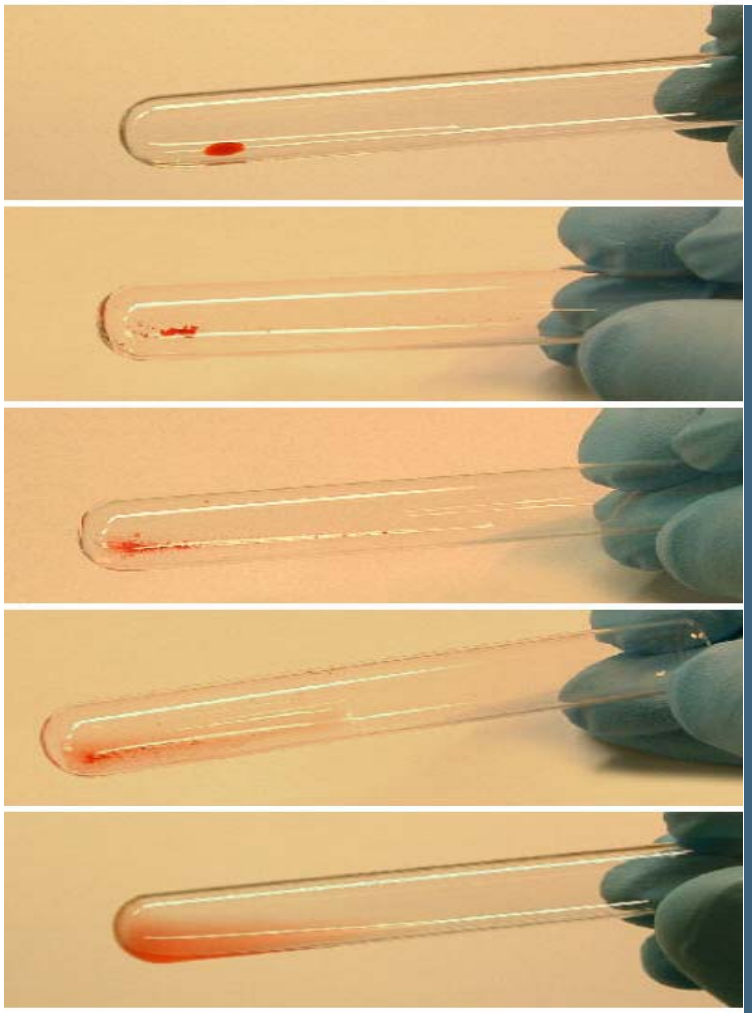
London Health Sciences Centre



Yikes! My units are incompatible

Presented by: Jeff Kinney, ART


IT'S STILL EARLY IN THE SEASON...
...UNFORTUNATELY
DOLISHAN
SUN MEDIA


web.ncf.ca/ai151/
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.
www.ComicStripGenerator.com

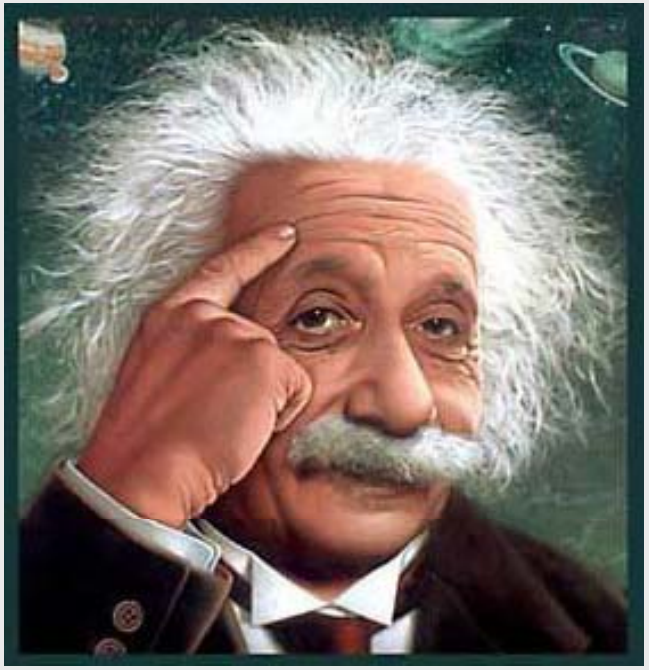
Objectives

- 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”



