



Seeing Beyond Limits



IMMUCOR.

To Boldy Go . . .

A journey from the Immunohematology test tube to
DNA Testing and Beyond

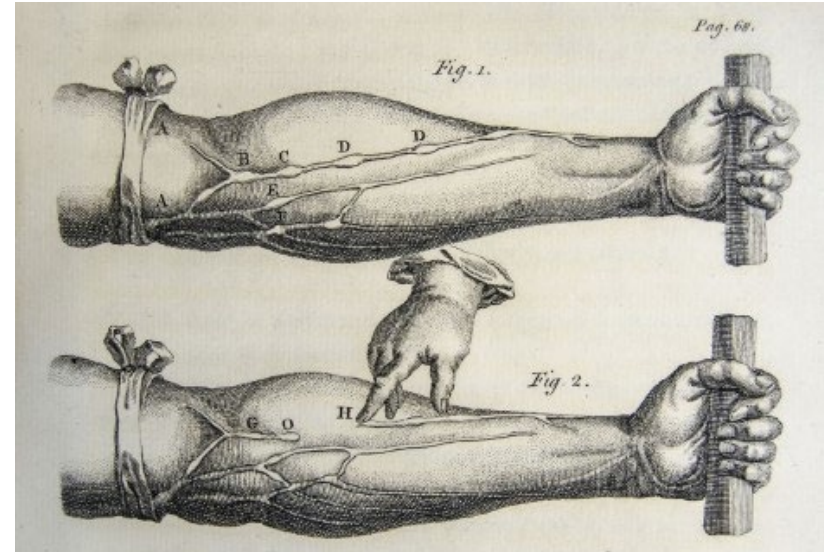
Objectives

- Examine and resolve a complex ABO Forward Type/Reverse Grouping Discrepancy
- Recall career opportunities in transfusion medicine today
- Discuss career opportunities ranging from hospital transfusion services to those in manufacturing, research and development and regulatory environments

Where We Have Been

1628

British physician William Harvey discovers the circulation of blood. The first known blood transfusion is attempted soon afterward.



Where We Have Been – cont'd

1818

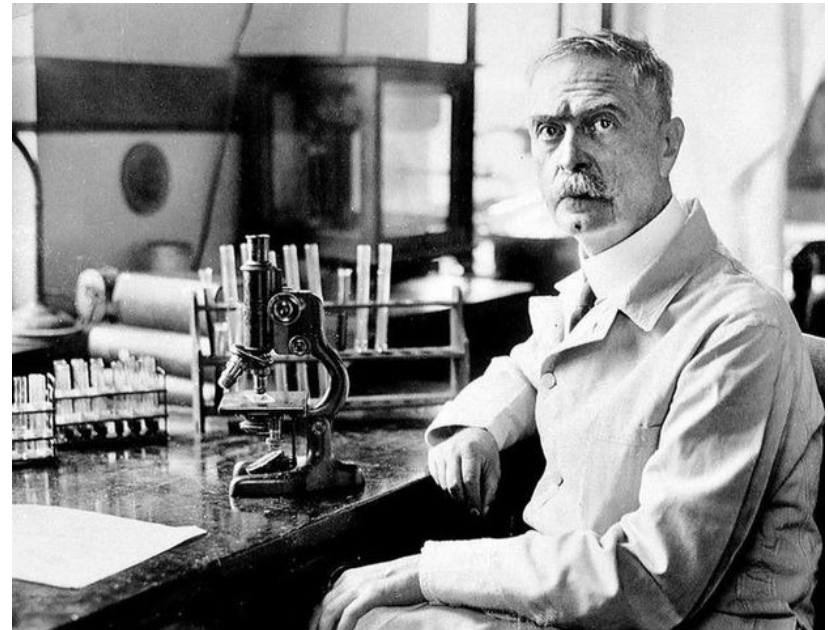
British obstetrician James Blundell performs the first successful transfusion of human blood to a patient for the treatment of postpartum hemorrhage.



