Resolution of ABO Discrepancies

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Objectives

1. Given the results of ABO typing, correctly identify if a discrepancy exists and if the source is most likely in the forward or reverse type.
2. Describe in detail several causes of ABO discrepancies due to the following:
   a) Weak or missing reactivity in the reverse typing.
   b) Unexpected reactivity in the reverse typing.
   c) Weak or missing reactivity in the forward typing.
   d) Unexpected reactivity in the forward typing.
3. Describe appropriate follow-up testing that is necessary in the resolution of ABO discrepancies.

ABO Discrepancies

- A very important part of pretransfusion testing involves detection, recognition, and resolution of ABO discrepancies.
- Discrepant results must be identified and the underlying causes investigated.

Troubleshooting Steps

- Step 1: Repeat the test.
  - Technical errors
    - Specimen mix-up
    - Forgot to wash cells
    - Incorrect cell suspension
    - Failure to add reagents or sample
    - Missed hemolysis reaction (read as negative)
    - Didn’t follow procedure
    - Incorrect centrifugation
    - Incorrect interpretation
- Step 2: Request a new specimen.

Troubleshooting Steps

- Read the Forward Type first.
  - Note: the Forward Type reactions may not be correct
- Look at the strongest reactions.
  - The strong vs. weak reactions can provide important clues.
- Any time you encounter a discrepancy of any kind:
  - 1.) Repeat the test to rule out technical errors.
  - 2.) If the results are the same, record the result as: Discrepant.

    Only type O, Rh-compatible blood should be issued until the investigation is completed.
  - 3). ALL discrepancies must be investigated and resolved before the correct ABO type can be resulted.

Reverse Type Discrepancies (Weak or Missing)

- Discrepancies in the reverse type are commonly encountered, and are generally due to weakly reacting or missing antibodies.

	Forward Type		Reverse Type	
	Anti-A	**Anti-B**	**A_1 Cells**	**B Cells**
Patient Result	0	3+	0	0

Weak or missing reactivity in reverse type

- Age related (<4-6 months old, elderly)
- Hypogammaglobulinemia
- Transplantation (Immunosuppressed)

Investigation:

- Room Temperature (RT) incubation and centrifuge again.
- This may allow the antibodies enough time to sensitize and form a lattice.
    - (Note: reverse type testing of of neonates is unnecessary)
- Record on the laboratory workup that you have done this.
- *Remember: if it wasn't documented, it wasn't done!*

Reverse Type Discrepancies (Extra)

- A_2 phenotype with Anti-A1
- Cold-reactive alloantibody (anti-M, anti-P_1, etc.)
- Cold-reactive autoantibody
- Pseudoagglutination due to rouleaux effect (hyperproteinemia)
- Transfusion of incompatible plasma components (mismatched platelets, etc.)
- Recent infusion of IVIG
- Serum antibody to reagent constituent

Resolution of A_2 with anti-A1

Forward Type

Reverse Type

Unexpected Reactivity with A1 Cells

Anti-A	Anti-B	A1 Cell	B Cell
4+	0	1+	4+

Most Anti-A reagents react strongly with A2 Cells

No unexpected antibodies detected

Antibody Screen	I.S. (RT)	AHG	Check Cells
Screening Cell 1	0	0	✓
Screening Cell 2	0	0	✓
Screening Cell 3	0	0	✓
Auto Control	0	0	✓

Anti-A1 lectin reacts with A1 cells only;
Is non-reactive with all other A subgroups

Lectin	Patient's Cells
D. biflorus	0

Most standard protocols require multiple reactive A1 cells and non reactive A2 cells to prove the presence of anti-A1

Cold-reactive alloantibody

Forward Type

Reverse Type

Unexpected Reactivity with A1 Cells

Anti-A	Anti-B	A1 Cell	B Cell
4+	0	1+	4+

Unexpected cold-reactive antibody detected;
Perform antibody identification panel