2+2=4

Auto vs Allo

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

Eluate:
- Non specific
- Strong reactions

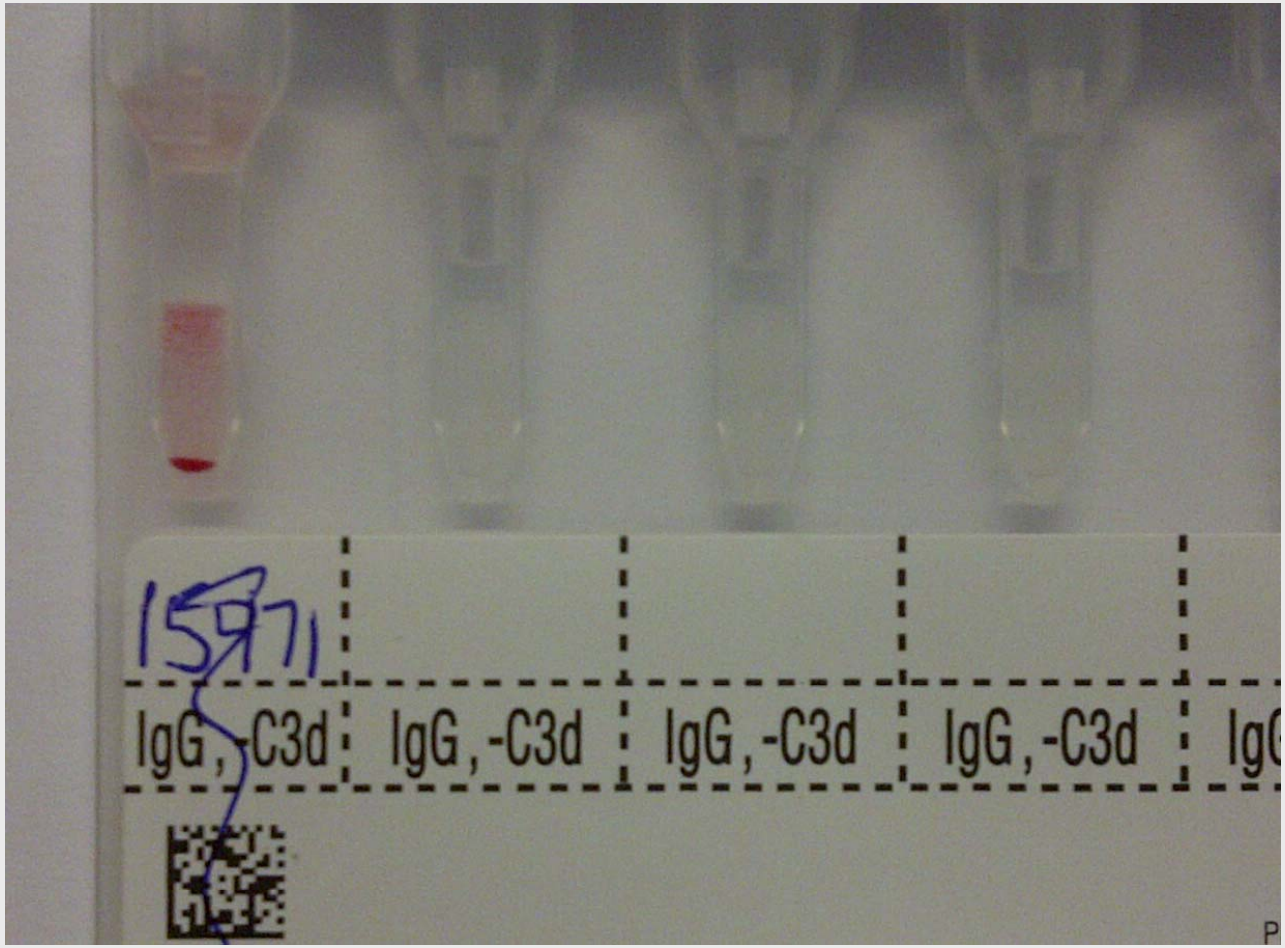
Phenotype:
- Ag positive

DAT:
- Variable – often negative
- Mixed field reaction

Eluate:
- Specific
- Variable reaction strengths

Phenotype:
- Ag negative

Mixed Field DAT



Auto vs Allo

Plasma Antibody:

- Usually consistent reaction strength
- Usually react with all cells tested except rare Rh null
- Occasionally may show specificity, e.g. Rh, Kell, Jk, LW, Sc, Gerbich etc.