Where We Have Been cont'd

1900

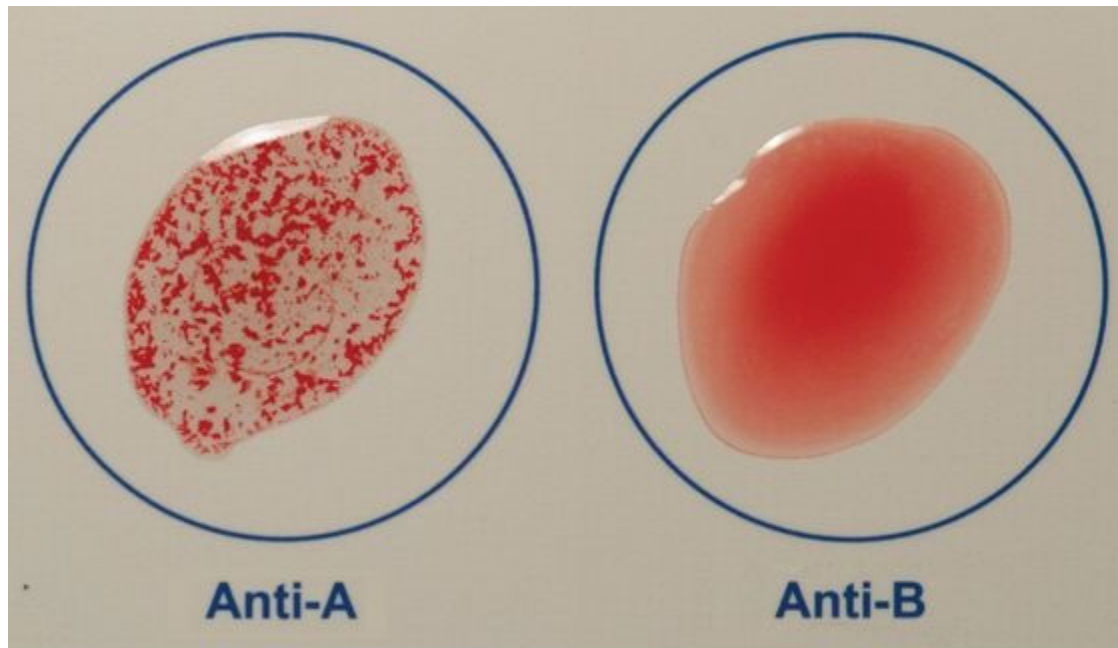
Karl Landsteiner, an Austrian physician, discovers the first three human blood groups, A, B, and C. Blood type C was later changed to O.



