Community Blood Center

ABO Discrepancies

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Objectives

- Identify and describe several causes of ABO discrepancies.
- List techniques used to resolve ABO discrepancies.
- Arrive at appropriate ABO interpretations based on laboratory results.

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ABO Discrepancies

- **Weak/missing reactivity**
  - Front type
  - Back type
- **Extra reactivity**
  - Front type
  - Back type

	Front type		Back Type	
	Anti-A	Anti-B	A1 cell	B cell
Weak reactivity	1+	0	0	4+
	0	0	2+	4+
Missing reactivity	0	0	0	4+
	0	4+	0	0
Additional reactivity	1+	1+	4+	4+
	4+	0	2+	4+

ABO Discrepancies: Where to begin?

Correct sample?? Technical error??

Patient History

- Very young/old: weak ABO antibodies
- Alloantibody that might interfere with reverse grouping
- Strong cold autoantibody
  - May interfere with both forward and reverse grouping
- Bone Marrow Transplant
- Recent transfusion
- Diagnosis
  - Weak antigens in leukemia, pregnancy, cord samples

ABO Discrepancies: How to resolve

Additional reagents

- Front type: Anti-A,B (different anti-A/B clones); Anti-A1
- Back type: A_2, O, autocontrol

Wash the cells

- Removes plasma antibodies/proteins
- Warm wash cells (37C saline): removes strong cold autos

Saline replacement

- Disperses rouleaux

Pre-warming

- Circumvent cold autoantibody or cold alloantibody interference

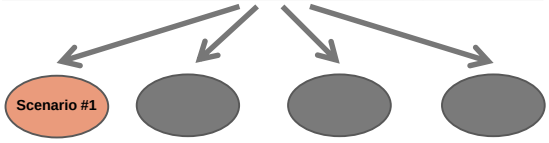
Increase sensitivity

- Increase plasma/cell ratio
- Increase incubation time
- Decrease temperature (ABO system "cold" reactive)
- Adsorption/Elution
  - # antigen/cells too low for agglutination
  - Demonstrate by adsorbing and eluting anti-A or B from cells
  - Rarely Used: replaced by molecular testing

Must run appropriate controls for any testing outside limits of package insert.

Case #1

Front Type				Back Type				
Anti-A	Anti-B	Anti-D	Rh Control	A_1	A_2	B	O	Auto control
4+	0	4+	0	1+	Not tested	4+	Not tested	Not tested



What's anti-A1 lectin?

What's the difference between A_1 and A_2 phenotypes?

- **Frequency in European population**
  - ~80% group A individuals A_1
  - ~20% group A individuals A_2
  - Other A subgroups rare (A_3, A_x, A_{el}, etc.)
- **Antigen differences**
  - Quantity of A antigens on cells
    - A_1 cells have approximately 5 times as many A antigens as A_2 cells
  - Qualitative differences
    - Antigens of A_1 individuals more branched
    - Why A subgroup individuals can make anti-A1.

Fung MK, Eder AF, Spitalnic SL, Westhoff CM. Technical Manual. 19th ed. Bethesda, MD: AABB; 2017: 271-272.

More about anti-A1

- **Detected in 1-8% of A_2 individuals, 22-35% of A_2B individuals**
  - Often "naturally occurring"
- **Usually IgM, reacts best at room temperature or below.**
  - Generally not considered clinically significant
- **Reports in literature of hemolytic anti-A1**

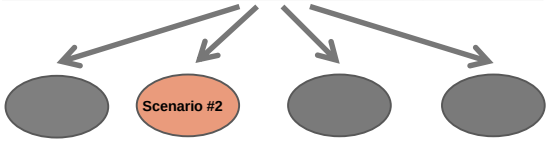
Helmich F, et al. Acute hemolytic transfusion reaction due to a warm reactive anti-A1. Transfusion. 2018;58;1163-1170.

- **Transfusion recommendations: XM compatible units**
  - A_2 RBCs
  - O RBCs

Fung MK, Eder AF, Spitalnic SL, Westhoff CM. Technical Manual. 19th ed. Bethesda, MD: AABB; 2017: 274.

