ABO Discrepancies
You got this!

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Objectives

- Define ABO discrepancy
- Explain the different categories of typing discrepancies
- Describe a few resolution techniques to resolve ABO discrepancies
- Evaluation case scenarios
A discrepancy exists when ..............

- The results of the red cell test (front type) do not agree with those of plasma test (back type).
- The current results do not agree with the historical blood type.
Categories

On a very basic level, an ABO discrepancy is classified in one of the following:

- Weak/Missing reactivity in the Cell (Forward) Typing (unexpected negative)
- Weak/Missing reactivity in the Plasma (Reverse) Typing (unexpected negative)
- Additional reactivity in the Cell (Forward) Typing (unexpected positive)
- Additional reactivity in the Plasma (Reverse) Typing (unexpected positive)
- Mixed-field reactivity in the Cell (Forward) Typing
- Mixed-field reactivity in the Plasma (Reverse) Typing
- Rh typing problems (We will not cover these today.)
Let’s take a moment to pause, wipe the sweat from your brow, and refocus.
Who are we serving:
Keep the patient’s needs in focus

*When a typing discrepancy is identified, it may be necessary to transfuse group O red cells as you complete the investigation.*

**Important Note:**

- **DO NOT** use the historical blood type for current red cell transfusions.
- Having that perfectly match red cell unit after the patient is deceased is **NOT** a blood bank success story.

Although, providing units when there is an unresolved discrepancy is not the best scenario, there are times that having blood to carry oxygen is more important to the patient’s survival.

- *The use of blood in these situations is a medical decision that must quickly weigh the pros and cons of the transfusion.*

**REMEMBER:** There are medical options and treatments for any subsequent transfusion reaction, but there is no medical treatments post death.
Let’s start
Step #1 when an ABORh discrepancy is encountered

Repeat the blood type test using the same sample with a new suspension of cells, new tubes and pipette fresh aliquots of reagents.

Contributors to many blood typing errors:

- **Improper technique**
  - **Solution:** Repeat the test and carefully follow procedure.

- **Clerical error**
  - **Solution:** Repeat the test, read and record reactions immediately. Don’t think you will remember them correctly.

- **Patient identification error**
  - **Solution:** Redraw the patient. Do not relabel or limit relabeling as defined in local procedures.

- **Specimen quality**
  - **Solution:** For fibrin/clots, you can try to use a wooden stick, rim the sample, respin, and retest. For interfering substances, recollect
For unresolved discrepancies, obtain patient history

- Age
- Diagnosis
- Transfusion/Hematopoietic progenitor transplant recipient
- Medications

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After the exclusion of technical error and equipped with patient history, how does one determine if the discrepancy is in the front or back type?

Evaluate the reactivity:

- Most often the stronger reactivity is correct, and the weaker reactivity is the reactivity that needs investigation.

Hint: Many times the discrepancy is in the back type.
Front Type Discrepancies
Forward (Front) type:

The three types of discrepancies that can be experienced in the forward type are:

- Missing or weak reactivity
- Extra reactivity
- Mixed-field reactivity
Missing and Weak Front Types
Case

- 85 year male
- No history at your hospital. But had heart surgery 10 years ago at a hospital on the east coast.
- Current diagnosis is AML (Acute Myeloid Leukemia)
- Order received for 2 units of irradiated RBC and 1 unit of apheresis platelets
- Type and screen results:
  - Anti-A: 0
  - Anti-B: 0
  - Anti-D: 0
  - D control: 0
  - A1 cell: 0
  - B cell: 0
  - Antibody screen is negative.
- You repeat the type and get the same results. Now what? Patient’s hgb is 5g/dL and platelet count is 3/mm$^3$. 
Forward type demonstrating missing or weak reactivity - Overview

Causes of apparent antigen loss (not a complete list)

- The inheritance of a weak ABO subgroup

- Cancer:
  - Some Leukemia's
  - Patients with primary pancreatic, ovary, and biliary tract cancer.

- HPC: hematopoietic progenitor cell transplant
  - Bone marrow
  - Mobilized peripheral blood
  - Umbilical cord blood


Back to the case

So.......... the patient has leukemia. Is it possible that there is a lost antigens?