ABO Forward Typing & Reverse Grouping Discrepancy

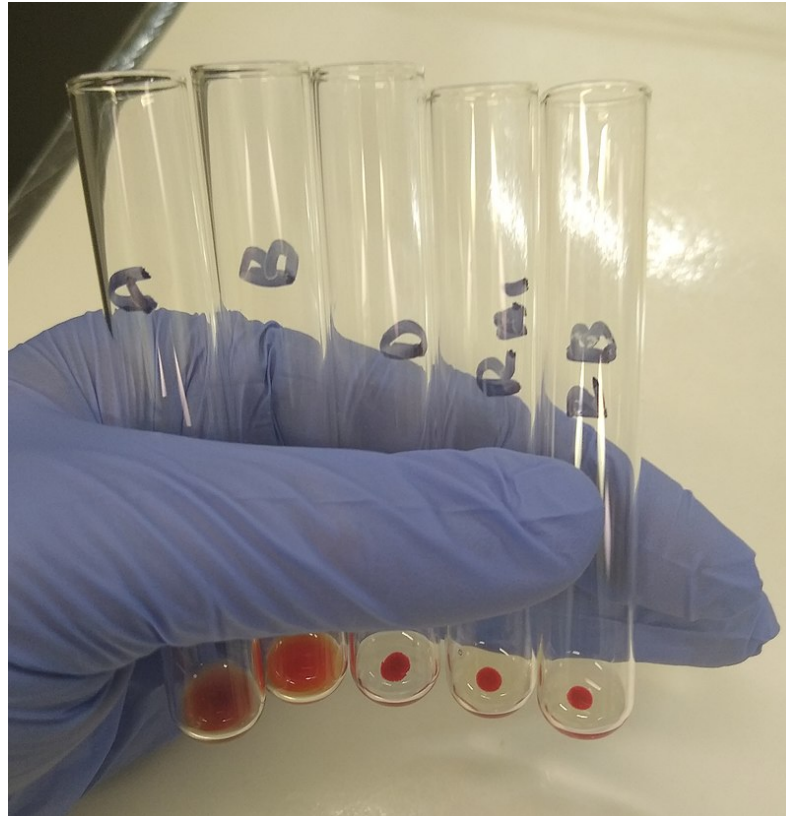


ABO Typing Options - Slide

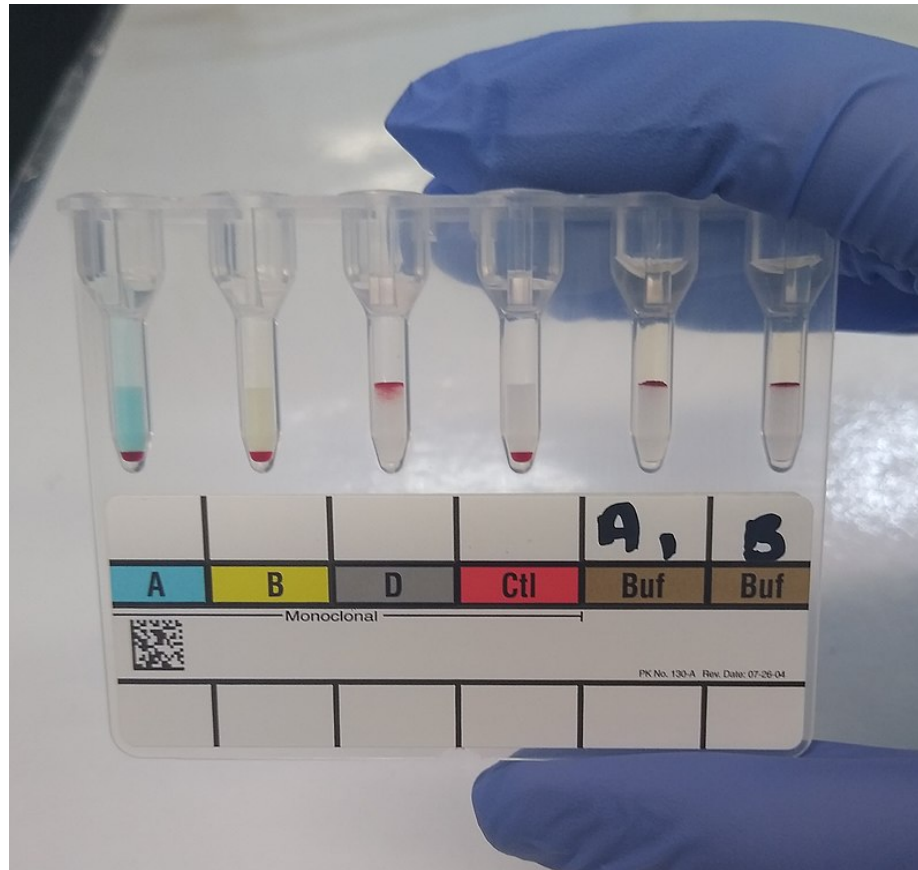


(b)