Antibody Screen	I.S. (RT)	AHG	Check Cells
Screening Cell 1	0	0	✓
Screening Cell 2	1+	0	✓
Screening Cell 3	0	0	✓
Auto Control	0	0	✓

Anti-A1 lectin reacts with A1 cells only;
Is non-reactive with all other A subgroups

Lectin	Patient's Cells
D. biflorus	4+

This pattern is an example of A_1 with unexpected, cold-reactive alloantibody

Cold-reactive autoantibody

Forward Type

Reverse Type

Unexpected Reactivity with A1 Cells

Anti-A	Anti-B	A1 Cell	B Cell
4+	0	1+	4+

Anti-A1 lectin reacts with A1 cells only;
Is non-reactive with all other A subgroups

Lectin	Patient's Cells
D. biflorus	4+

Pan-reactivity at room temperature

Antibody Screen	I.S. (RT)	AHG	Check Cells
Screening Cell 1	1+	0	✓
Screening Cell 2	1+	0	✓
Screening Cell 3	1+	0	✓
Auto Control	1+	0	✓

This pattern is an example of A_1 with cold-reactive autoantibody

Pseudoagglutination due to rouleaux effect

Forward Type

Reverse Type

Unexpected Reactivity with A1 Cells

Anti-A	Anti-B	A1 Cell	B Cell
4+	0	1+	4+

Rouleaux usually disappears after wash steps

Antibody Screen	I.S. (RT)	AHG	Check Cells
Screening Cell 1	1+	0	✓
Screening Cell 2	1+	0	✓
Screening Cell 3	1+	0	✓
Auto Control	1+	0	✓

Anti-A1 lectin reacts with A1 cells only;
Is non-reactive with all other A subgroups

Lectin	Patient's Cells
D. biflorus	4+

Reactions appear "stringy;" rouleaux effect can be seen under microscopic evaluation

Saline Replacement

- ✓ Set up reverse type testing as you usually would.
- ✓ Perform immediate spin (I.S.) centrifugation
- ✓ Before shaking the tubes, carefully remove all the plasma/serum with transfer pipette.
- ✓ Replace plasma with 2 drops of normal saline.
- ✓ Read reactions as you usually would.

- Principle of the test: if antibody-antigen lattice formation has occurred during the I.S. phase, it will remain undisturbed when you remove the plasma/serum. This should only remove interfering proteins that cause a false positive reaction.

Previous sample after saline replacement

Forward Type

Reverse Type

Pseudoagglutination disappears after saline replacement

Anti-A	Anti-B	A1 Cell	B Cell
4+	0	0	4+

Antibody Screen	I.S. (RT)	AHG	Check Cells
Screening Cell 1	0	0	✓
Screening Cell 2	0	0	✓
Screening Cell 3	0	0	✓
Auto Control	0	0	✓

Reverse Type Discrepancies

- ~~A_2 phenotype with Anti-A1~~
- ~~Cold-reactive alloantibody (anti-M, anti-P_1, etc.)~~
- ~~Cold-reactive autoantibody~~
- ~~Pseudoagglutination due to rouleaux effect (hyperproteinemia)~~
- Transfusion of incompatible plasma components (mismatched platelets, etc.)
- Recent infusion of IVIG
- Serum antibody to reagent constituent

Case 1

Forward Type

Reverse Type

Anti-A	Anti-B	A1 Cell	B Cell
0	3+	3+	1+

What is the person's most likely type?
Which reaction(s) is/are suspect?
What further test(s) should be performed?

Case 1

Forward Type

Reverse Type

Anti-A	Anti-B	A1 Cell	B Cell
0	3+	3+	1+

- No rouleaux observed under microscopic investigation
- Results are most consistent with cold-reactive autoantibody
- Perform cold panel to identify specificity
- Possible cold autoadsorption
- Pre-warmed technique?