- You repeat the test and let the front type sit at room temperature for 30 minutes, spin, and read. The following results are obtained:
  - Anti-A: 2+
  - Anti-B: 2+
  - Anti-D: 0
  - D control: 0
  - A1 cells: 0
  - B cells: 0

Discrepancy resolved. The patient is an AB negative.
Cancer and ABO antigen loss

- Several leukemia types can show weakened or mixed field reactivity in the ABO forward type.
  - For example, AML and CML

- Primary pancreatic, ovary, and biliary tract cancer may demonstrate a weak or missing front type.
<table>
<thead>
<tr>
<th>Red Cell Phenotype</th>
<th>Anti-A</th>
<th>Anti-B</th>
<th>Anti-A,B</th>
<th>A1 Cells</th>
<th>B cells</th>
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</thead>
<tbody>
<tr>
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<td>4+</td>
<td>0</td>
<td>4+</td>
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<td>1+ to 2+</td>
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<td>0/2+*</td>
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<tr>
<td>Bm</td>
<td>0</td>
<td>0/±</td>
<td>0/±</td>
<td>4+</td>
<td>0</td>
</tr>
</tbody>
</table>

*Occurrence of anti-A1 is variable in these phenotypes

Routinely used to complete forward type

Investigative use in discrepancy resolution

Routinely used to complete reverse type

Knowing the expected reaction and the expected strength of the reaction will help you logically and systemically resolve ABO discrepancies.
Hematopoietic progenitor cell transplant

- Antigen loss, antigen gain, antibody loss, or antibody gain.

- Follow site specific standard operating procedure for recommendations.
Overview of techniques to resolve decreased reactivity in the front type:

Resolution of an apparent loss of antigen

- Use washed red cells that are properly suspended to the correct concentration and repeat the test.

  If you still have a discrepancy ......................

- Incubate the front type tubes at room temperature for 5 to 30 minutes. Spin and read.

  If you still have a discrepancy .................

- Incubate the front type tubes at 4°C for 15 to 30 minutes.
  
  - You must include a control and the control must be negative at the 4°C for the results to be valid.

  - Patient’s washed red cells mixed with 6% albumin may act as the control.

    - The control is to detect spontaneous or autoagglutination.

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- Technical Manual, Method 2-5
Mixed-Field Front Type Discrepancy
Front type: Mixed-field reactivity

- Leukemia, previously discussed
- Out of group transfusions
- Transplant
- Fetomaternal hemorrhage
- Chimerism
- ABO subgroups (for example, A₃) may also give the appearance of mixed-field reactivity.
  - Newborns
- Hematopoietic progenitor cell transplant
  - Bone marrow
  - Peripheral blood
  - Umbilical cord blood

Extra Reactivity in the Front Type
Causes of extra reactivity

- Rouleaux
- Cold agglutinin
- Positive DAT causing spontaneous agglutination
- Out of group transfusion
- Hematopoietic progenitor cell transplant (HPC)
Forward type: Extra reactivity

Extra reactivity in the front type still can be resolved without to much difficulty if you remember a few basic things.

- Repeat the test, starting from the beginning to rule out technical or clerical error.

- If using plasma to make your cell suspension, repeat the test using a saline washed cell suspension.

- If you have multiple testing platforms, use a different platform.
Forward type: Extra reactivity

Rouleaux

- Evaluate for rouleaux by using a microscope. Rouleaux has a classic coin stacking appearance.
- Resolution: Wash the patient’s cells with saline, resuspend, then retest.
Forward type: Extra reactivity

Cold agglutinin:

- Specimen will appear ‘chunky’ as you observe it in the collection tube.

Resolution:

- Test soon after collection.
- Use warm saline to wash the cells and make the cell suspension.
- Warm saline + put back in heat block
Spontaneous agglutination

- Red cells having a positive direct antiglobulin test (DAT), (IgG/C3, IgG, C3) can agglutinate in the presence of protein or other potentiating media that is added to antiserum diluents.

Resolution:

- Wash the cells several times with saline.
- If unable to resolve, send to the reference laboratory.

Stop for a theory refresher:

So, you are asking yourself, why can we use warm saline to wash the red cells but can’t warm the back type prior to doing a lot of work?

- In the cell type (front or forward type), we are only looking at the antigens on the cells.

- In the plasma typing (back or reverse type), you are looking for antibodies.
Out of group transfusions

- Usually these appear as mixed field not extra reactivity. If extra reactivity is present in the front type, investigate transfusion history. Notify a caregiver and pathologist if an incompatible transfusion was given.

Hematopoietic progenitor cell transplant (HPC)

- Bone marrow
- Peripheral blood
- Umbilical cord blood
Back Type Discrepancies
Category: Back Type discrepancies

Remember, history will help you focus your work efforts

Subcategories:
- Missing reactivity/weak reactivity
- Extra reactivity (includes mixed-field reactivity)
Pause for a review
Antibody production usually begins after birth.

- Anti-A and Anti-B levels change throughout life.

- Antibody production usually begins after birth in response to environmental stimuli.

- Isotypes associated with the back type.

**References**

Weak or missing reactivity in the back type
Resolution steps for weak or missing reactivity in the back type

Fixing this type of discrepancy may be easier than you think.

► {Do first} Increase antigen-antibody contact time.

► {Do second} When using tube methodology, increase the plasma to cell ratio.