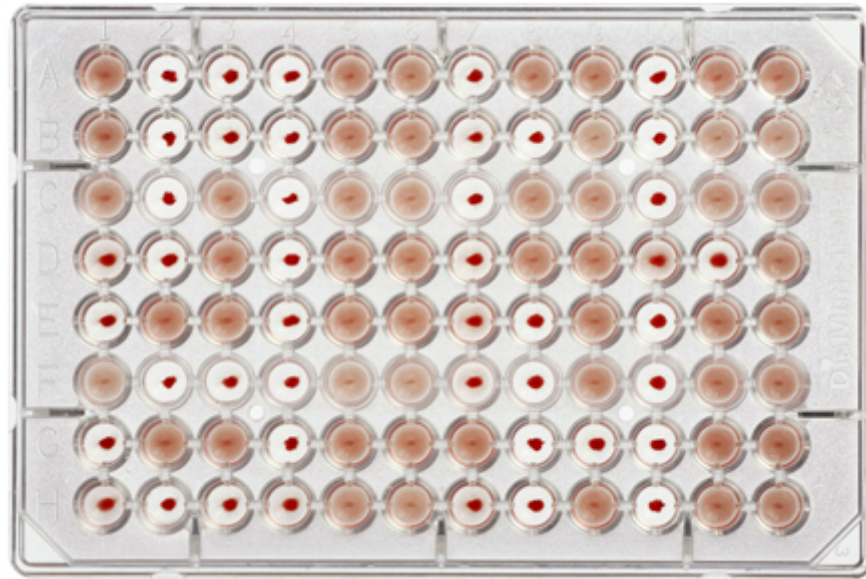
ABO Typing Options - Tube



ABO Typing Options – Gel Column



ABO Typing Options – Microwell



ABO Typing Reagents

- Anti-A
- Anti-B
- Anti-A,B
- Anti-A₁
Lectin/Monoclonal
- A₁ Cells
- B Cells
- O Cells
- A₂ Cells
- Group O Screening
Cells

Case Study

- Type and Screen ordered on a pregnant patient in the ER
- Patient fell off a ladder while washing pollen off of the windows of her house
- First pregnancy
 - 4 months into pregnancy with no complications
- No previous records
- Medications
 - Iron supplement only

Type and Screen Results

Anti-A	Anti-B	Anti-D	A ₁ Cells	B Cells
4+	0	3+	1+	3+

Interpretation:

ABO Type:

Rh Type:

Type and Screen Results

Anti-A	Anti-B	Anti-D	A ₁ Cells	B Cells
4+	0	3+	1+	3+

Interpretation:

ABO Type: ???

Rh Type:

Type and Screen Results

Anti-A	Anti-B	Anti-D	A ₁ Cells	B Cells
4+	0	3+	1+	3+

Interpretation:

ABO Type: ???

Rh Type: Positive

Antibody Screen Results – Tube Testing

Screening Cell	37/LISS	Anti-IgG	Checkcell	Interpretation
1	0	0	2+	Negative
2	0	0	2+	Negative
3	0	0	2+	Negative

0

No agglutination/No Hemolysis

1 – 4+

Agglutination

Interpretation: Negative

Problem

Anti-A	Anti-B	Anti-D	A ₁ Cells	B Cells
4+	0	3+	1+	3+

Sample forward types as a group A

Sample reverse groups as a group O

Observation:

The reaction against the A₁ Cells is 1+



The Mystery is Afoot



Next Steps

ACTION	REASON
Repeat the testing with the same sample	To exclude the possibility of a technical error during testing
Wash the RBCs and retest	Removes possible interference from suspended RBCs caused by abnormal concentrations of proteins or infused macromolecular solutions
Test for unexpected RBCs reacting at Room Temperature	Room Temperature antibodies may react with the reverse grouping

Repeat Testing with Patient's Washed RBCs

Anti-A	Anti-B	Anti-D	A ₁ Cells	B Cells
4+	0	3+	1+	3+

Now What? What about a subgroup of A?

1. Type patient's Cells with Anti-A₁ Lectin
2. Reverse group the patient's plasma against A₂ Cells

Anti-A	Anti-B	Anti-D	Anti-A ₁	A ₁ Cells	B Cells	A ₂ Cells
4+	0	3+	0	1+	3+	0

Patient is A₂

Have we solved our mystery? (Resolved the ABO Discrepancy?)



