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Community Blood Center

Module 2: ABO System and Discrepancies

New York Blood Center · IBR Innovative Blood Resources · Rhode Island Blood Center · Blood Bank of Delmarva

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EXPANDING OUR ORGANIZATION TO MEET CLINICAL, CELLULAR AND TRANSFUSION PRODUCT AND SERVICE NEEDS FOR PATIENTS. NOW PROVIDING ALMOST ONE MILLION BLOOD PRODUCTS, OVER 450,000 LABORATORY AND MULTI-ASSAY INFECTIOUS DISEASE TESTS AND OVER 12,500 SPECIALTY CLINICAL PROCEDURES ANNUALLY TO HOSPITALS NATIONWIDE.

Comprehensive Cell Solutions · National Center for Blood Group Genomics · National Cord Blood Program

New York Blood Center · Community Blood Center · IBR Innovative Blood Resources · Rhode Island Blood Center · Blood Bank of Delmarva

Objectives

- Discuss characteristics of the ABO blood group system, including antigens, antibodies, and genetics. → **Introduction**
- Identify and describe several causes of ABO discrepancies, and list techniques used to resolve ABO discrepancies.
- Arrive at appropriate ABO interpretations based on laboratory results. → **ABO Case Studies**
- Discuss causes of RhD discrepancies, and describe testing used to arrive at appropriate RhD interpretations. → **RhD discussion & Cases**

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→ **ABO Case Studies**

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ABO: Why 1st Blood System Described?

Unique characteristics

- Landsteiner's rule: "naturally occurring antibodies to antigens missing from cells
- Complete agglutinins
- Antibodies reactive over wide thermal range
  - RT
- Antigen present in large numbers on cells

Detected without modern technology

- Centrifugation
- Antiglobulin reagents

Landsteiner Noble Prize 1933

http://www.virology.ws/2008/12/19/100th-anniversary-of-the-isolation-of-poliovirus/landsteiner2/

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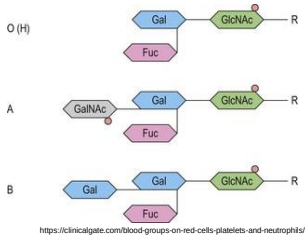
ABO Antigens: the basics

- Levels increase from birth to 3 years of age and then remain stable
- Expressed on RBC, platelets, T and B cells, most body tissues
- Soluble forms in plasma, saliva, most body fluids (depending on secretor status of individual)



https://clinicalgate.com/blood-groups-on-red-cells-platelets-and-neutrophils/

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ABO Antigen Density

Common blood groups A_1, A_2, B, O and AB

- ~ 10^6/cell = Easily detected by agglutination tests

Adult cells > cord

- ~50% fewer antigens on cord cells
- Cord lack branched sugars carrying ABH antigens
- Still easily detected with modern reagents

$A_1 > A_2$

- A_1 five times more antigens than A_2
- Antigens on A_2 cells still easily detected

Weaker subgroups of A or B (AB)

- Rare
- 700 – 50,000/cell
- May not be detectable in agglutination tests
  - Cause of ABO discrepancies
- Continuum from strong to weak expression

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ABO Antibodies

- Appear at 3-6 months; only 15% do not have detectable antibody by 6 months
  - Landsteiner noted "fetal blood contained no agglutinins"
- Titers peak at 5-10 years
- "Naturally occurring" due to environmental stimuli
- Most IgM (except anti-A,B in group O)

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ABO Genetics

- Over 200 alleles at ABO locus
- Handful convey common blood groups A_1, A_2, B, O, AB in most individuals
  - >99% group A are A_1 or A_2
- Most are rare and associated with weak or null phenotypes
  - Weak: A subgroups, B subgroups
  - Null: O

https://ijcnlp2008.org/dna-clipart.html

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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+

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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

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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

If transfusion is needed urgently, and there is an ABO discrepancy, must transfuse **group O RBCs, group AB plasma**

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

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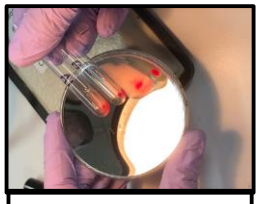
Case #1

Front Type



Back Type

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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 #1

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What's anti-A1 lectin?

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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.

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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.

