

Introduction to the ABO Blood Group

Justin R. Rhees, M.S., MLS(ASCP)^{CM}, SBB^{CM}







Objectives

- Describe the biochemistry and production of the A, B, and H antigens.
- 2. Compare and contrast the subgroups of the A and B blood types.
- 3. Describe two lectins that can be used to aid in correct ABO typing.
- 4. Given the results of forward and reverse ABO typing, correctly interpret the patient's ABO group and identify patterns of discrepancy.

ABO Typing





Anti-B: 0

A antigen detected

ABO Typing





Anti-A: 0 Anti-B: 0

Neither A nor B antigens detected

ABO Typing





Anti-A: + Anti-B: +

A and B antigens detected

Anti-A	Anti-B	Туре
+	0	А

Anti-A	Anti-B	Туре
0	+	В

Anti-A	Anti-B	Туре
+	+	AB

Anti-A	Anti-B	Туре
0	0	0







Plasma or serum (Contain antibodies)

Do not form antibodies to A or B antigens

Erythrocytes (Express antigens)

Type AB



Forward Type

Anti-A against Pt. RBC	Anti-B against Pt. RBC		

Forward Type

Anti-A against Pt. RBC	Anti-B against Pt. RBC	Pt. Plasma against A RBC	Pt. Plasma against B RBC	

Forward Type

Anti-A against Pt. RBC	Anti-B against Pt. RBC	Pt. Plasma against A RBC	Pt. Plasma against B RBC	Interp.
+	0	0	+	А

Forward Type

Anti-A against Pt. RBC	Anti-B against Pt. RBC	Pt. Plasma against A RBC	Pt. Plasma against B RBC	Interp.
0	+	+	0	В

Forward Type

Anti-A against Pt. RBC	Anti-B against Pt. RBC	Pt. Plasma against A RBC	Pt. Plasma against B RBC	Interp.
+	+	0	0	AB

Forward Type

Anti-A against Pt. RBC	Anti-B against Pt. RBC	Pt. Plasma against A RBC	Pt. Plasma against B RBC	Interp.
0	0	+	+	Ο

ABORh



Basic ABO Biochemistry:

ABH antigen formation

- Mendelian
 - A and B are codominant
- Chromosome 9
 - Over 200 alleles have been identified at the ABO locus!
- O gene is an amorph
 - O/O inheritance produces O phenotype

Inheritance question

- My mother and father are both A positive
- My sister is O negative
- Possible?

	Α	Ο
А	A/A	A/O
0	A/O	0/0

Inheritance question

- My mother and father are both A positive
- My sister is O negative
- Possible?

	Α	0
А	A/A	A/O
0	A/O	0/0

	D	d
D	D/D	D/d
d	D/d	d/d

D=Rh antigen d=lack of Rh antigen

Basic ABO Biochemistry:

ABH antigen formation

- Glycosyltransferases: add sugars to a basic precursor substance.
- 37th day of fetal life.
- Neonate: 25-50% antigen sites on RBC
- How are these antigens formed?



H antigen







O/O genes?

Result: Lots of unmodified H antigens on the RBC



hh genotype

- The *H* gene is present in more than 99.99% of the population. (*HH* or *Hh*)
- The *hh* genotype is therefore extremely rare.
- Known as O_h or the "Bombay" phenotype, (*hh*) individuals may inherit ABO genes, but because the H antigen is not formed, no ABO expression can occur.

Genes: *h/h*, *A/B*



Neither A nor B antigens detected

O_h Bombay phenotype

- First reported by Bhende in 1952 in Bombay, India. Approx 130 cases worldwide have been reported.
- because of *hh* inheritance, ABO cannot be expressed.
- No reactions with anti-A, anti-B, or anti-H
- Bombay individuals produce anti-A, anti-B, anti-A,B, and anti-H. They ABO type as O, but cannot receive O blood. Why?
 - A: Type O has the highest amount of H. Transfusion of type O blood would cause an immediate hemolytic transfusion reaction.
 - O_h individuals should only receive O_h donor blood

Anti-H lectin

- A *lectin* is a protein that is capable of binding to a carbohydrate.
- A lectin with anti-H specificity can be derived from the seeds of the Ulex europaeus plant



Common gorse, Ulex europaeus Photo credit: Creative Commons https://commons.wikimedia.org/wiki/File:Ulex_europaeus_flowers.jpg

Anti-H lectin will agglutinate Group O cells, but not O_h (Bombay) cells

Early transfusion attempts



• 1667 Jean-Baptiste Denis transfused blood from a calf into "madman" Antoine Mauroy.

Image source: Wellcome Library Attribute: https://wellcomecollection.org/works/jj7nx247?query=blood+transfusion

Last half of 19th Century





J. H. Aveling 'Immediate Transfusion' Image Source: Wellcome Collection

Image Source: Science Museum, London Photo and Image Attributions: https://wellcomecollection.org/works/bndyugwh?query=blood+transfusion

• 1873, F. Gesellius estimated that 56% of transfusions ended in death

Karl Landsteiner



W. Frandsteiners

- Karl's serum agglutinates my cells.
- My serum does not agglutinate Karl's cells.
- What are the possible blood types?