Case #1

Front Type				Back Type				
Anti-A	Anti-B	Anti-D	Rh Control	A_1	A_2	B	O	Auto control
4+	0	4+	0	1+	Not tested	4+	Not tested	Not tested



Does the patient's plasma contain cold autoantibody?

		Rh					Kell		Duffy		Kidd		MNS				Results		
		D	C	E	c	e	K	k	Fy^a	Fy^b	Jk^a	Jk^b	M	N	S	s	5' RT	LISS 37C	LISS IAT
1		+												+	+	+	1+	0	0
2		+												+	0	+	1+	0	0
3		+	0	+	+	0	0	+	+	0	+	+	+	0	+	+	1+	0	0
4		+	0	0	+	+	0	+	0	0	+	0	+	+	0	+	1+	0	0
5		0	+	0	+	+	0	+	+	0	+	0	+	+	0	0	1+	0	0
6		0	0	+	+	+	0	+	0	+	+	+	0	+	0	+	1+	0	0
7		0	0	0	+	+	+	+	0	+	+	0	+	0	+	+	1+	0	0
8		0	0	0	+	+	0	+	+	+	0	+	0	+	+	+	1+	0	0
9		0	0	0	+	+	0	+	+	+	0	+	+	0	0	+	1+	0	0
10	I-	+	+	0	0	+	0	+	+	0	+	+	+	+	+	0	0	0	0
11		+	0	0	+	+	+	+	0	0	+	+	0	+	+	+	1+	0	0
Auto																	1+	0	0

1 nonreactive cell at RT: I-negative cell

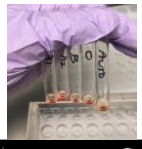
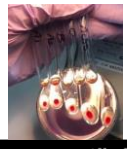
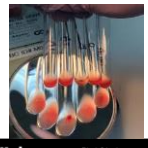
Autocontrol reactive at 5' RT

Cold Screen

Cold Screen		
	30' RT	30' 4C
SCI	2+	4+
SCII	2+	4+
I-negative	0	3+
Auto	2+	4+

What is pre-warming?

- Incubate all reagent red cells and patient plasma (and pipet) at 37C prior to testing
- Add patient plasma to reagent cells quickly without changing the temperature of the testing environment from 37C
- Incubate all tubes at 37C (~30 min)
- DO NOT CENTRIFUGE! (centrifugation will quickly cool the sample in the tubes)
- Shake and read settled tubes

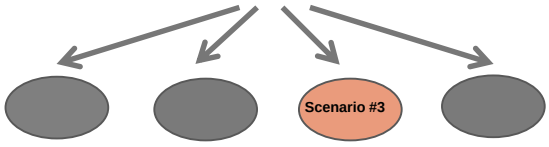
One word of caution!

NEVER utilize pre-warm testing unless you know what you are pre-warming!!

(demonstrate that the patient has cold autoantibody prior to pre-warming)

Case #1

Front Type				Back Type				
Anti-A	Anti-B	Anti-D	Rh Control	A_1	A_2	B	O	Auto control
4+	0	4+	0	1+	Not tested	4+	Not tested	Not tested



Scenario #3

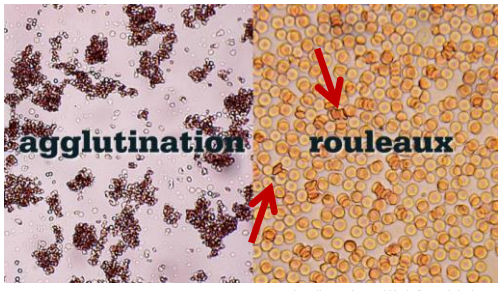
Front Type				Back Type				
Anti-A	Anti-B	Anti-D	Rh Control	A_1	A_2	B	O	Auto control
4+	0	4+	0	1+	1+	4+	1+	1+

These same results (same as scenario #2) may be due to rouleaux!!

