Yikes! My units are incompatible

Presented by: Jeff Kinney, ART
IT'S STILL EARLY IN THE SEASON...

...UNFORTUNATELY
He really thought the Maple Leafs would make the playoffs this year.

Well the poor lad will have lots of company where he's going.
Objectives

- Compare and contrast the laboratory investigation of pan reacting allo and autoantibodies

- List possible options for identifying alloantibodies in patients with warm autoantibodies

- State why one cannot give “least incompatible” RBCs to patients with warm autoantibodies
Why do we need to Differentiate?

- Provide physician with results for a potential diagnosis
- Allo – provide antigen negative
- Auto – provide phenotypically similar
- Crossmatch/Transfuse compatible or incompatible
“A man should look for what is, and not for what he thinks should be”
Auto vs Allo

**DAT:**
- Positive – all cells are coated with IgG and or C3

**Eluate:**
- Non specific
- Strong reactions

**Phenotype:**
- Ag positive

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**DAT:**
- Variable – often negative
- Mixed field reaction

**Eluate:**
- Specific
- Variable reaction strengths

**Phenotype:**
- Ag negative
Mixed Field DAT
<table>
<thead>
<tr>
<th>Auto</th>
<th>vs</th>
<th>Allo</th>
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<tbody>
<tr>
<td><strong>Plasma Antibody:</strong></td>
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<tr>
<td>- Usually consistent reaction strength</td>
<td>- May be consistent reaction strength if high incident</td>
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<tr>
<td>- Usually react with all cells tested except rare Rh null</td>
<td>- Variable reaction strength</td>
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<tr>
<td>- Occasionally may show specificity, e.g. Rh, Kell, Jk, LW, Sc, Gerbich etc.</td>
<td>- Dosage</td>
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<td>- Multiple antibodies</td>
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Testing to Differentiate

- **Autoadsorptions**
  - Confirm presence of auto antibody
  - Identify alloantiobodies

- **Alloadsorptions**
  - Identify alloantibodies

- **Dilutions**
  - Identify alloantibodies

- **Methodology**
  - Identify alloantibodies

- **Chemical/Enzyme treated**
  - Identify alloantibodies
  - Aid in selecting testing methodology
Effects of Enzymes (ficin, papain)

- Enhanced
  - Rh, Kidd, Lewis, P1, Vel, LW, Jra, I, Auto

- No Change
  - Kell, Lutheran, Dombrock, Cromer, Diego

- Negative/Weakened
  - Duffy, MN, JMH, Knops, Ch/Rg, In

- Variable
  - S, s, Yta

*Majority – depends on specificity
Effects of 0.2M DTT

- **No Change**: Rh, Kidd, Lewis, P1, MNSs, Jra, Csa, Gerbich, Vel, I, Auto*
- **Weakened**: Knops, Cromer, Diego, Lutheran, Dombrock
- **Destroyed**: Kell, LW, Scianna, Indian, JMH, Yta
- **Enhanced**: Kx

*Majority - depending on specificity*
Auto/Allo Adsorption Testing Procedures

- ZZAP (Commercial reagent  W.A.R.M)
- PEG
- Enzyme
What is ZZAP

- Sulphydryl/enzyme solution containing dithiothreitol (DTT) and ficin or cysteine activated papain solution
- Used for the removal of IgG antibody and complement from sensitized red cells
- Enzyme treatment allows for red cells to more efficiently adsorb auto antibody
Autoologous Adsorption

- **2-stage procedure**
  - Treatment of red cells to remove IgG antibody
  - Adsorb autoantibody from plasma

- **QC – Treat a K+ and Fy+ red cell with reagent**
  - Phenotype the treated cell for the K antigen and the appropriate Fy antigen – both Ag’s are destroyed by the treatment
  - Test DAT post treatment – should be weakened/negative
Divide treated cells into equal aliquots
- Strength of plasma antibody may indicate number of adsorptions required
- Usually in a ratio of 1:1 or 1:2 cells: serum incubate at 37°C for 30 minutes
- Harvest adsorbed plasma and repeat with new aliquot of cells

Perform DAT on cells used for adsorption
- Macroscopic – continue adsorptions
- Microscopic – test adsorbed plasma
Testing adsorbed plasma

- Objective #1: to determine if autoantibody has been sufficiently removed while limiting waste of adsorbed plasma
  
  - Test autologous DAT negative cells, or
  
  - Test phenotypically matched panel cell, or
  
  - Test R1R1, K− & R2R2, K− panel cell
Testing Adsorbed Plasma

- **Objective #2**: exclude or identify clinically significant antibody(ies)
  - Test additional panel cells
  - Exclude CSA’s with negative results
Why perform alloadsorptions?

