Tales from the Blood Bank *(Case Studies)*

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Objectives

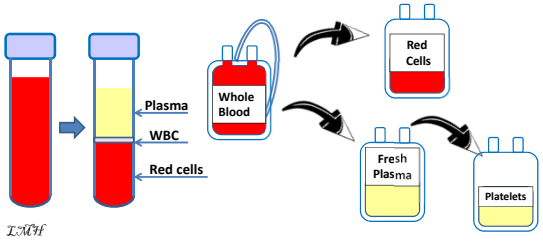
- Summarize the pretransfusion process
- Illustrate expected results of ABO/Rh typing and recognize discrepancies
- Interpret results of antibody screening tests and recognize unexpected results

Pretransfusion compatibility testing

- **Series of serologic and nonserologic procedures and processes designed to ensure best possible results of transfusion including but not limited to:**
  - Patient identification
  - Clerical checks and records checks
  - ABO testing and Rh grouping
  - Antibody screening
  - Crossmatch and blood selection

Blood Transfusion

- Injection of blood or blood components into the bloodstream after hemorrhage/loss during surgery or trauma to restore oxygen carrying capacity or provide clotting factors or platelets



Blood typing

- ABO system is the most important of all blood groups in transfusion practice

•Karl Landsteiner (1901) inadvertently performed the first forward and reverse grouping that is now done on all blood donors and patients

Importance of ABO typing

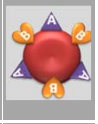
- ABO is the only blood group system in which an individual will predictably have potent antibody to any antigen
  - A N-acetylgalactosamine
  - B D-galactose
  - H L-fucose

  } Carbohydrate antigens
- ABO incompatible blood transfusion is the most common cause of life-threatening hemolytic transfusion reactions

Rh typing

- Presence or absence of the protein antigen- D on the RBC determines the Rh type
  - **Approx. 85% of general population is Rh positive**
  - Individuals who lack D *may form* anti-D if exposed to antigen by transfusion or pregnancy
    - D antigen may be weakly expressed and require additional testing at 37°C and AHG for detection (weak D testing) with an Rh control

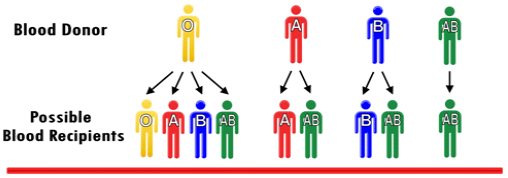
ABO types

	O	A	B	AB
Antigens				
Antibodies	Anti-A Anti-B	Anti-B	Anti-A	Ø

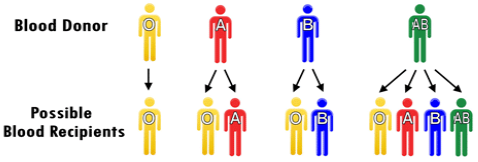
ABO Racial Bias

ABO Group	Whites	Blacks	American Indians	Asians
O	45	49	79	40
A	40	27	16	28
B	11	20	4	27
AB	4	4	<1	5

