What’s Your Type?
Solving ABO Discrepancies

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## “Normal” ABO Typing

<table>
<thead>
<tr>
<th>Forward / Front Typing (Patient Cells)</th>
<th>Reverse / Back Typing (Patient Serum)</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Anti-A</strong></td>
<td><strong>Anti- B</strong></td>
</tr>
<tr>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>4+</td>
<td>0</td>
</tr>
<tr>
<td>0</td>
<td>4+</td>
</tr>
<tr>
<td>4+</td>
<td>4+</td>
</tr>
</tbody>
</table>
What Makes an ABO Discrepant?

• “Forward” and “Reverse” types do not “match”
• Unexpected reactions
  – Extra Reactions
  – Missing Reactions
  – Unusual Reactions
• Reactions that do not match patient history
Investigate Thoroughly

• Check for technical errors
  – Check for proper sample type
  – Was proper procedure followed?

• Check patient history
  – Recent transfusions
  – Diagnosis
  – Previous problems
  – Age of patient
Request for New Specimen

• Anytime there is doubt about specimen identification, get a new sample

• It doesn’t matter if you obtain the correct blood type on a specimen if it is from the wrong patient!!

• AABB has strict guidelines for patient identification
Common Technical Errors

- Sample mix-up
- Cell suspensions too light or too heavy
- Clerical errors
- Missing hemolysis as a positive reaction
- Failure to add reagents
- Contaminated reagents
- Failure to follow mfg. instructions
- Warming during centrifugation
Categories of Discrepancies

• Discrepancies of the Forward typing
  – Too many reactions (Extra)
  – Too few reactions (Weak or Missing)
  – Mixed field reactivity

• Discrepancies of the Reverse typing
  – Too many reactions (Extra)
  – Too few reactions (Weak or Missing)
Discrepancies of the Forward Typing

- Involve problems with the cell typing
- Less commonly seen than serum problems
- In the majority of cases, the forward typing is usually the correct typing
Problems in the Forward Type

- Too many reactions
  - Rouleaux
  - Cold autoantibodies coating cells
  - Acquired B-like antigens
  - Cells are “polyagglutinable”
Too Many Reactions In Forward Type Rouleaux – High Protein Problem

- Appears as weak (loose) agglutination
  - Stacked coins under the scope
- Seen in people with Multiple Myeloma, Waldenstrom’s Macroglobulinemia or other plasma abnormalities
- High proteins coat the cells and makes them very sticky
- Wharton’s Jelly on cord blood samples
- Plasma expanders such as Dextran & PVP
True Agglutination vs Rouleaux
Resolution of Rouleaux in the Cell Typing

• Wash patient’s cells 4-6 times with saline ➔ repeat test
  – True agglutination does not wash away
  – Rouleaux is dispersed; no agglutination upon repeat testing

• All cord blood samples need to be washed 4-6x before testing because **Wharton’s Jelly** causes spontaneous “agglutination”

• Rouleaux also messes up reverse typing - we will talk about this later
Too Many Reactions in Forward Type: Cold Autoantibodies Coating Cells

- Patient’s with strong cold agglutininins will have their own cells coated with autoantibody (usually autoanti-I) and have spontaneous agglutination
- Check patient diagnosis and IAT result
- Wash patient’s cells 4-6x with warm saline → repeat ABO
- Cold auto Abs also mess up reverse typing – more later
Too Many Reactions in Forward Type: Acquired B Phenomenon

- Often associated with disease or infection of the digestive tract (ie. Colon cancer)

- The group A patient looks like an AB
  - Reactions with Anti-B are usually weak

- Check patient diagnosis as part of resolution
Too Many Reactions in Forward Type: Acquired B Phenomenon

- Bacterial enzymes “eat away” the N-acetyl part of the group A immunodominant sugar changing it into D galactosamine, which cross-react with anti-B antisera

\[
\text{N-acetyl-D-galactosamine} \rightarrow \text{D-galactosamine}
\]

- \(A_1\) Cells:
  - 0
- \(B\) Cells:
  - 4+
- Anti-A:
  - 4+
- Anti-B:
  - 1+
Too Many Reactions in Forward Type: Polyagglutination

- Patient’s cells have become T-activated (polyagglutinable) and react with all sources of human serum
- Resolve by using monoclonal reagents (from mouse)
- Extremely rare occurrence
Missing (or Weak) Reactions in the Forward Typing

- Subgroups of A or B
- Disease related (weakened)
  - Leukemia
  - Hodgkin's Disease
  - Check patient diagnosis

<table>
<thead>
<tr>
<th>Anti-A</th>
<th>Anti-B</th>
<th>$A_1$ Cells</th>
<th>B Cells</th>
</tr>
</thead>
<tbody>
<tr>
<td>1+/0</td>
<td>0</td>
<td>0</td>
<td>4+</td>
</tr>
</tbody>
</table>
Subgroups of A