► {Do third} Use both methods together.
Continued: Resolution of weak or missing reactivity in the back type

- Place the tubes in the refrigerator for 3-30 minutes then spin and read.

- Use fresh donor segments from Type A neg and Type B neg units as back typing cells.

Extra reactivity and mixed-field reactivity
ABO discrepancies caused by unexpected plasma reactions are not uncommon.

- Common causes of extra reactivity in plasma grouping are:
  - Cold autoantibodies
  - Rouleaux
  - Cold-reactive alloantibodies
  - A and B subtypes
STOP - don’t just prewarm

- Prewarm is a wonderful way to eliminate cold reactions,
  **BUT**
- Prewarm technique should only be used AFTER you understand what you are prewarming.

**WHY?**
- Panagglutination removed with pre-warm technique. Patient transfused with 2 RBCs. Patient died 6-8 hours post transfusion.
- Patient’s antibody reactivity was eliminated with prewarm technique. Patient had a hemolytic transfusion reaction (HTR).

Back type: Extra reactivity - continued
(may include mixed-field reactivity)

Looking at the patient history can help you focus your work efforts. This is especially true when you have extra reactivity in the back type.

- Review the patient’s diagnosis:
  - Increased protein levels in plasma may result in rouleaux.
  - Look for auto-immune processes.
- Evaluate for transfusions: Remember, all allo-antibodies start out in the IgM phase.
  - For group A and AB, consider anti-A1.
- Evaluate for out of group transfusion: Remember to look for out of group plasma products.
- Is the patient a Hematopoietic progenitor cell transplant (HPC) recipient?
- Medications
  - Did the patient receive IVIG?
Back type: Extra reactivity
(may include mixed-field reactivity)

Rouleaux

Resolution: Perform Saline replacement technique.
Is the patient type A or AB?

Consider anti-A1

Anti-A1 is found in the serum of 1-8% of A2 individuals and 22%-35% of A2B individuals.

Resolution: Use A2 cells for the back type, and type the patient’s cells with A1 Lectin.

Cold antibody evaluation.
  ▶ Cells used in cold antibody evaluation:
    ▶ Screen cell I/II
      ▶ May use mini/full panel if underlying alloantibody is suspected
    ▶ Auto Control (AC)
    ▶ A1 cells
    ▶ B cells
    ▶ Type O cord cells
  ▶ Incubation time: Immediate spin and then room temperature incubation.

Not rouleaux nor anti-A1, now what?
Cold alloantibody

- Auto control at immediate spin and room temp will be negative.
- Cord cells may be reactive.
- Immediate spin and/or room temperature screening cells will be selectively positive.
- Panel will define the antibody.

**Resolution:**
- Place the back type tubes in the 37°C heat block
- Use back typing cells negative for the antigen
Back type: Extra reactivity  
(may include mixed-field reactivity)

**Cold autoantibody**

- The auto control is typically positive at immediate spin and/or room temperature.
- Screening cells are positive.
- Back typing cells may be positive.
- Cord cells are weakly reactive or negative if the reactivity is due to an auto-anti-I.

**Resolution:**

- Once the auto-antibody has been identified, placing the tubes in the 37°C heat block may help resolve the ABO discrepancy.
Back type: Extra reactivity - continued
(may include mixed-field reactivity)

Hematopoietic progenitor cell transplant
- Follow site specific standard operating procedure for instructions.

Out of group transfusion
- Review transfusion history to identify.
  - Look for out of group plasma transfusions.
- If unable to resolve the discrepancy, continue with Group O red cells and AB plasma.
Other weird stuff that may cause a back type discrepancy
Back type: Extra reactivity - continued (may include mixed-field reactivity)

- IVIG
- Antibodies to the reagent constituent
- ABO Subgroups
ABO Discrepancies
Case Scenarios
Case Scenario #1

**Patient Initial Reactivity:**
Front type = A pos
Back type = O
Antibody screen = negative

<table>
<thead>
<tr>
<th></th>
<th>Immediate Spin</th>
</tr>
</thead>
<tbody>
<tr>
<td>Anti-A</td>
<td>4+</td>
</tr>
<tr>
<td>Anti-B</td>
<td>0</td>
</tr>
<tr>
<td>Anti-D</td>
<td>4+</td>
</tr>
<tr>
<td>A1 cells</td>
<td>1+</td>
</tr>
<tr>
<td>B cells</td>
<td>4+</td>
</tr>
</tbody>
</table>

Thinking through the logic of the blood type discrepancy:
1. The weaker reactivity is probably the discrepant reaction
2. Antigens rarely change
3. Antibodies come and go
Keep the patient’s needs the center of your work

The first set of questions is about the welfare of patient:

- How is the patient doing?
- Are they hemodynamically stable?
- How much time do you have to resolve this discrepancy?
- Will emergency issued products be needed?
Initial evaluation

It appears that you have extra reactivity in the back type.