Red Blood Cell Transfusions



Platelet & Plasma Transfusions

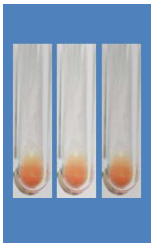


www.communityblood.org

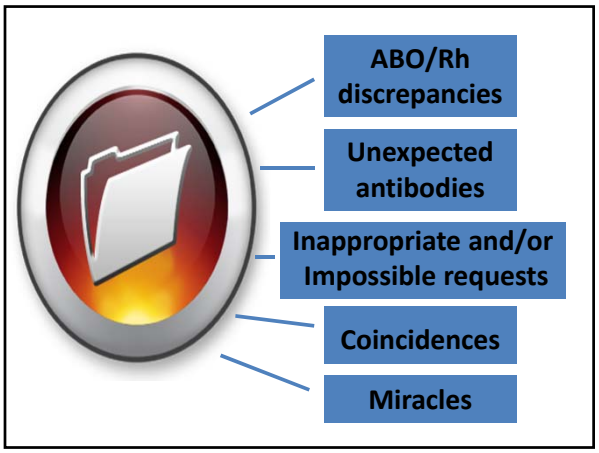
Known antisera to detect cell antigens

Known cells to test for serum antibodies

Antibody screens



No agglutination or hemolysis
NEGATIVE



ABO Discrepancies

- Weak or missing reactivity in cell grouping (antigens)
- Extra reactivity in cell grouping (antigens)
- Weak or missing reactivity in serum grouping (antibodies)
- Extra reactivity in serum grouping (antibodies)

All ABO discrepancies should be resolved before transfusion or until resolved give only type O red cells and AB plasma.

DISCREPANCIES IN ABO FORWARD AND REVERSE GROUPING

Missing or weak antigens
- Subgroup of A or B
- Suppression (A antigen may be suppressed in AB person)
- Pregnancy- decrease A transferase
- Disease change- leukemia, Hodgkin's disease

Extra antigen
- Rouleaux (unwashed cells)
- Acquired antigen – A or B

Missing or weak antibody
- Newborns
- Elderly persons
- Hypogammaglobulinemia
- Chimeras

Extra antibody
- Rouleaux
- "Unexpected" antibody reacting with A or B cells
- Anti-A1 in A subgroup individual

ABO Discrepancy Chart

Cause	Anti-A	Anti-B	A1 cells	B cells	O cells	Resolution
Massive transfusion	1+	0	Wk	4+	0	History, saliva inhibition
Leukemia	0-1+	0	0	1+	0	Inc. RT 30'
Acquired B	4+	1-2+	0	4+	0	Mod BS-1 lectin; Acidify anti-B
Rouleaux	4+	2+	2+	4+	2+ @ RT and 37C	Wash cells, saline replace
A2 w/ anti-A1	2+	0	1+	4+	0	Anti-A1 lectin and A2 cells
Unexpected RT antibody	4+	0	1+	4+	1+@ RT	Antibody ID
Newborns	3+	0	0	0		Reverse not performed
Elderly	3+	0	0	0	0	Age, RT for30'
Hypogamma-globulinemia	3+	0	0	0	0	Diagnosis; imm levels, inc.@ RT for 30 min

87 YO man in surgery, no transfusions

Cell typing?

Cell testing				Serum testing	
Anti-A	Anti-B	Anti-D	Rh control	A1 cells	B cells
					
			ND		

Additional cell testing required?

Serum typing?

Pertinent patient information

Probable cause of discrepancy?

Resolution step/s?

ABO Discrepancy Chart

Cause	Anti-A	Anti-B	A1 cells	B cells	O cells	Resolution
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Acquired B	4+	1-2+	0	4+	0	Mod BS-1 lectin; Acidify anti-B
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Newborns	3+	0	0	0		Reverse not performed
Elderly	3+	0	0	0	0	**Age, RT for 30'**
Hypogammaglo bulinemia	3+	0	0	0	0	Diagnosis; imm levels, inc.@ RT for 30 min

Resolution Testing

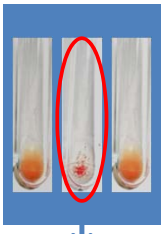
Cell testing		Serum testing 5-10" Rm Temp		Patient Info
Anti-A	Anti-B	A1 cells	B cells	
				87 YO Male No Transx
				

Is the discrepancy resolved?

What is the patient's true blood type?

Positive screens

Agglutination or hemolysis **POSITIVE**

Antibody Identification

Antibody identification panel

Case 1

A shipment of ten type "A positive" units of red blood cells was received from our blood supplier for stock. In accordance with AABB *Standards,* the receiving institution of red cell shipments is required to reconfirm the labeled ABO of each unit and the Rh type of all Rh-negative units.

A 3-5% suspension of red cells was prepared from a segment of each unit for cell (forward) typing with anti-A and anti-B.