- In vitro phenomenon due to abnormal patient plasma protein concentration
- Can be seen in any test involving patient plasma (including back type)
- "Stack of coins," refractile aggregation of RBCs
- Can be mistaken for agglutination macroscopically

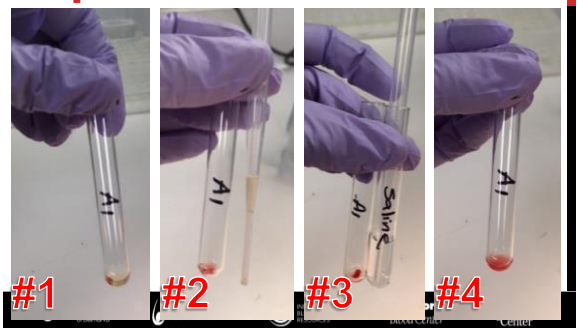
Fung MK, Eder AF, Spitalnic SL, Westhoff CM. Technical Manual. 19th ed. Bethesda, MD: AABB; 2017: 370-371.

Can you see the difference?



https://www.atdove.org/video/saline-agglutination-test

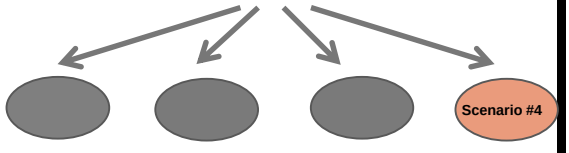
Steps to saline replacement…



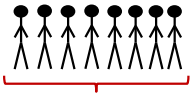
Steps to saline replacement…

Case #1

Front Type				Back Type				
Anti-A	Anti-B	Anti-D	Rh Control	A_1	A_2	B	O	Auto control
4+	0	4+	0	1+	Not tested	4+	Not tested	Not tested



Cells for back type: Pooled



Pooled!

Identifying the alloantibody

	Rh					Kell		Duffy		Kidd		MNS				Results		
	D	C	E	c	e	K	k	Fy^a	Fy^b	Jk^a	Jk^b	M	N	S	s	5' RT	LISS 37C	LISS IAT
1	+	+	0	0	+	0	+	+	+	+	+	+	+	+	+	1+	0	0
2	+	+	0	0	+	+	+	0	+	0	+	0	+	0	+	0	0	0
3	+	0	+	+	0	0	+	+	0	+	+	+	0	+	+	2+	1+	1+
4	+	0	0	+	+	0	+	0	0	+	0	+	+	0	+	1+	0	0
5	0	+	0	+	+	0	+	+	0	+	0	+	+	0	0	1+	0	0
6	0	0	+	+	+	0	+	0	+	+	+	0	+	0	+	0	0	0
7	0	0	0	+	+	+	+	0	+	+	0	+	0	+	+	2+	1+	1+
8	0	0	0	+	+	0	+	+	+	0	+	0	+	+	+	0	0	0
9	0	0	0	+	+	0	+	+	+	0	+	+	0	0	+	2+	1+	1+
10	+	+	0	0	+	0	+	+	0	+	+	+	+	+	0	1+	0	0
11	+	0	0	+	+	+	+	0	0	+	+	0	+	+	+	0	0	0
Auto																0	0	0

Scenario #4

Front Type				Back Type				
Anti-A	Anti-B	Anti-D	Rh Control	A_1	A_2	B	O	Auto control
4+	0	4+	0	1+	1+	4+	1+	0

Once the antibody is identified, resolve the typing discrepancy by…

- Prewarming the back type
- Using RBCs for your back type testing that don't express the corresponding antigen
  - For example, M-negative A_1 cells & M-negative B cells
  - Enzyme-treated cells

Review of the 4 scenarios:

Front Type				Back Type				
Anti-A	Anti-B	Anti-D	Rh Control	A_1	A_2	B	O	Auto control
4+	0	4+	0	1+	Not tested	4+	Not tested	Not tested

← Discrepant Results!

Review of the 4 scenarios:

Front Type				Back Type				
Anti-A	Anti-B	Anti-D	Rh Control	A_1	A_2	B	O	Auto control
4+	0	4+	0	1+	Not tested	4+	Not tested	Not tested
				1+	0	4+	0	0

← Discrepant Results!

Possible A subgroup with anti-A1
- Test patient cells with anti-A1 lectin.

Review of the 4 scenarios:

Front Type				Back Type				
Anti-A	Anti-B	Anti-D	Rh Control	A_1	A_2	B	O	Auto control
4+	0	4+	0	1+	Not tested	4+	Not tested	Not tested
				1+	0	4+	0	0
				1+	1+	4+	1+	1+

← Discrepant Results!