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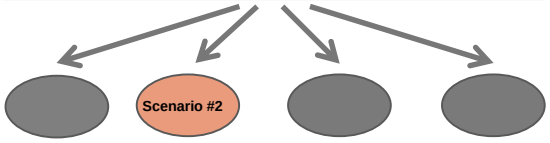
Case #1

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



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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	+	+	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

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COLD SCREEN

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

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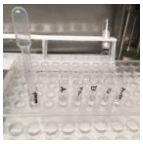
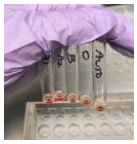
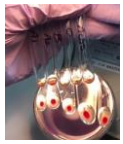
What is pre-warming?

- 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)

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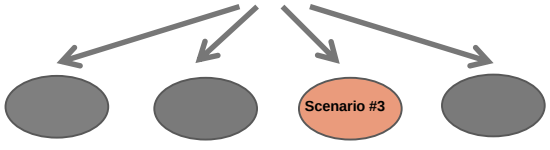
Case #1

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



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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.

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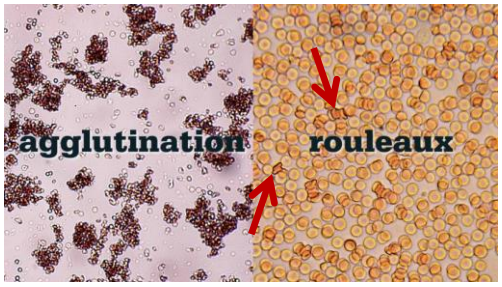
Can you see the difference?



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

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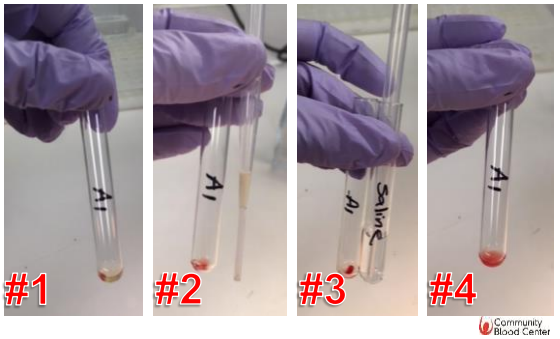
Steps to saline replacement…



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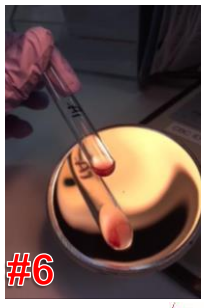
Steps to saline replacement…



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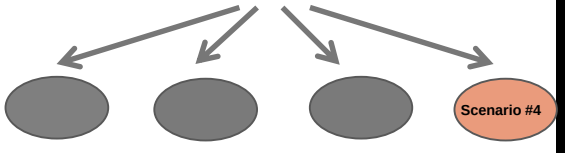
4+	0	4+	0	1+	Not tested	4+	Not tested	Not tested



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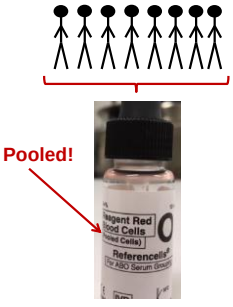
Cells for back type: Pooled



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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

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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!**

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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.

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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

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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

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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!

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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+

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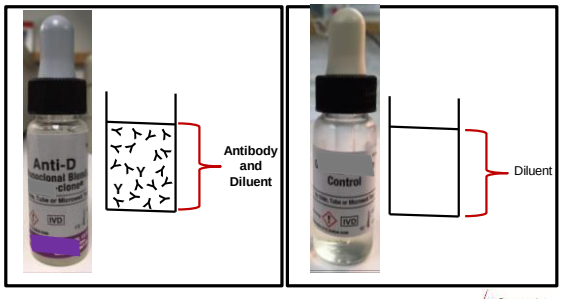
Wait… What is the Rh control?



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Strong cold agglutinins

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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

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Mixed field

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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

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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

ABO Discrepancies

With a partner, use the choices available in the boxes to explain and resolve the following ABO discrepancies. You may use any answer more than once or not at all.