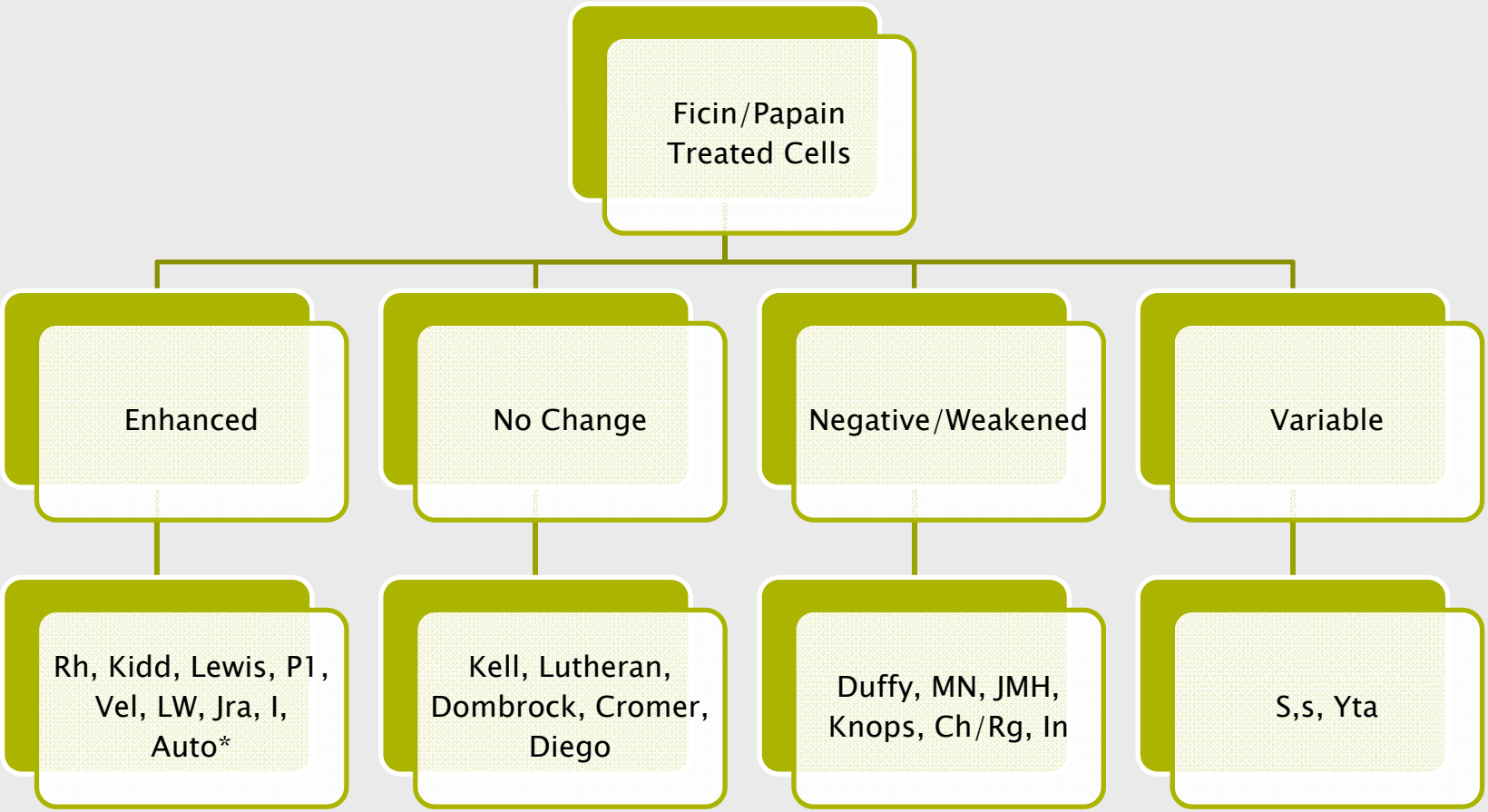
Plasma Antibody:

- May be consistent reaction strength if high incident
- Variable reaction strength
  - Dosage
  - Multiple antibodies