Karl Landsteiner



W. Frandsteiners

- Karl's serum agglutinates my cells.
- My serum does not agglutinate Karl's cells.
- What are the possible blood types?

Karl is type O. I am type A, B, or AB Karl is type A or B. I am type AB

A Subgroups

• A subgroups:

 $-A_1 A_2 A_3 A_x A_{end} A_m A_y A_{el}$ etc.

- Approx. 80% of type A individuals are A₁
- Approx. 20% of type A individuals are A₂
- The remaining subgroups comprise 1%

A_1 and A_2

 Inheritance of an A₁ gene elicits production of *high concentrations* of

 $\alpha \text{-} 3 \text{-} \text{N-} acetylgalactosaminyltransferase}$

- Converts almost all of the H precursor structure to A₁ antigens.
- A₁ antigens are more highly branched than the "common A" structure shown previously
- A₂ type has fewer antigens per cell, only exist as "common A"

A_1 and A_2

- The immunodominant sugar on *both* A₁ and A₂ RBCs is *N*-acetyl-galactosamine; however, there are subtle antigenic differences which cause the body to discern self from non-self.
- A₁: 810,000 to 1,170,000 antigen sites
- A₂: 240,000 to 290,000 antigen sites

A_1 and A_2

- A₁ subgroup has both "common A" and A₁ antigens. Most of the H antigens have been converted.
- A₂ subgroup has only "common A" antigens.
 More unconverted H antigens.





Anti-A₁

- Because approximately 20% of type A individuals are A₂, we sometimes encounter anti-A₁ in transfusion medicine.
- Anti-A₁ is non-RBC Immune, IgM, and usually cold reacting. It is only considered clinically significant if it is reactive at 37° C.
- Anti-A₁ is produced by approx. 1-8% of A₂ individuals.

ABO Discrepancy

Forward Type		Туре	Revers	se Type
				_

Anti-A	Anti-B	A ₁ Cell	B Cell
+	0	+	+

Anti-A₁ Lectin

- A purified extract made from the seeds of the *Dolichos biflorus* plant agglutinate red blood cells with A₁ antigens present.
- Note: there is no anti-A₂ lectin. Why?

Blood Group	Antigen Present	Anti-A (Anti-A plus Anti-A ₁)	Anti-A ₁ Lectin
A ₁	A ₁ A	+	+
A ₂	Α	+	0

Weak subgroups of A

• As stated before, the prevalence of A subgroups of A weaker than A₁ and A₂ is less than 1%

Subgroup	Laboratory Results	Number of A antigenic sites
A ₃	Mixed field reaction with anti-A and most anti-A,B reagents	35,000 per RBC
A _x	Characteristically not agglutinated with anti-A but do agglutinate with most examples of anti-A,B	4000
A _{end}	Mixed field reaction with anti-A and anti-A,B. A_{end} is inherited as an allele at the ABO locus. Anti- A_1 is found in some sera. Only H is found in secretions.	3500
A _m	Characteristically not agglutinated, or very weakly agglutinated by all anti-A and anti-A,B reagents. Usually do NOT produce anti-A ₁ in sera.	200-1900

Weak subgroups of A

- Fewer antigen sites on the RBC means weaker reactions with antisera.
- It is possible for an A_x donor to be mistyped as
 O. This unit could then be transfused into an
 O recipient, who has anti-A,B. The anti-A,B antibody in the recipient could agglutinate and lyse the donor A_x RBCs and cause intravascular hemolysis.

Weak subgroups of B

- Subgroups of B are very rare and less frequent than A subgroups.
 - B, B₃, B_x, B_m, B_{el}, etc.

AB subgroups

• AB individuals can demonstrate subgroups of A, B or both

 $-A_1B$, A_2B , A_xB , A_1B_{el} , etc.

Reactivity of anti-H lectin

$O > A_2 > B > A_2B > A_1 > A_1B > O_h$ (Bombay)

Greatest amount of H

Least amount of H

ABO Discrepancies

- All ABO Discrepancies must be resolved prior to reporting a patient or donor ABO group.
- Why investigate these discrepancies?



Is there anything wrong with this picture?

Mixed Field (MF) Reaction



Control tubes

Patient tubes

Case Study



A technologist reads and reports a patient's blood type:

Anti-A	Anti-B	Anti-D	A1 Cell	B Cell
3+	3+	4+	0	0

Interpretation: AB Positive

Case Study



A technologist reads and reports a patient's blood type:

Anti-A	Anti-B	Anti-D	A1 Cell	B Cell
3+	3+	4+	0	0

Interpretation: AB Positive The sample is from an A positive patient undergoing a type B negative BMT. Lack of visible reverse type is due to immunosuppression. According to our protocols, the patient should be supported on irradiated, washed, O negative red cells.

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

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

Forward '	Type
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Anti-A	Anti-B	A1 Cell	B Cell
+	+	0	0

Forward	Type
---------	------

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

Forward Type

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

What do vampires put on their steak?



Answer: A₁

Image credit: Creative Commons

Attribute: https://commons.wikimedia.org/wiki/File:Little-vampire.svg

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