Possible Explanations

Cold Autoantibody
Recent Transfusion
Cold reacting alloantibody
A subgroup with anti-A1
BMT (group A+ to O+)
Rouleaux
Immunosuppression

Ways to Resolve

Increase incubation time of back type
Prewarm back type
Inquire about patient history
No further testing required
Saline replacement of backtype
Warm wash front type
Decrease incubation temperature
Identify alloantibody
Test with anti-A1 lectin

	Front Type: Testing patient cells					Back Type: Testing patient plasma						
	Anti-A	Anti-B	Anti-A,B	Anti-D	Rh Cont.	A1 cells	A2 cells	B cells	O cells	Auto Cont.	Possible Explanation	Ways to Resolve
1	4+	0	4+	4+	0	1+	0	4+	0	0	•	•
2	0	4+	4+	4+	0	4+	3+	1+w	1+w	1+w	• •	• •
3	0	0	0	4+	0	0	0	3+	0	0	• •	• •

Objectives

- Discuss characteristics of the ABO blood group system, including antigens, antibodies, and genetics. → Introduction
- Identify and describe several causes of ABO discrepancies, and list techniques used to resolve ABO discrepancies.
- Arrive at appropriate ABO interpretations based on laboratory results. → ABO Case Studies
- Discuss causes of RhD discrepancies, and describe testing used to arrive at appropriate RhD interpretations. → RhD discussion & Cases

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RhD discrepancies

Causes:

- Incorrect sample/technical error
- Unusual RhD antigen expression
  - Weak D
  - Partial D
- Difference in antibody clones in different reagents
  - Antibodies to different epitopes of D antigen
- Different methodologies
  - Exampe: IS vs IAT
- Patient history
  - BMT? Transfusion?

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More on RhD discrepancies

Narrow specificity of monoclonal Rh reagents

- Single epitope vs entire antigen

Expected that cells with unusual antigens (RhD variants) will react differently with different monoclonal antisera

- Strong, weak or negative reactions
- Patients/donors may “change” Rh type if different reagent used

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RhD discrepancies: strategies

Patient race

- Different RhD variants are more prevalent in individuals in specific ethnic groups

Test with multiple reagents containing different anti-D clones and human polyclonal anti-D

- Known reaction pattern with some common partial Ds
- Run controls
- Record lot number and reagent ID (e.g., 114757 IM Series 4)
  - Human polyclonal anti-D
  - IM Series 4 and 5 anti-D
  - Gammaclone anti-D
  - Ortho Bioclone anti-D

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RhD discrepancies: strategies

Test with Partial RhD Typing Kit

- Panel of 12 monoclonal anti-Ds
- Classify 16 different partial or weak D depending on reactivity

http://quotient-merged.gethifi.com/us/products/pipeline-products/specialty-kits

Test for Cc, Ee, and low prevalence antigens, if applicable

- See *FactsBook*

Molecular testing

- Definitive way to accurately determine RhD status

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Case 1

Memorial Hospital reports a patient previously typed as Rh positive in 2002 now appears Rh negative

Questions

- Race of patient?
- History of BMT???
- What reagent/method used in 2002?
- What reagent/method used currently?

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Case 1

Memorial Hospital reports a patient previously typed as Rh positive in 2002 now appears Rh negative

Questions

- Race of patient? **African American**
- History of BMT??? **No**
- What reagent/method used in 2002? **Gammaclone anti-D/tube-immediate spin reading only**
- What reagent/method used currently? **Ortho gel**

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IRL Testing

Different Anti-D reagents					
	IM Gamma-clone IS/IAT	IM Series 4	IM Series 5	Ortho Bioclone	Human Poly-clonal IS/IAT
Patient	Pos/Neg	Neg	Neg	Neg	Neg/Neg
Pos cont	Pos	Pos	Pos	Pos	Pos
Neg cont	Neg	Neg	Neg	Neg	Neg

Ortho Gel not available

Other Rh Antigens				
	Anti-C	Anti-E	Anti-c	Anti-e
Patient cells	0	0	4+	4+

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Reactivity of FDA licensed Anti-D with Unusual (Variant) D Antigens

Reagent	IgM	IgG	Normal D Pos IS test	DVI IS/weak D	DBT IS/weak D	D^{Har} Whites IS/weak D	Crawford Blacks IS/weak D
Gammaclone	GAMA401	F8D8	Pos	Neg/Pos	Pos	Pos	Pos /Neg
Immucor Series 4	MS201	MS26	Pos	Neg/Pos	Pos	Pos	Neg
Immucor Series 5	Th 28	MS26	Pos	Neg/Pos	Pos	Pos	Neg
Ortho Bioclone	MAD2	Poly-clonal	Pos	Neg/Pos	Neg/Pos	Neg/Neg	Neg
Ortho Gel	MS201	NA	Pos	Neg	Pos	Pos	Neg
Polyclonal			Pos	Neg/Pos	Neg/Neg	Neg/Neg	Neg/Neg

Community Blood Center

Reactivity of FDA licensed A… with Unusual (Variant) D Antigen

Same pattern as our patient!