Possible rouleaux
Possible cold auto
- Examine microscopically
- Saline replacement if rouleaux
- Prewarm if cold auto

Review of the 4 scenarios:

Front Type				Back Type				
Anti-A	Anti-B	Anti-D	Rh Control	A_1	A_2	B	O	Auto control
4+	0	4+	0	1+	Not tested	4+	Not tested	Not tested
				1+	0	4+	0	0
				1+	1+	4+	1+	1+
				1+	1+	4+	1+	0

← Discrepant Results!

Cold-reacting alloantibody
- Identify the alloantibody
- Prewarm back type
- Use antigen negative cells to resolve

Case #2

Front Type				Back Type				
Anti-A	Anti-B	Anti-D	Rh Control	A_1	A_2	B	O	Auto control
0	0	4+	0	0	0	0	0	0

Ways to promote/strengthen reactivity of back type:

- Increase incubation time at 22C
- Decrease temperature*
- Increase plasma:cell ratio (use 4 drops of plasma & 1 drop of cells in each tube)

* Be careful: Many individuals have cold autoantibodies!

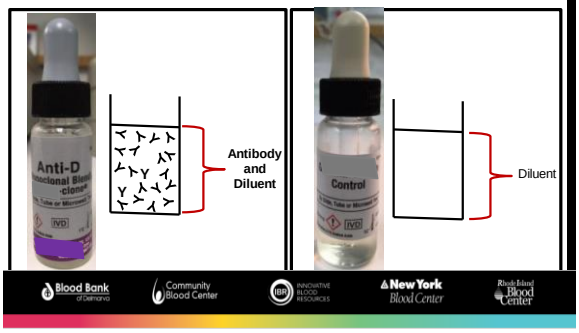
Case #2

Front Type				Back Type				
Anti-A	Anti-B	Anti-D	Rh Control	A_1	A_2	B	O	Auto control
0	0	4+	0	0	0	0	0	0
				2+	1+	2+	0	0

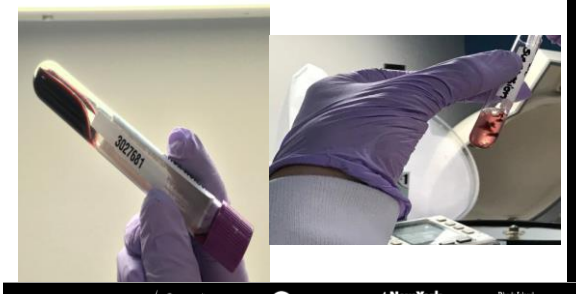
4 drops plasma:1 drop cells, 15 minute 22C

Patient blood type: O+

Wait… What is the Rh control?



Strong cold agglutinins



Case #3

Front Type				Back Type				
Anti-A	Anti-B	Anti-D	Rh Control	A_1	A_2	B	O	Auto control
4+	4+	4+	4+	4+	4+	4+	4+	4+
4+	0	4+	0	←Repeat testing following 4X warm wash				
Prewarming the back type →				0	0	2+	0	0

Patient blood type: A+

Cold adsorption:

Adsorb cold autoantibody at 4C, test adsorbed plasma in the back type

Mixed field

Case #4

Front Type				Back Type				
Anti-A	Anti-B	Anti-D	Rh Control	A_1	A_2	B	O	Auto control
4+mf	0	4+	0	0	0	3+	0	0

What is the best explanation for these results?

~~Patient is group O+ and received A+ blood~~

Patient is group A+ and received O+ blood

Patient is group A+ and received O- blood

Patient is group A+ and received A- blood

Case #4

Front Type				Back Type				
Anti-A	Anti-B	Anti-D	Rh Control	A_1	A_2	B	O	Auto control
4+mf	0	4+	0	0	0	3+	0	0

Looks like group A

Case #4

Front Type				Back Type				
Anti-A	Anti-B	Anti-D	Rh Control	A_1	A_2	B	O	Auto control
$4+^{mf}$	0	4+	0	0	0	3+	0	0

