Antibody Identification

Part 1: The Basics

A Blood Bank Guy Video Podcast

D. Joe Chaffin, MD
March 2012

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, prenatals, 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

**R1:** DCE r': dCE
**R2:** De r": dE
**R3:** Dce r : dce
**R4:** DCE r*: dCE

**Other stuff:**
- Donor ID
- Special Antigen Type

Co(b+)

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

D, C, c, E, e, Fy^a, Fy^b, Jk^a, Jk^b, K, k, Le^a, Le^b, M, N, P1, S, s

Pt. phenotype results

Jk(a+b–): Assume "double dose" Jk^a
Jk(a+b+): "Single dose" Jk^a and Jk^b
Jk(a–b+): Assume "double dose" Jk^b
This is tube testing!
LISS ✓
Albumin ✓
Saline ✓
PEG ✓
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

- Solid-phase testing

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<th>Kidd</th>
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This is almost always:
- Gel testing
  OR
- Solid-phase

All other results are suspect

If the AC is positive:

• 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!
**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**

- 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

**General Process**

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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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### Results

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**

- Check history
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**General Process**

- Try first for a single antibody to explain all reactions

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Results:

1. Gel or solid-phase most likely (probably gel)
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### General Process

- Try first for a single antibody to explain all reactions
- Failing that...
- Depending on pattern, hypothesize:
  - Two antibodies in same phase

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1. Gel or solid-phase most likely (probably gel)
2. No autoantibody obvious
3. Variable pattern; multiple antibodies most likely
1. Tube testing for sure
2. No autoantibody obvious
3. Highly variable pattern, both warm and cold

**Results**

- **Colds**: Le^a, Le^b, M, N, P
- **Fy^b**

**General Process**

- Try first for a single antibody to explain all reactions
- Failing that...
- Depending on pattern, hypothesize:
  - Two antibodies in same phase
  - One warm and one cold antibody
  - Consider multiple warm and colds
- Next podcast!!
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
  - “Autoadsorption”

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**Adsorption**

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

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**Elution**

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

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**Proteolytic Enzymes**

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

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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
## 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

---

### Results

<table>
<thead>
<tr>
<th>Cell</th>
<th>Rh-hr</th>
<th>Kell</th>
<th>Duffy/Kidd</th>
<th>Pton</th>
<th>MNS</th>
<th>Lewis</th>
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</tr>
</tbody>
</table>

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