• $A_1$ (80%) $A_2$ (20%)

• Other rare subgroups: <1%
  - $A_3$ – mixed field reactivity
  - $A_x$ – positive with anti-A,B, not anti-A
  - $A_{el}$ – only detected by adsorption/ elution procedures
  - Several others
A₁ vs. A₂ (or Other Subgroups)

Reactions of Patients Red Cells with

<table>
<thead>
<tr>
<th>Blood Group</th>
<th>Antigen Present</th>
<th>Anti-A (Anti-A plus Anti-A₁)</th>
<th>Anti-A₁ lectin</th>
</tr>
</thead>
<tbody>
<tr>
<td>A₁</td>
<td>A₁ A</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>A₂</td>
<td>A</td>
<td>+</td>
<td>0</td>
</tr>
</tbody>
</table>

**FIGURE 6-9** A₁ versus A₂ phenotypes.

## Subgroups of A

<table>
<thead>
<tr>
<th></th>
<th>Anti-A</th>
<th>Anti-B</th>
<th>Anti-A,B</th>
<th>Anti-$A_1$ lectin</th>
<th>Anti-H lectin</th>
<th>Can Form Anti-$A_1$</th>
</tr>
</thead>
<tbody>
<tr>
<td>$A_1$</td>
<td>4+</td>
<td>0</td>
<td>4+</td>
<td>4+</td>
<td>0</td>
<td>No</td>
</tr>
<tr>
<td>$A_2$</td>
<td>4+</td>
<td>0</td>
<td>4+</td>
<td>0</td>
<td>3+</td>
<td>Yes (about 8% will have naturally occurring anti-$A_1$)</td>
</tr>
<tr>
<td>$A_3$</td>
<td>2$^+mf$</td>
<td>0</td>
<td>3$^+mf$</td>
<td>0</td>
<td>4+</td>
<td>Yes</td>
</tr>
<tr>
<td>$A_x$</td>
<td>0</td>
<td>0</td>
<td>2+</td>
<td>0</td>
<td>4+</td>
<td>Yes</td>
</tr>
<tr>
<td>$A_{el}$</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>4+</td>
<td>Yes</td>
</tr>
</tbody>
</table>
Resolution of Missing Forward Type

- Subgroups of A: test patient cells with $A_1$ lectin (Dolichos Biflorus). Only $A_1$ phenotype will agglutinate.

- Subgroups of B: extremely rare.

- Secretor studies, adsorption/elution procedures or serum transferase studies can be performed if indicated.
  - These are the MOST SENSITIVE tests.

- Test with human Anti-A,B: will be reactive with subgroups $A_x$ and $B_x$. 
Mixed Field Reactivity in Forward Typing**

- Recent transfusion of “out-of-group” blood – most common cause of “mf”
- Bone Marrow Transplants (BMT)
- Exchange transfusions (SCD or HDFN)
- Fetal-maternal bleeding (rarely noticeable)
- $A_3$ or $B_3$ subgroups
- Chimeras

**Check patient history for resolution of this category**
Summary of Cell Problems

• Most common causes:
  – Rouleaux – wash x6 with saline
  – Cold auto – warm wash x6 with saline
  – Mixed field – check patient history
Discrepancies of the Reverse Typing – Serum Problems

- **Missing** serum reactions:

<table>
<thead>
<tr>
<th></th>
<th>Anti-A</th>
<th>Anti-B</th>
<th>A₁ Cells</th>
<th>B Cells</th>
</tr>
</thead>
<tbody>
<tr>
<td>Missing</td>
<td>4+</td>
<td>0</td>
<td>0</td>
<td>0</td>
</tr>
</tbody>
</table>

- **Extra** serum reactions:

<table>
<thead>
<tr>
<th></th>
<th>Anti-A</th>
<th>Anti-B</th>
<th>A₁ Cells</th>
<th>B Cells</th>
</tr>
</thead>
<tbody>
<tr>
<td>Extra</td>
<td>4+</td>
<td>0</td>
<td>2+</td>
<td>4+</td>
</tr>
</tbody>
</table>
Reverse Testing Problems: Weak or Missing Reactions

- **Age of patient**
  - Elderly people have depressed Ab production
  - Newborns don’t make Abs until 3-6 months (any Abs seen are of maternal origin)

- **Disease states**
  - Hypo- or Agammaglobulinemia
  - Chronic Lymphocytic Leukemia (CLL)
  - Lymphomas; malignant lymphoma
  - Immunosuppressive drugs

- **True Chimera-twins**
Resolution of *Weak* or *Missing* Abs

- Enhance the reactivity of the IgM Abs
  - Increase serum to cell ratio
  - Incubate for 10’-30’ at room temp
  - Incubate for 10’-30’ at 4C (must run autocontrol or group O cells for negative control)
  - Use of enzyme-treated reverse cells
Reverse Testing Problems: *Extra Reactivity*