Antibody Screen	I.S. (RT)	AHG	Check Cells
Screening Cell 1	1+	0	✓
Screening Cell 2	1+	0	✓
Screening Cell 3	1+	0	✓
Auto Control	1+	0	✓

Case 2

Forward Type

Reverse Type

Anti-A	Anti-B	A1 Cell	B Cell
4+	3+	1+	1+

What is the person's most likely type?
Which reaction(s) is/are suspect?
What further test(s) should be performed?

Case 2

Forward Type

Reverse Type

Anti-A	Anti-B	A1 Cell	B Cell
4+	3+	1+	1+

- No rouleaux observed under microscopic investigation
- Results are most consistent with cold-reactive alloantibody
- Perform antibody identification (include immediate spin (I.S.) room temperature (RT) phase to identify specificity)
- Antigen type reagent A1 and B cells

Antibody Screen	I.S. (RT)	AHG	Check Cells
Screening Cell 1	0	0	✓
Screening Cell 2	1+	0	✓
Screening Cell 3	1+	0	✓
Auto Control	0	0	✓

Case 3

Forward Type

Reverse Type

Anti-A	Anti-B	A1 Cell	B Cell
3+	3+	1+	0

What is the person's most likely type?
Which reaction(s) is/are suspect?
What further test(s) should be performed?

Case 3

Forward Type

Reverse Type

Anti-A	Anti-B	A1 Cell	B Cell
3+	3+	1+	0

Antibody Screen	I.S. (RT)	AHG	Check Cells
Screening Cell 1	0	0	✓
Screening Cell 2	0	0	✓
Screening Cell 3	0	0	✓
Auto Control	0	0	✓

Case 3

Forward Type

Reverse Type

Anti-A	Anti-B	A1 Cell	B Cell
3+	3+	1+	0

Lectin	Patient's Cells
D. biflorus	0

Antibody Screen	I.S. (RT)	AHG	Check Cells
Screening Cell 1	0	0	✓
Screening Cell 2	0	0	✓
Screening Cell 3	0	0	✓
Auto Control	0	0	✓

Most likely: A_2B with anti-A1

Case 4

Forward Type

Reverse Type

Anti-A	Anti-B	A1 Cell	B Cell
3+	0	1+	3+

What is the person's most likely type?
Which reaction(s) is/are suspect?
What further test(s) should be performed?

Antibody Screen	I.S. (RT)	AHG	Check Cells
Screening Cell 1	0	0	✓
Screening Cell 2	0	0	✓
Screening Cell 3	0	0	✓
Auto Control	0	0	✓

Case 4

Forward Type

Reverse Type

Anti-A	Anti-B	A1 Cell	B Cell
3+	0	1+	3+

Lectin	Patient's Cells
D. biflorus	3+

Antibody Screen	I.S. (RT)	AHG	Check Cells
Screening Cell 1	0	0	✓
Screening Cell 2	0	0	✓
Screening Cell 3	0	0	✓
Auto Control	0	0	✓

Case 4

Forward Type

Reverse Type

Anti-A	Anti-B	A1 Cell	B Cell
3+	0	1+	3+

No unexpected antibodies detected

Antibody Screen	I.S. (RT)	AHG	Check Cells
Screening Cell 1	0	0	✓
Screening Cell 2	0	0	✓
Screening Cell 3	0	0	✓
Auto Control	0	0	✓

Patient is A1

Lectin	Patient's Cells
D. biflorus	3+

Check patient history for recent transfusion of ABO incompatible plasma products, infusion of IVIG, or investigate possible antibody to A1 reagent constituent.

Forward Type Discrepancies (Weak or Missing Reactions)

- Weak or missing RBC activity
  - Weak ABO subgroups
  - Leukemia/malignancy
  - Transfusion of group O red cells
  - Bone Marrow Transplant

Weak or missing reactivity in the forward type

Anti-A	Anti-B	A1 Cell	B Cell
0	0	0	3+

Possible weak subgroup of A (A_x, etc.)