- Recent transfusion may prohibit availability of autologous cells
- In severe cases of AIHA insufficient autologous red cells may be available for auto adsorption procedure
- Alloadsorptions tend to be more efficient at autoantibody removal
  - Autoantibodies can sometimes have a specificity against a weak or depressed antigen expression on patients own cells
Alloadsorptions using ZZAP

- Only used for the detection of alloantibody
- Historically use R1R1, R2R2, rr
- Removal of autoantibody coating the red cells not required
- K,Fy,MNSs system antigens are denatured and will not be present on treated cells thus allowing easier cell selection for the adsorptions
- Additional high incident antigens are destroyed. E.g. LW, Yta, JMH
- Enzyme treatment of cells will usually enhance uptake of autoantibody
Allogeneic Procedure

- Same as autoadsorption

- Perform DAT post adsorption to ensure IgG antibody is being removed from plasma
  - Macroscopic – continue adsorptions
  - Microscopic – test adsorbed plasma
Testing adsorbed plasma

- To determine if autoantibody has been sufficiently removed while limiting waste of adsorbed plasma
  - Test allogeneic adsorbing cell that is untreated
  - Test autologous DAT negative cells, or
  - Test phenotypically matched panel cell, or
  - Test R1R1, K− & R2R2, K− panel cell
Why I like ZZAP

- Adsorbed plasma can be tested in Gel
  - Perfect when limited sample available or sample redraw not required

- Can be used for both auto and alloadsorptions

- Testing DAT on adsorbed cells QC’s effectiveness of procedure and minimizes waste on adsorbed plasma
Adsorbed cells can possibly be re-used for additional adsorptions after treatment with chloroquine

- Reagent is commercially available and easy to use
- Inexpensive
- In-house reagent can be made if commercial reagent not available
- Can be used for cold adsorption studies
PEG Adsorptions

- 20% PEG Solution
- No pretreatment of cells required
- Used for both auto or alloadsorptions
- Some reports of weakening of loss of alloantibody
- Adsorbed plasma cannot be tested in Gel
- Larger volume of sample required
Crossmatch

- Process in place to detect ABO incompatibility
- Adsorbed vs unadsorbed plasma
- Crossmatch method may determine serological compatibility
  - i.e. LISS vs Gel
- Phenotypically matched; Rh, K, Kidd etc.
- Antigen negative if antibody has specificity?
Terminology

Irregardless ➔ Regardless

Unthaw ➔ Thaw

Least Incompatible ➔ Incompatible
The term “least incompatible” unit should be placed in the garbage heap of serologic terminology. It is not defined in transfusion medicine nomenclature; it is undoubtedly used differently by various transfusion services; its use does not convey information regarding the extent of compatibility testing performed; and finally, the term implies that this is an acceptable alternative to adequate serologic evaluation prior to transfusion of patients with AIHA.”
Case Study

- 87 y/o female
- Lymphoma
- Transfusions History
  - 2 u Feb 2012
  - 2 u Oct 2011
  - 2 u Nov 2009
  - 2 u Oct 2002
- Referred sample for pos DAT and nonspecific antibody in plasma
Investigation

- DAT: 4+ IgG only
- Eluate: nonspecific 4+
- Plasma antibody: 2+

- Ficin Panel 0–1+

- Select cells: Ch/Rg−, Knops−, In(a−), Yt(a−), JMH−, Ge−2–3–4

- LISS tube panel: weak+
- Phenotype: looking for evidence of dual cell population (mixed field)
Investigation Continued…. 

- EGA treated patient cells: performed auto control 2–3+

- PEG autoadsorption: adsorbed plasma was nonreactive with only one adsorption

- PEG alloadsorption: adsorbed plasma was nonreactive with only one adsorption, all common CSA’s excluded

Final Interpretation: Warm auto antibody with no CSA’s detected
Questions?