- Step 1:
  - Evaluate sample quality.
  - Repeat with a new aliquot of washed patient’s cells, fresh reagents, and plasma.

- Step 2: Obtain patient history.

<table>
<thead>
<tr>
<th></th>
<th>Immediate Spin</th>
</tr>
</thead>
<tbody>
<tr>
<td>Anti-A</td>
<td>4+</td>
</tr>
<tr>
<td>Anti-B</td>
<td>0</td>
</tr>
<tr>
<td>Anti-D</td>
<td>4+</td>
</tr>
<tr>
<td>A1 cells</td>
<td>1+</td>
</tr>
<tr>
<td>B cells</td>
<td>4+</td>
</tr>
</tbody>
</table>

Repeated test results
Patient History

- Male, 40 years old.
- Transfused 5 years ago after a motor vehicle accident.
- No medication.
- Diagnosis: GI bleed.

What does this history tell us?
1. Sex and transfusion history.
2. Medication list and diagnosis were not significant.
Big picture

The screen is negative with a blood type discrepancy which appears to be due to extra reactivity in the back type.

What does this reactivity pattern tell us?

- The reactivity likes colder temperatures. (no reactivity in the screen)
- You are most likely dealing with an IgM antibody or a cold reactive IgG.
- Start to consider antibodies that have this reactivity pattern when you start your evaluation.
Immediate spin reaction:

Hint: If you do not have A2 cells, type A negative cord cells can be substituted to help with your investigation. Babies type as A subtype. Adult levels of the ABO expression are generally present by age 2 to age 4.

<table>
<thead>
<tr>
<th></th>
<th>IS</th>
<th>37°C 5-10 min</th>
<th>1. Spin tube (Do not shake) 2. Put tube at 37°C for 5-10 min. 3. Read and record results</th>
<th>PW SAL AHG</th>
<th>CC</th>
<th>PW PEG AHG</th>
<th>CC</th>
</tr>
</thead>
<tbody>
<tr>
<td>A1 Cells</td>
<td>1+</td>
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<td>A2** Cells</td>
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<td>B Cells</td>
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</tbody>
</table>

What does this reactivity pattern tell us?
1. Whatever is reacting is on all the cells except for the cord cells.
2. The auto control is positive!!! Since the patient has NOT been transfused in the last 3 months, you should be thinking about cold auto-antibodies.
3. Cord cell have little-i. Adults have big-I.
4. This appears to be a cold reactive auto anti-I.
Logical thought process: The negative serologic results in the cord cells at immediate spin and a history of NO recent transfusions will allow us to warm the cell to 37°C.

<table>
<thead>
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<th></th>
<th>IS</th>
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<th>CC</th>
</tr>
</thead>
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<tr>
<td>A1 Cells</td>
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<td>B Cells</td>
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<td>0</td>
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</tbody>
</table>

• **Conclusion:** Cold Auto Antibody in the plasma. The patient’s blood type is A Pos.
Case Scenario #2
Case Scenario #2

Front type = 0 positive
Back type = A
Antibody screen = negative

Think through the logic of this blood type discrepancy:

- The weak reactivity is probably the discrepant reaction.
- Antigens rarely change.
- Antibodies come and go.

<table>
<thead>
<tr>
<th>Immediate Spin</th>
<th>Anti-A</th>
<th>Anti-B</th>
<th>Anti-D</th>
<th>Monoclonal Control</th>
<th>A1 cells</th>
<th>B cells</th>
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</thead>
<tbody>
<tr>
<td></td>
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<td>0</td>
<td>4+</td>
<td>0</td>
<td>0</td>
<td>4+</td>
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</tbody>
</table>
Initial Evaluation

- It appears that you are missing reactivity in the front type.
- Step 1: Repeat test using saline washed cells.
- You obtain a valid blood type.

<table>
<thead>
<tr>
<th></th>
<th>Immediate Spin</th>
</tr>
</thead>
<tbody>
<tr>
<td>Anti-A</td>
<td>2+</td>
</tr>
<tr>
<td>Anti-B</td>
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<td>Anti-D</td>
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<td>Monoclonal Control</td>
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<td>A1 cells</td>
<td>0</td>
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<td>B cells</td>
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</tbody>
</table>

Repeated test results
Why did this technique work?

Let’s look at the patient’s history.

- Female, 65 years old, 3 pregnancies in the past, youngest child is 30 years old.
- Never transfused.
- Medication: daily multi-vitamin.
- Diagnosis: newly diagnosed with pancreatic cancer.

What does this history tell us?

Diagnosis is significant

- Pancreatic cancer is associated with the appearance of lost ABO antigens.
  - Testing interference: Secretion of soluble ABO antigens.
Questions?