- Test cells with anti-A,B reagent
- May require genotype testing to confirm

Leukemia/malignancies can result in temporary loss of expression of ABO antigens

- Check patient's diagnosis/history

Recent massive transfusion of group O RBCs

- Check transfusion history

Bone Marrow Transplant

- Possible Group A patient receiving Group O BMT
- Check patient's diagnosis/history

Forward Type Discrepancies (Extra Reactions)

- Extra reactions in the forward type
  - Autoagglutinins/excess protein coating the cells
  - Unwashed cells: plasma proteins
  - Transplantation of out-of-group Bone Marrow
  - Acquired B antigen
  - B(A) Phenomenon
  - Out-of-group transfusion

Extra reactivity in the forward type

Anti-A	Anti-B	A1 Cell	B Cell
1+	1+	3+	3+

Autoagglutinins/excess protein coating the cells

- Check patient's diagnosis/history
  - Waldenstrom's Macroglobulinemia
  - Multiple Myeloma
  - Recent infusion of high molecular weight volume expander
- May need to wash cells multiple times and retest
- Perform Direct Antiglobulin Test (DAT) including Saline Control

Extra reactivity in the forward type

Anti-A	Anti-B	A1 Cell	B Cell
1+	0	4+	4+

Transplantation of out-of-group Bone Marrow

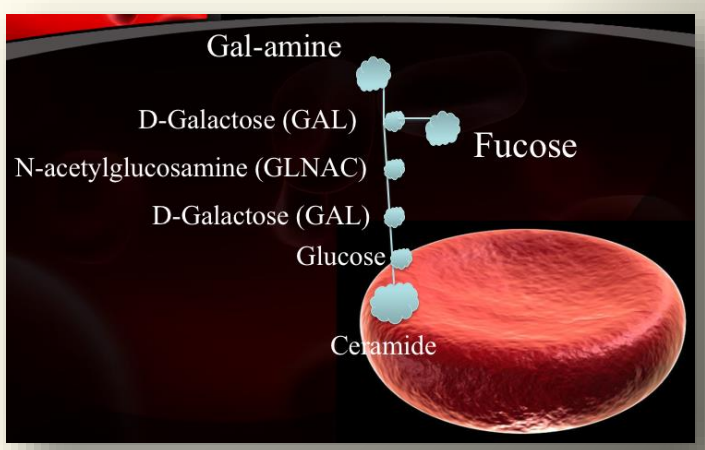
- Check patient's diagnosis/history
- Possible Mixed Field reactivity?

Extra reactivity in the forward type

Anti-A	Anti-B	A1 Cell	B Cell
4+	1+	0	4+

Possible Acquired B Phenomenon

- Check patient's diagnosis/history
  - Transient
  - Group A individuals can acquire "B-like antigen"
  - Despite the reactivity in the forward type, the patient's serum will not react with autologous red cells (patient does not have anti-A)



Extra reactivity in the forward type

Anti-A	Anti-B	A1 Cell	B Cell
4+	1+	0	4+

- Acquired B Phenomenon can occur in the setting of infection by gastrointestinal bacteria.
  - Enteric bacteria can possess the deacetylase enzyme capable of converting A antigen to a B-like analog.
- To resolve: RBCs can be tested using a different monoclonal anti-B reagent or acidified (pH 6.0) human anti-B.
  - Human anti-B will not react with acquired B antigen.
  - The ability of monoclonal anti-B to recognize acquired B should be noted in the manufacturer's insert.