Reagent	IgM	IgG	Normal D Pos IS test	DVI IS/weak D	DBT IS/weak D	D^{Har} Whites IS/weak D	Crawford Blacks IS/weak D
Gammaclone	GAMA401	F8D8	Pos	Neg/Pos	Pos	Pos	Pos /Neg
Immucor Series 4	MS201	MS26	Pos	Neg/Pos	Pos	Pos	Neg
Immucor Series 5	Th 28	MS26	Pos	Neg/Pos	Pos	Pos	Neg
Ortho Bioclone	MAD2	Poly-clonal	Pos	Neg/Pos	Neg/Pos	Neg/Neg	Neg
Ortho Gel	MS201	NA	Pos	Neg	Pos	Pos	Neg
Polyclonal			Pos	Neg/Pos	Neg/Neg	Neg/Neg	Neg/Neg

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Case 1: Review

African American patient

Reactive with Gammaclone anti-D

- IgM clone strongly reactive at IS with low incidence Crawford, not D antigen, on cells
- IgG clone negative, i.e., weak D test neg

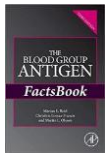
Nonreactive with Ortho Gel

Other reagents tested consistent with Crawford

Crawford seen with Ce/ce and ce/ce phenotypes

Consider Rh negative for transfusion

- Can make multiple Rh system antibodies – see *FactsBook*



https://www.amazon.com/Blood-Group-Antigen-FactsBook/dp/0124158498

We would confirm this with *RHD* genotype- use serology and molecular testing together!

Case 2

A donor's historical ABO/Rh is A negative from 3 donations in the 1990s. She was tested at her doctor's office and told she was Rh positive

Questions

- Race of Donor?
- History of BMT???
- What reagent/method used in 1990s?
- What reagent/method used currently?

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Case 2

A donor's historical ABO/Rh is A negative from 3 donations in the 1990s. She was tested at her doctor's office and told she was Rh positive

Questions

- Race of Donor? **Caucasian**
- History of BMT??? **No**
- What reagent/method used in 1990s? **Immucor human polyclonal anti-D/PK7300**
- What reagent/method used currently? **Ortho gel**

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IRL Testing

Different Anti-D Reagents					
	IM Gamma-clone	IM Series 4	IM Series 5	Ortho Bio-clone IS/IAT	Human Poly-clonal IS/IAT
Patient	Pos	Pos	Pos	Neg/Neg	Neg/Neg
Pos cont	Pos	Pos	Pos	Pos	Pos
Neg cont	Neg	Neg	Neg	Neg	Neg

Ortho Gel not available

Other Rh Antigens				
	Anti-C	Anti-E	Anti-c	Anti-e
Patient cells	0	0	4+	2+

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Reactivity of FDA licensed Anti-D with Unusual (Variant) D Antigens

Reagent	IgM	IgG	Normal D Pos IS test	DVI IS/weak D	DBT IS/weak D	D^{Har} Whites IS/weak D	Crawford Blacks IS/weak D
Gammaclone	GAMA401	F8D8	Pos	Neg/Pos	Pos	Pos	Pos /Neg
Immucor Series 4	MS201	MS26	Pos	Neg/Pos	Pos	Pos	Neg
Immucor Series 5	Th 28	MS26	Pos	Neg/Pos	Pos	Pos	Neg
Ortho Bioclone	MAD2	Poly-clonal	Pos	Neg/Pos	Neg/Pos	Neg/Neg	Neg
Ortho Gel	MS201	NA	Pos	Neg	Pos	Pos	Neg
Polyclonal			Pos	Neg/Pos	Neg/Neg	Neg/Neg	Neg/Neg

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Reactivity of FDA licensed [illegible] with Unusual (Variant) D Antigens

Same pattern as our patient!