RBCs that express A antigen agglutinate

Patient cells

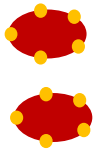
RBCs that don't express A antigen don't agglutinate

Donor cells

Case #4

Front Type				Back Type				
Anti-A	Anti-B	Anti-D	Rh Control	A_1	A_2	B	O	Auto control
$4+^{mf}$	0	4+	0	0	0	3+	0	0

All RBCs express D antigen



Patient cells

Donor cells

Case #4

Front Type				Back Type				
Anti-A	Anti-B	Anti-D	Rh Control	A_1	A_2	B	O	Auto control
$4+^{mf}$	0	4+	0	0	0	3+	0	0

Patient is group A+ and received O+ blood

Important things to know!!!

- ✓ Transfusion of non ABO-identical RBCs affects the **FRONT** type
- ✓ Transfusion does not usually interfere with the back type
- ✓ Use the mixed-field reactions to determine what type of blood patient received

Weak A subgroup

Front Type					Back Type				
Anti-A	Anti-B	Anti-A,B	Anti-D	Rh Control	A_1	A_2	B	O	Auto control
0	0	1+	3+	0	0	0	4+	0	0

Weak A subgroups:
- Examples: A_3, A_x, A_m
- Infrequently encountered

Resolving the discrepancy:
- Anti-A,B reagent
- **Genomic testing**
- Adsorption/elution studies (using polyclonal anti-A)

What do we mean by adsorption/elution?

Polyclonal anti-A

Weak A subgroup RBCs

What do we mean by adsorption/elution?

Polyclonal anti-A

Weak A subgroup RBCs

Test eluate against group A cells

Recent massive transfusion with group O RBCs

In cases of massive transfusion, it is possible to entirely replace patient blood volume with transfused cells

Front Type				Back Type				
Anti-A	Anti-B	Anti-D	Rh Control	A_1	A_2	B	O	Auto control
0	0	3+	0	0	0	4+	0	0

A sample taken shortly after may type group O in the front type (only donor cells detected)

Recent transfusion rarely affects the back type

- These results could indicate a group A patient (according to back type) who recently has been massively transfused with group O RBCs

Get patient history!!!

Hematopoietic Stem Cell/ Bone Marrow Transplant

Front Type				Back Type				
Anti-A	Anti-B	Anti-D	Rh Control	A_1	A_2	B	O	Auto control
0	0	3+	0	0	0	4+	0	0

Patient's blood type may change

- Example: Group A patient receives transplant from group O donor
- May receive patient sample during engraftment
  - Both donor and recipient cell populations detected
  - Mixed field results
- If transplant successfully engrafts
  - Patient will assume donor blood type
  - May not develop antibodies to antigens his/her cells formerly expressed

Review: Common ABO Discrepancies

Discrepancy		Cause	Resolution
Back type	Extra reactions	A subgroup with anti-A1	Test plasma with A_2 cells, test cells with anti-A1 lectin
		Rouleaux	Confirm rouleaux microscopically, saline replacement
		Cold autoantibody	Pre-warm back type
		Cold alloantibody	Identify the alloantibody, then pre-warm back type or use antigen negative cells for back type
	Missing reactions	Neonate	Back type not performed <4 months
		Immunosuppression	Incubate back type at room temperature, increase plasma:cell ratio, decrease temperature
Front type	Extra reactions	Cold agglutinin coating red cells	Wash cells with warm saline
	Missing reactions	Weak A or B subgroups	Test cells with anti-A,B, adsorption/elutions, genomic testing
	Mixed-field reactions	Multiple cell populations in sample due to recent transfusion/Bone marrow transplant	Acquire patient history
Special considerations		• Bone Marrow Transplant • Recent massive transfusion with group O RBCs	Acquire patient history

Review: ABO discrepancies

- Weak (≤ 2+) reactivity should be investigated
- Decide if missing or additional reactivity present
- Decide if problem is in front or back type
- Use the appropriate tool(s) to investigate
- If testing outside the parameters of the package insert, **RUN APPROPRIATE CONTROLS**

Objectives

- Identify and describe several causes of ABO discrepancies.
- List techniques used to resolve ABO discrepancies.
- Arrive at appropriate ABO interpretations based on laboratory results.

References

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