Antibody Identification
Part 1: The Basics
A Blood Bank Guy Video Podcast

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March 2012

• Prerequisites
• Geography of a panel
• Antibody ID method
• Case examples

Prerequisites
• Blood Group Overview
• General facts
• Podcast from December 2011
• Pretransfusion Testing
• Testing methodologies
• Antibody screen
• Podcast from February 2012

When Do We I.D.?
• Following a positive antibody screen
• Patients, prenatsals, donors
• When testing suggests a new antibody
• To confirm a previously identified antibody (per facility SOP)
Definitions

- Alloantibody
  - Antibody against RBC antigens not present on patient’s own RBCs
- Autoantibody
  - Antibody against RBC antigens present on patient’s own RBCs

What’s a Panel?

- Just an expanded antibody screen
- Uses group O reagent RBCs
- RBCs from 8-20 donors
- Patient serum or plasma
- IS / 37°C / AHG if tubes
- AHG only if gel or solid phase
- Reactions documented on a sheet that outlines every RBC’s phenotype

Geography

- Let’s take a close look at a panel
- Important to know your way around
- There are variations, but this is a general guide
A series of 8-20 group O donor reagent red cells, each tested for all main antigens (“phenotyped”)

Rh-hr (or “Hr”) = Modified Wiener Rh genotype for donor

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Other stuff:
- Donor ID
- Special Antigen Type

Pt. phenotype results

| PC | + | 0 | + |

**Full donor phenotype**

D phenotype for each donor, C phenotype for each donor, etc...

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Jk(a+b–): Assume “double dose” Jk<sup>a</sup>  
Jk(a+b+): “Single dose” Jk<sup>a</sup> and Jk<sup>b</sup>  
Jk(a–b+): Assume “double dose” Jk<sup>b</sup>
**IS** = Immediate spin  
**37** = 37°C Incubation  
**AHG** = Anti-human globulin  
**IAT** = Indirect antiglobulin test  
(=AHG)

This is tube testing!  
- LISS ✅  
- Albumin ✅  
- Saline ✅  
- PEG ✅

This could be:  
- Tube testing  
- Gel testing  
- Solid-phase testing
IS = Immediate spin
37 = 37°C Incubation
AHG = Anti-human globulin
IAT = Indirect antiglobulin test (=AHG)

This could be:
- Tube testing
  OR
- Gel testing
  OR
- Solid-phase testing

All other results are suspect
If the AC is positive:

Ya Gotta Have a Plan...
- Consistent approach minimizes error
- Most are simple, but cutting corners increases risk for dumb mistakes
- Use this or your own system, but use the same approach every time!

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General Process

- Check history
- Check autocontrol
- Look at general pattern
- Look at what’s NOT there (cross-outs)
- Look at what IS there
- Use special techniques as necessary
- Ensure statistical significance

History

- Both in real life and on exams
- History can give you a clue and keep you from doing something dumb
- Up to 70% of cases impacted by history

History

- Clinical history examples:
  - Anti-D in pregnant pt; consider RhIG
  - Recent bacterial infection; consider antibiotic induced warm autoab
  - Recent viral illness; consider auto-anti-I or -i (consider age)
  - Recent transfusion; consider newly developing antibody
  - ITP; consider IV RhIG in D+ patient

History

- Clinical history examples:
  - Consider racial profiling (in a good way, of course!)
  - African-Americans: Lack of Duffy antigens
  - Asians: Almost all D+
  - Whites: May lack high freq antigens
History

- Consider serologic history, if known
- Previous phenotyping will help, but be careful!
- Transplants, transfusions, errors
- In real world, can use this info for targeting the panel and cells chosen

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Positive Autocontrol

- More details next time...
- Question 1: Is DAT positive?
- Question 2: What’s the patient history?
- Possibilities include:
  - Autoantibodies (warm and cold)
  - Recent transfusion/DHTR
  - Drug-induced issues
  - Passively acquired antibodies
General Process

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Pattern

- Are reactions:
  - Uniform or variable?
  - Against all, most, or rare cells?
  - Present in what phases?

Variability

- Uniform reactions suggest a single antibody
- Variable reactions suggest either:
  - Multiple antibodies OR
  - Single antibody with dosage

Cells Reactive

- With negative autocontrol...
  - A mixture of reactive and nonreactive cells suggests:
    - A single alloantibody OR
    - Multiple alloantibodies
    - A single reactive cell suggests:
      - A single alloantibody against a low-prevalence antigen
Cells Reactive

- With negative autocontrol...
- All or virtually all cells reactive suggests:
  - Multiple alloantibodies
  - A single alloantibody against a high-prevalence antigen

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### General Process

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### Cross-outs

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1. Gel or solid-phase most likely (probably gel)
2. No autoantibody obvious
3. Uniform pattern; single antibody most likely
“Cross-outs”

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General Process

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1. Gel or solid-phase most likely (probably gel)
2. No autoantibody obvious
3. Uniform pattern; single antibody most likely

Try first for a single antibody to explain all reactions
General Process

- Try first for a single antibody to explain all reactions
- Failing that...
- Depending on pattern, hypothesize:
  - Two antibodies in same phase
1. Tube testing for sure
2. No autoantibody obvious
3. Highly variable pattern, both warm and cold
**General Process**

- Check history
- Check autocontrol
- Look at general pattern
- Look at what’s NOT there (cross-outs)
- Look at what IS there
- Use special techniques as necessary
- Ensure statistical significance

**Phenotyping**

- Helps confirm identification of alloantibody by demonstrating lack of antigen
- Is a TOOL in confirmation, not sole measure of confirmation

**Adsorption**

- Removing antibodies from sample by incubation with antigen-positive RBCs
Adsorption

- Removing antibodies from sample by incubation with antigen-positive RBCs

“Alloadsorption”

Antik- K+C+S–
Anti- C K+C+S–
Anti- S K+C+S–

“Adsorbed Serum”

Elution

- Removal of RBC-bound antibodies
- Heat, cold, chemical (glycine)

“Elution”

Anti-K
Anti-C

Proteolytic Enzymes

- Enzymes such as ficin and papain may change Ag expression/Ab binding

General Process

- Check history
- Check autocontrol
- Look at general pattern
- Look at what’s NOT there (cross-outs)
- Look at what IS there
- Use special techniques as necessary
- Ensure statistical significance
Probability

- Basic idea: Ensure what you are seeing is not pure chance
- Traditional interpretation:
  - Ag present: 3 positive reactions
  - Ag absent: 3 negative reactions
- AABB Standards for IRLs, 7th ed:
  - 5.3.3 requires only 2 of each reaction to assign specificity
  
Let’s do some together!

1. Gel or solid-phase most likely (probably gel)
2. No autoantibody obvious
3. Uniform pattern; single antibody most likely

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Thanks!

- Monica LaSarre (Bonfils)
- Tuan Le (Bonfils)
- Colleen Chiappa (Bonfils)
- Kevin Elman (N. Co. Med Center)
- Cami Melland (Bonfils)
- The immortal Connie Howard (Walter Reed)

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