG antibodies from sensitized RBCs
  - Antibodies attached to RBCs are released, concentrated, and purified
  - Recovered ab is an eluate
- Various methods:
  - Temperature variation
  - pH manipulation
  - Chemical
Warm Auto Resolution

Eluate testing
- Usually reactive with all cells tested
- Nonreactive eluates can also occur
  - Antibodies to medications
  - Nonspecific binding of proteins to RBC membranes

Recently transfused patients
- Eluate may show alloab specificity
Antibody removal from serum to RBCs carrying the corresponding antigen

- Serum is incubated with appropriate RBCs under optimal conditions
  - Warm – 37°C
  - Cold – 4°C

Types
- Autoadsorption
- Allogeneic adsorption
Autoadsorption

- Limited by transfusion history and amount of sample
  - Patients transfused in the last 3 months do not qualify
  - Severely anemic patients may not have enough RBCs to carry out procedure

- Cells are treated to remove bound autoab
  - DTT
  - CDP
  - WARM
Allogenic Adsorption

- **Alloadsorption**
  - Cells phenotypically similar to patient are used
  - Enzyme treatment removes some antigens to aid in matching and enhances ab uptake

- **Differential or “Triple” Adsorptions**
  - Used when patient’s phenotype is unobtainable
  - Three separate adsorptions performed
    - $R_1R_1$, $R_2R_2$, $rr$ cells
Antibodies to Reagents and Drugs

Possible causes:
- Patient antibody may combine with a dye, drug or chemical in the reagent to form antibody complexes
- Chemicals may bind to the cells
- Cell membrane may be modified so that spontaneous agglutination occurs
Neutralization:

- Uses soluble antigen to inhibit reactivity of certain antibodies
- Soluble antigen added to serum sample
- Mixture incubated at room temp. to neutralize (by occupying antigen-binding sites)
- Neutralized serum tested against RBC’s
- Must have control (saline)…why???

**Table 11–4. Sources of Substances for Neutralization for Certain Antibodies**

<table>
<thead>
<tr>
<th>Antibody</th>
<th>Substance</th>
</tr>
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<tbody>
<tr>
<td>Anti-P₁</td>
<td>Hydatid cyst fluid, pigeon droppings,</td>
</tr>
<tr>
<td></td>
<td>turtledoves’ egg whites</td>
</tr>
<tr>
<td>Anti-Lewis</td>
<td>Plasma or serum</td>
</tr>
<tr>
<td>Anti-Chido, Anti-Rodgers</td>
<td>Plasma or serum</td>
</tr>
<tr>
<td>Anti-Sd₁</td>
<td>Urine</td>
</tr>
</tbody>
</table>
Effect of Chemicals on Antigens:

- Various chemicals can inactivate antigen activity; good for making cells that lack certain antigens
  - Ficin and Papain
  - DTT (dithiothreitol) inactivates Kell
  - ZZAP inactivates Kell and M,N,S, Fya, Fyb
  - Chloroquine diphosphate (CDP) inactivates Bg
  - AET (aminoethylisothiouronium) inactivates Kell
**Chloroquine diphosphate**
- Used to remove IgG antibodies from surface of red cells while leaving the red cell antigens intact to phenotype (antigen type cells with positive DAT)
- Incubate 2 hours at room temp. or 30 minutes @ 37°C

**ZZAP**
- Mixture of DTT and papain that removes antibody from sensitized cells and enzyme treats them at the same time (RBC’s remain intact)
- Should not be used for phenotyping (enzyme treated-false positive or false negative)
Titer:

- To measure the quantity of antibody
- Test two-fold serial dilutions of serum against antigen-positive red cells
- Reciprocal of the highest dilution showing visible agglutination is the antibody titer
- Most often used to monitor quantity of antibody in woman’s serum during pregnancy
- Considered significant = fourfold increase (4 to 16)
Cell Separation

- Separation of transfused cells from autologous red cells by centrifugation
- **Principle**: newly formed autologous red cells have a lower specific gravity than transfused cells and can be separated by centrifugation
- Autologous cells conc. at top of microhematocrit tube
- Better separation 3 days post transfusion
- Some red cell antigens not expressed as strongly on reticulocytes

AABB Technical manual pg. 685
my brain is experiencing technical difficulties
please stand by...