Testing to Differentiate

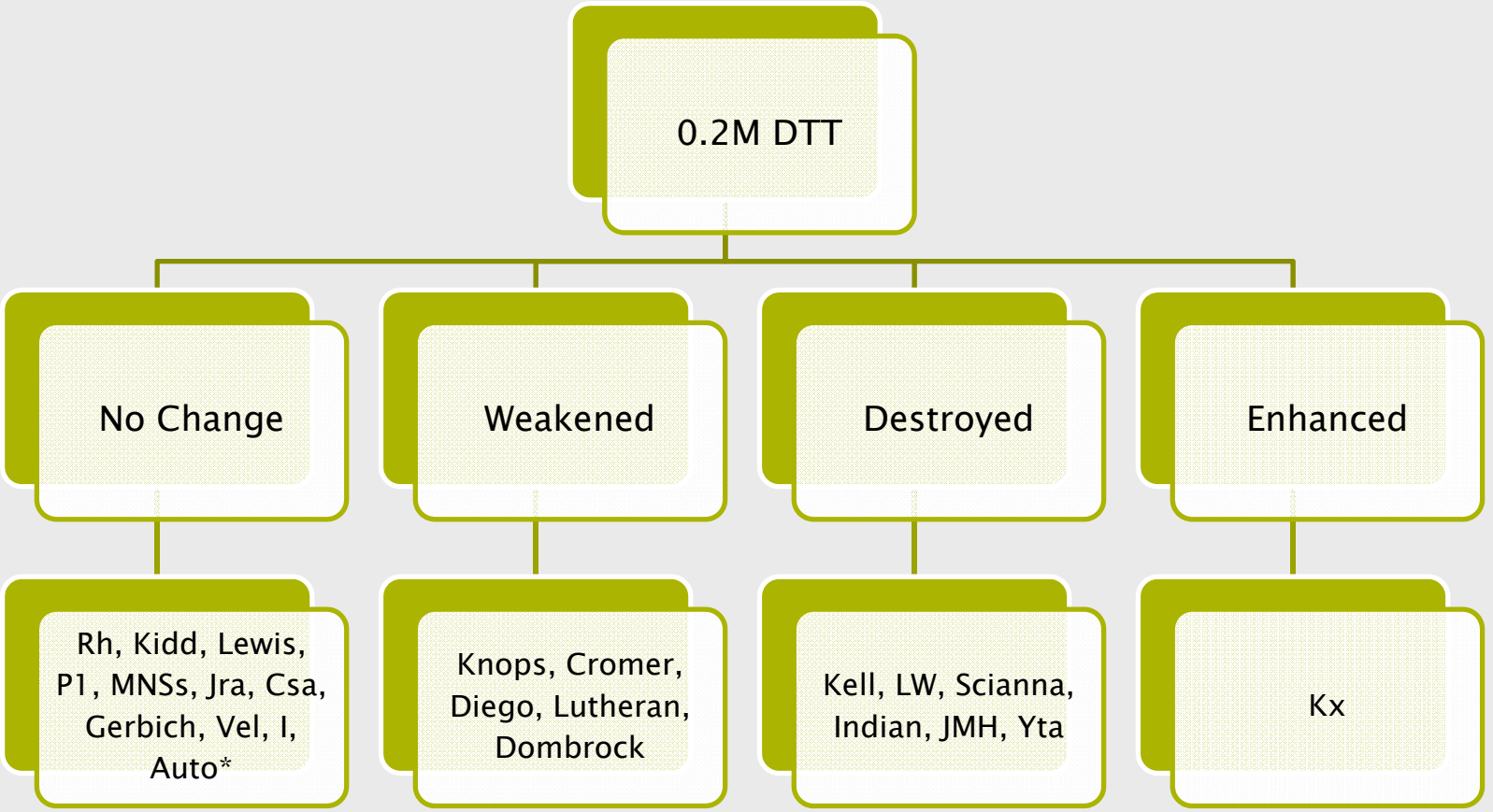
- Autoadsorptions
  - Confirm presence of auto antibody
  - Identify alloantiobodies
- Alloadsorptions
  - Identify alloantibodies
- Dilutions
  - Identify alloantibodies
- Methodology
  - Identify alloantibodies
- Chemical/Enzyme treated
  - Identify alloantibodies
  - Aid in selecting testing methodology

Effects of Enzymes (ficin, papain)



*Majority – depends on specificity

Effects of 0.2M DTT



*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

Autologous 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

.....continued

- **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

….continued

- 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

LEAST INCOMPATIBLE

- Immune Hemolytic Anemias 2nd Edition
  - Lawrence D Petz, George Garratty

"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
  - 2u Feb 2012
  - 2u Oct 2011
  - 2u Nov 2009
  - 2u 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?