Units	1	2	3	4	5	6	7	8	9	10
Anti-A	4+	4+	3+	4+	4+	**0**	4+	3+	4+	4+
Anti-B	0	0	0	0	0	**0**	0	0	0	0
Interp	A	A	A	A	A	**O**	A	A	A	A

Repeat testing- same results

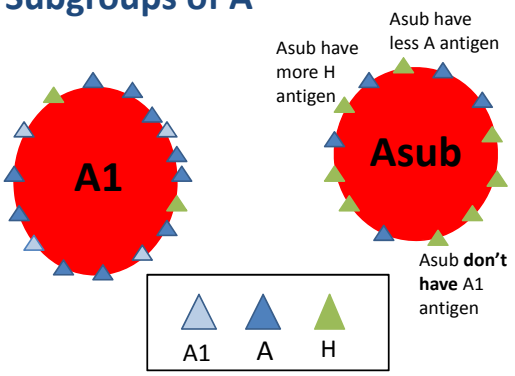
Unit #6 was pulled for additional testing.

Repeat testing of washed cell suspension and serum testing on segment with prolonged room temperature

Patient cells against known antisera			Patient serum against known cells	
Anti-A	Anti-B	Anti-A,B	A1 cells	B cells
				
0	0	0	0	4+

O **A**

Subgroups of A



Subgroups of A

- Two principle subgroups of A are A1 and A2
  - Serological distinction based on reactivity with Anti-A1 (Dolichos biflorus lectin or human Anti-A1)
  - **80%** of the A and AB population are A1 or A1B; the remaining 20% are A2 and A2B
- Difference between A1 and A2 is **quantitative and qualitative ; some subgroups form anti-A1**
- Weaker subgroups of A are > 1% of population

Characteristics of weak subgroups of A:

Use the following steps to help differentiate the subgroups of A:

1. Use A1-lectin to differentiate A_1 cells from others- agglutinates A1 only
2. Look for weaker or mixed field reactions
3. Look for anti-A_1 in serum (serum reacts with A_1 cells but not A_2 cells)
4. Look at strength of reactions with anti-A,B or with lectin-H

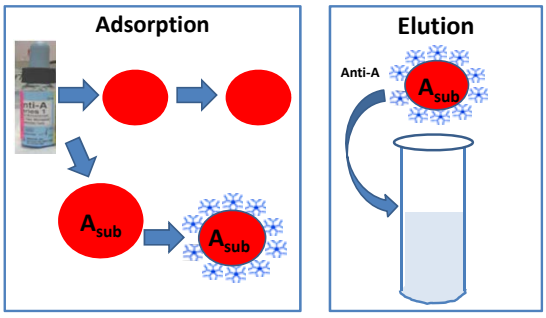
	Reagents				Antibodies in serum		
Phenotypes	**Anti-A**	**Anti-B**	**Anti-A,B**	**Anti-H**	**Anti-A**	**Anti-B**	**Anti-A1**
A3	++mf	0	++mf	3+	No	Yes	Sometimes
Ax	Wk/0	0	2+	4+	0/wk	Yes	Almost always
Aend	wk mf	0	Wk mf	4+	No	Yes	Sometimes
Am*	0/wk	0	0/+	4+	No	Yes	No
A_Y*	0	0	0	4+	No	Yes	No
Ael*	0	0	0	4+	Some	Yes	Yes

0 = negative; mf =mixed-field agglutination; wk =weak
*A specificity demonstrated only by absorption/elution procedures

Adapted from Harmening

Practical importance of weaker subgroups

- If a blood donor is mistyped as O, unit could cause transfusion reaction if given to an O recipient



Resolution

- Donor unit appeared to be from a possibly A_Y and was approporiately labeled by the
- Blood supplier uses alternate technology for standard donor unit typing (solid phase) and had noted no discrepancy
- Segments retained for the next victim…..

Case 2 A routine type and screen was submitted on a 46 year-old male who was scheduled for minor surgery. Records check showed no previous blood bank history.