- **Rouleaux**
- **Autoantibodies**
  - Autoanti-I
  - Autoanti-H
  - Autoanti-IH
- **Alloantibodies**
  - Anti-A₁
  - Anti-M (very common)
  - Anti-N, -P₁, -Leᵃ, -Leᵇ, -Rhs, -K
Reverse Testing Problems: Rouleaux

- Due to high protein problems

- You can’t “wash” the rouleaux away in the reverse or you wash away the antibody

- Resolve with *Saline Replacement Technique*
Saline Replacement Technique

- Set up reverse typing as usual
- Spin tubes
- Take a pipette and remove/discard plasma from each tube
- “Replace” plasma in each tube with 2 drops saline
- Spin & read

**NOTE** - True agglutination remains; rouleaux disappears
Reverse Testing Problems: Autoantibodies – Anti- I, Anti- H or Anti- IH

• Auto anti-I or –H or –IH
  – Cord cells are I-; adult cells are I+
  – A₁ cells are H-; O cells are H+

• If autoantibody is present, screening cells (I & H positive) and autocontrol will be reactive; reverse cells with be reactive if auto anti-I or -IH

• Resolve with pre-warm testing
  – Can also run a “short cold panel” using A₁ vs O cells and adult vs cord cells
Reverse Testing Problems: Alloantibodies

- Cold Alloantibodies?
  - “LMNOP”

- Anti-A$_1$
  - Made by subgroups of A other than A$_1$
  - Usually is naturally occurring
  - Reverse A$_1$ cells will be unexpectedly positive
  - Screen cells I & II will be negative, as will the autocontrol
Reverse Testing Problems: Alloantibodies

• Resolution of anti-A₁
  – Prove A subgroup: type cells with anti-A₁ lectin (need to run QC for antisera)
  – Run A₁ and A₂ cells to prove antibody reacts selectively with A₁ cells and not A₂ cells (need to prove by 3+3 rule)

• Report as Aₘₐₜ or Aₘₜ B with anti-A₁

• Transfuse with A₂ or O cells until anti-A₁ is not demonstrating
Reverse Testing Problems:
Alloantibodies- Other Cold Allos

• Anti-Le\textsuperscript{a}, -Le\textsuperscript{b}, -M, -N, -P\textsubscript{1}
  – Cold allo antibodies seen at IS phase with reverse cells
  – Antibody Screen (I & II) will also be positive at IS, with \textit{negative autocontrol}

• Anti-Rh’s, -K
  – Rarely, these can be seen at the IS phase with reverse cells
  – Antibody Screen will be positive here, too, with a \textit{negative autocontrol}
Reverse Testing Problems: Resolution for Other Cold Allos

- Run an antibody identification panel to prove the antibody 3+3

- Ag type patient to prove he can make the Ab (he should be Ag-)

- Repeat reverse typing with A₁ and B cells that are negative for the Ag (ie. Anti-M ID’d, test with M- reverse cells)
Summary

- Forward and reverse typing don’t “match”
- ABO Discrepancies must be resolved before transfusing or must transfuse with group O
- Problem is usually with the “weak reactions” if all cells are positive
- Forward typing is usually the correct type – more problems with serum
Case #1

• An 83 y.o. male is in for a pacemaker implantation. A type and screen is ordered. There is no patient history in the computer. The following results are observed:

<table>
<thead>
<tr>
<th>Anti-A</th>
<th>Anti-B</th>
<th>Anti-D</th>
<th>A⁺ Cells</th>
<th>B Cells</th>
<th>I - Gel</th>
<th>II - Gel</th>
</tr>
</thead>
<tbody>
<tr>
<td>4⁺</td>
<td>0</td>
<td>4⁺</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
</tr>
</tbody>
</table>

• What is the discrepancy?
• What are possible causes?
• How would you resolve?
Case #1

• Weak or missing reactions with the reverse cells – Forward type is A; reverse is AB

• Elderly; newborn; leukemia/lymphomas; hypo/agammaglobulinemia; chimera

• Resolution
  – Increase serum:cell (add 1-2 drops of serum)
  – Incubate at RT for 15 minutes ➔ respin
  – If still negative, add autocontrol and incubate at 4C for 15C
  – Reference labs my ficin treat reverse cells and repeat typing
# Case #1 Resolution

<table>
<thead>
<tr>
<th></th>
<th>Anti-A</th>
<th>Anti-B</th>
<th>Anti-D</th>
<th>A1Cells</th>
<th>B Cells</th>
<th>I (Gel)</th>
<th>II (Gel)</th>
</tr>
</thead>
<tbody>
<tr>
<td>4+</td>
<td>0</td>
<td>4+</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td></td>
<td>0</td>
<td></td>
<td>0</td>
<td>1+</td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