Extra reactivity in the forward type

Anti-A	Anti-B	A1 Cell	B Cell
1+	4+	4+	0

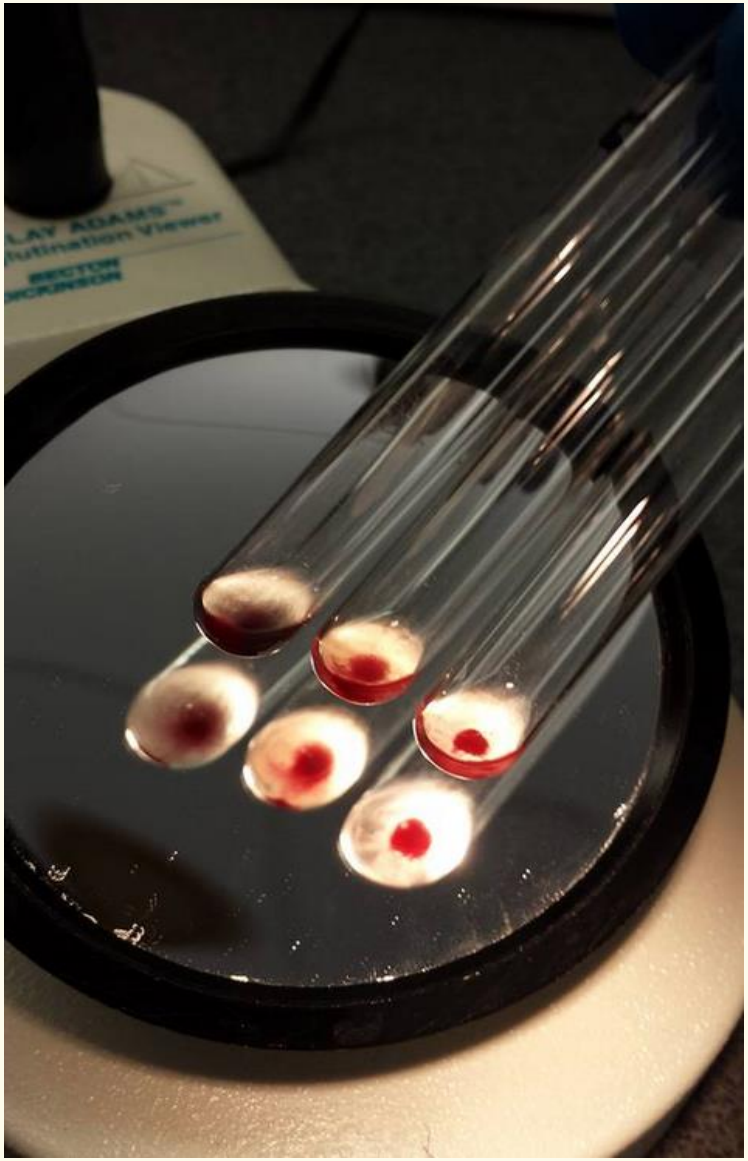
- B(A) phenotype is an autosomal dominant phenotype
- Weak A expression on group B red cells.
- Amino acid polymorphisms of the *B* gene are responsible: alpha-3-D-galactosyltransferase can use UDP-N-acetylgalactosamine, which adds some GalNAc to the H antigens.
- Weak, extra reaction with anti-A <2+

Extra reactivity in the forward type

Anti-A	Anti-B	A1 Cell	B Cell
3+ MF	2+MF	0	3+

- Possible out-of-group RBC transfusion:
  - (Possible group B RBC transfused to group A recipient!)

Mixed Field



Mixed Field (MF) Reaction

Control tubes

Patient tubes

Question 1

Forward Type		Reverse Type	
Anti-A	**Anti-B**	**A1 Cell**	**B Cell**
1+	0	3+	3+

Question 2

Forward Type		Reverse Type	
Anti-A	**Anti-B**	**A1 Cell**	**B Cell**
4+	1+	0	3+

Question 3

Forward Type		Reverse Type	
Anti-A	Anti-B	A1 Cell	B Cell
1+	3+	3+	0

Question 4

Forward Type		Reverse Type	
Anti-A	Anti-B	A1 Cell	B Cell
0	1+	3+	0

References

- Harmening DM, Ed. *Modern Blood Banking and Transfusion Practices*, 6th Ed. F. A. Davis Company, Philadelphia. 2012.
- Fung MK, Eder AF, Spitalnik SL, Westhoff CM. *AABB Technical Manual*, 19th Ed. AABB Press. 2017