Reagent	IgM	IgG	Normal D Pos IS test	DVI IS/weak D	DBT IS/weak D	D^{Har} Whites IS/weak D	Crawford Blacks IS/weak D
Gammaclone	GAMA401	F8D8	Pos	Neg/Pos	Pos	Pos	Pos /Neg
Immucor Series 4	MS201	MS26	Pos	Neg/Pos	Pos	Pos	Neg
Immucor Series 5	Th 28	MS26	Pos	Neg/Pos	Pos	Pos	Neg
Ortho Bioclone	MAD2	Poly-clonal	Pos	Neg/Pos	Neg/Pos	Neg/Neg	Neg
Ortho Gel	MS201	NA	Pos	Neg	Pos	Pos	Neg
Polyclonal			Pos	Neg/Pos	Neg/Neg	Neg/Neg	Neg/Neg

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Case 2: Partial D Typing Kit Results

Kit ID	Anti-D Cell Line	Weak D Type 1 and 2[a]	DII & DNU	DIII	DIV	DV[a]	DCS	DVI	DVII	DOL	DFR	DIIIH	DAR[2]	DAR-E	DHR[2] & DAU-4	DBT	Rh^{Hr}	Pos Cont.	Neg Cont.	Case 2
A	LHM7656	+	+	+	+	+/0	+	0	+	+	+	+	+	0	0	0	(+)0	3+	0	0
B	LHM7659	+	+	+	0	+	+	+	+	+	+	+	+	+	+	0	0	3+	0	0
C	LHM174?02	(+)0	+	+	0	0	+	0	+	0	0	+	0	0	0	0	0	3+	0	0
D	LHM502B	+	+	+	+	+	+	0	+	+	+	+	+	+	+	0	0	3+	0	0
E	LHM169/01	+	+	+	0	0	+	0	+	+	+	+	0	0	0	0	0	3+	0	0
F	ESD1	+	+	+	0	+	+	+	+	+	+	+	+	+	+	0	0	3+	0	0
G	LHM7655	+	+	+	0	+	+	+	+	+	+	+	+	+	+	0	0	3+	0	0
H	LHM7764	+	0	+	0	+	+	+	+	+	+	+	+	+/0	0	0	0	3+	0	0
I	LHM7645	(+)0	+	+	0	0	0	0	+	0	0	0	0	0	0	0	0	3+	0	0
J	LHM5919	+	+	+	+	+	+	0	0	0	0	(+)	0	(+)	+	+	0	3+	0	0
K	LHM169/00	+	+	+	+	+	+	0	+	+	+	+	+	0	0	0	0	3+	0	0
L	LHM571?7	+	+	+	+	+	0	0	+	+	0	+	+	0	0	+	0	3+	0	0

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Case 2: Review

Caucasian patient/donor

Testing consistent with RH33/D^{Har}

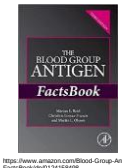
- Reactive with IM Gammaclone anti-D, Series 4, Series 5, Ortho Gel
- Nonreactive with Human polyclonal
- Partial D Typing Kit RhD^{Har}

RH33/D^{Har} seen with c+, weak e+; see *FactsBook*

Consider Rh negative for transfusion

Blood center follow-up:

- Former donor units labeled as RhD-negative

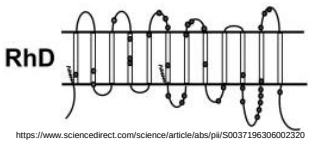


https://www.amazon.com/Blood-Group-Antigen-FactsBook/dp/0124158498

We would confirm this with *RHD* genotype- use serology and molecular testing together!

RhD discrepancies: take home message

- Most donor/patients carry common Rh genes, express normal antigen on cells and react well with all commercial anti-D reagents
- Cells from individuals with unusual genes, expressing unusual antigens will react differently with different monoclonal antisera
- Serologic testing and molecular testing used together to accurately determine RhD status



https://www.sciencedirect.com/science/article/abs/pii/S0037196306002320

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Objectives

- **Discuss characteristics of the ABO blood group system, including antigens, antibodies, and genetics.** → **Introduction**
- **Identify and describe several causes of ABO discrepancies, and list techniques used to resolve ABO discrepancies.**
- **Arrive at appropriate ABO interpretations based on laboratory results.**

→ **ABO Case Studies**

- **Discuss causes of RhD discrepancies, and describe testing used to arrive at appropriate RhD interpretations.** → **RhD discussion & Cases**

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References

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

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

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New York Blood Center

IBR INNOVATIVE BLOOD RESOURCES

Rhode Island Blood Center

Blood Bank of Delmarva

| SAVEALIFENOW.ORG