Added 2 more drops serum to reverse typing; incubated 10 minutes; respun
Case # 2

A 28 y.o. female is life-lined to your facility following a car accident. She received 2 units of O Neg blood in transport. A type and crossmatch for 4 units is requested. You get the following results:

<table>
<thead>
<tr>
<th></th>
<th>Anti-A</th>
<th>Anti-B</th>
<th>Anti-D</th>
<th>A₁ Cells</th>
<th>B Cells</th>
<th>I - Gel</th>
<th>II - Gel</th>
</tr>
</thead>
<tbody>
<tr>
<td>4+mf</td>
<td>0</td>
<td>4+mf</td>
<td>0</td>
<td>4+</td>
<td>0</td>
<td>0</td>
<td>0</td>
</tr>
</tbody>
</table>

- What is the discrepancy?
- What are possible causes?
- How would you resolve?
- What type of blood would you give?
Case #2

- Mixed field reactivity seen in anti-A and anti-D tubes
- Subgroup of A (A₃); receipt of out of group blood; chimera; BMT; exchange transfusion or fetal-maternal bleed
- History check to confirm receipt of blood. Since you have no BB records, you still must give group O blood until you know for sure her true blood type. To find this out you would have to do a cell separation to separate donor blood from patient blood.
- Once blood type is confirmed, transfuse with type specific
Case #3

- A 63 y.o. female oncology patient is to receive 2 units of leukoreduced packed cells as an outpatient. She has a history of being A Positive and has received platelet transfusions for the past 2 weeks. This is her first red cell transfusion. Her results are as follows:

<table>
<thead>
<tr>
<th>Anti-A</th>
<th>Anti-B</th>
<th>Anti-D</th>
<th>A₁ Cells</th>
<th>B Cells</th>
<th>I - Gel</th>
<th>II - Gel</th>
</tr>
</thead>
<tbody>
<tr>
<td>4+</td>
<td>0</td>
<td>4+</td>
<td>2+</td>
<td>4+</td>
<td>0</td>
<td>0</td>
</tr>
</tbody>
</table>

- What is the discrepancy?
- What are possible causes?
- How do you resolve? What type to transfuse?
Case #3

- **Extra** reactivity in the reverse grouping with the $A_1$ Cells
- Possible Causes: rouleaux; subgroup of A that has made anti-$A_1$; passive anti-A from platelet txns; autoantibodies; alloantibodies
Case #3

• Resolution:
  – Verify types of platelet transfusions (if group O platelets were received, this may be passive Anti-A)
  – Perform antibody screen and if positive perform ABID (think LMN_P)
  – If antibody screen is negative, think anti-A₁
    • Type with anti-A₁ lectin
    • Type with 2 more sources of A₁ vs A₂ cells (if available) for 3+3 proving
    • Transfuse with A₂ cells or group O cells
Case #4

A 74 y.o. male has a T&C for 2 units ordered. He has no blood bank history but his admitting diagnosis is Multiple Myeloma. You get the following results:

<table>
<thead>
<tr>
<th>Anti-A</th>
<th>Anti-B</th>
<th>Anti-D</th>
<th>A₁ Cells</th>
<th>B Cells</th>
<th>I - Gel</th>
<th>II - Gel</th>
</tr>
</thead>
<tbody>
<tr>
<td>2+</td>
<td>4+</td>
<td>4+</td>
<td>4+</td>
<td>3+</td>
<td>0</td>
<td>0</td>
</tr>
</tbody>
</table>

- What is the discrepancy?
- What are possible causes?
- How would you resolve?
- What type of blood would you transfuse?
Case #4

• Can’t tell which is correct type but there are extra reactions present

• Rouleaux; cold autoantibody ➔ can effect both forward and reverse typing

• Resolution:
  – For cells:
    • Warm wash x 6 will take care of both rouleaux & cold auto
  – For serum:
    • Saline replacement for rouleaux
    • Pre-warm technique for cold

• Transfuse type specific, or group O if unable to resolve; may need blood warmer if it is a cold auto
ABO Resolutions in YOUR Lab

• Check patient history
• Repeat your patient blood type
• Redraw patient if necessary
• Run an antibody screen at room temperature, including an autocontrol
ABO Resolutions in *YOUR* Lab

- **To Eliminate** extra reactions:
  - Wash patient cells and repeat forward typing
  - Prewarm reactions if you have extra reactions in both forward and reverse
  - Perform Saline Replacement Test

- **To Enhance** weak or missing reactions:
  - Add extra serum to reverse tubes to enhance a weak reaction
  - Allow tubes to sit in your rack for 10-30 minutes and respin
Questions?

Says here you're a type O.

That must be a typo.