Patient cells against known antisera			Patient serum against known cells	
Anti-A	**Anti-B**	**Anti-D**	**A1 cells**	**B cells**
4+	2+	0	0	4+
	AB		A	

ABO Discrepancy Chart

Cause	Anti-A	Anti-B	A1 cells	B cells	O cells	Resolution
Acquired B	4+	1-2+	0	4+	0	Mod BS-1 lectin; Acidify anti-B

•Acquired B phenomena caused modification of the blood group A sugar by bacterial enzymes
- N-acetyl-D-galactosamine into D-galactosamine
- •Pseudo-B antigen cross-reacts with anti-B antisera and disappears after recovery

Acidified anti-B did not react with the patient's cells.

Resolution

- Patient was blood type A negative and was exhibiting acquired-B phenomena due to septicemia (confirmed by patient chart review).
- Blood for transfusion should be that appropriate for type A individuals (A or O red cells, A or AB plasma).
- Subsequent testing on later specimens typed appropriately

Case 3

A specimen from a 19 year old AA male patient was submitted for Type and Screen with direct antiglobulin testing (DAT). The patient's suspected diagnosis was possible PNH (paroxymal nocturnal hemoglobinuria) but this was not proven.

Direct antiglobulin test		
Polyspecific coombs	Anti-IgG	Anti-C3
0	0	0

	Patient cells against known antisera			Patient serum against known cells	
	Anti-A	Anti-B	Anti-D	A1 cells	B cells
1	0	4+	4+	4+	0
2	0	4+	2+	4+	0
3	0	4+	wk	4+	0
4	0	4+	0*	4+	0

Weak D negative at AHG*

Resolution

- Patient demonstrated total loss of D antigen by conventional test methods. This loss was not due to transfusion with Rh negative blood or bone marrow transplant.
- All subsequent Rh typing remained negative.
- Literature review……

Many individuals with spontaneous Rh phenotype splitting or progressive Rh antigen loss were found to suffer from hematologic disease, such as acute or chronic myelogenous leukemia, myeloproliferative disease, or myelodysplastic syndrome, in the majority of cases without detectable cytogenetic abnormalities of chromosome 1. However, spontaneous appearance of D-negative RBCs was also observed in originally D-positive healthy subjects or patients with nonhematologic disease.

Case 4 Pooled batch testing of scavenged Chemistry specimens detected several common antibodies and *one* interesting find.

- 45 YO Caucasian female HMC patient /no blood bank history
  - Type O positive
  - Antibody screens and all panel cells tested 4+ positive at AHG but autocontrol was negative.

- Combination of multiple alloantibodies and/or antibody to high frequency antigen suspected
  - Reacted with all rare antigen neg cells
  - Patient cells tested pos for high frequency antigens LSUHSC had antisera for testing
  - Testing abandoned due to……

- Three days later, notified that a white female HMC patient being transferred from another facility for treatment.
  - Type ***O negative*** with ***antibody to the high frequency antigen, Vel***
  - Two frozen rare, O negative, Vel negative rbc units were stocked at the blood center for her possible transfusion
  - Our workup detected antibody reactive 4+ at AHG with all screens and negative autocontrol on this patient.

Resolution

- Serum A was tested against the cells of the transfer patient (B)
  - --- **NEGATIVE**
- Serum B tested against the cells of the chemistry patient (A)
  - --- **NEGATIVE**
  - Conclusion
    - **Both sera had anti-Vel**

Significance of anti-Vel

- Vel is a high frequency antigen variably expressed on rbcs of >99% of individuals in all populations
  - Vel negative RBCs found in 1 in 4000 people
    - Found in 1 in 1500 Norwegians and Swedes
    - Weakly expressed on cord cells
- IgM or IgG antibody associated with no to severe transfusion reactions and HDFN

Marion E. Reid, Christine Lomas-Francis. The Blood Group Antigen FactsBook, Second Edition (Factsbook). (Academic Press, 2003). Pages 503 - 504.

Obtaining rare units

- Blood supplier — No antigen negative units/donors
- Rare donor files — Limited units/ shipping delay
- Full siblings