Is this patient an A_2 individual with an Anti- A_1 causing the reverse group discrepancy?

Have we followed all of the clues and ruled out the basic possibilities?

Have we solved our mystery? (Resolved the ABO Discrepancy?)



What about the AABB
guidance?

“Test for unexpected RBCs
reacting at Room
Temperature”

Review the Antibody Screen

Screening Cell	37/LISS	Anti-IgG	Checkcell	Interpretation
1	0	0	2+	Negative
2	0	0	2+	Negative
3	0	0	2+	Negative

Screening Cell	Immediate Spin
1	1+
2	2+
3	0

The Antigen List of the Antibody Screen

PANOSCREEN Master List

412-13

IMMUCOR, INC. Norcross, GA 30071 USA

US LICENSE NO: 886

LOT NO: 02416

EXPIRES: 2021/03/19

PANOSCREEN
VAL

PANOSCREEN

Donor			Rh - Hr		Kell					Duffy		Kidd		Lewis		P	MN			Luth-eran		Xg											
Barcode #	Add'l Typings	Rh Donor ID	D	C	c	E	e	C ^w	K	k	Kp ^a	Kp ^b	Js ^{a*}	Js ^b	Fy ^a	Fy ^b	Jk ^a	Jk ^b	Le ^a	Le ^b	P ₁	M	N	s	s	Lu ^a	Lu ^b	Xg ^{a*}					
I 198416		R1R1 B10123	+	+	0	0	+	0	0	+	0	+	0	+	0	0	+	0	+	0	+	+	0	+	0	+	0	+	+				
II 298416	Yt(b+)	R2R2 C4053	+	0	+	+	0	0	0	+	0	+	0	+	0	+	0	0	0	+	+	+	0	+	0	0	0	+	0				
III 398416		rr G1692	0	0	+	0	+	0	+	+	0	+	0	+	0	+	+	+	+	0	+	0	+	0	+	0	+	+					

* Indicates those antigens whose presence or absence may have been determined using only a single example of a specific antibody.
An antigen designated with a 'w' represents a weakened expression of the antigen that may or may not react with all examples of the corresponding antibody.



Immediate Spin Antibody Panel

Rh - Hr						Kell						Duffy		Kidd		Lewis		P	MN				Lutheran		Xg	PATIENT'S SERUM TEST RESULTS TEST METHODS			
D	C	c	E	e	C'	K	k	Kp ^a	Kp ^b	Js ^{a+}	Js ^b	Fy ^a	Fy ^b	Jk ^a	Jk ^b	Le ^a	Le ^b	P ₁	M	N	S	s	Lu ^a	Lu ^b	Xg ^{a+}				
+	+	0	0	+	0	0	+	0	+	0	+	+	+	0	+	0	+	+	+	0	+	0	+	+	+	1			
+	+	0	0	+	+	0	+	0	+	0	+	+	0	+	0	0	+	+	+	0	0	+	0	0	+	2			
+	0	+	+	0	0	0	+	0	+	0	+	+	+	+	0	0	+	+	0	+	0	+	0	0	0	3			
+	0	+	0	+	0	0	+	0	+	0	+	+	0	+	0	0	0	0	+	0	0	+	0	0	+	4			
0	+	0	0	+	0	0	+	0	+	0	+	+	+	+	0	0	+	+	0	+	0	+	0	0	+	5			
0	0	+	+	+	0	+	0	0	+	0	+	+	0	0	+	+	+	+	+	0	+	0	0	+	6				
0	0	+	0	+	0	+	+	0	+	0	+	0	+	0	+	0	+	+	+	0	+	0	0	0	+	7			
0	0	+	0	+	0	0	+	0	+	0	+	+	0	+	0	0	+	0	0	+	0	+	0	0	+	8			
0	0	+	0	+	0	0	+	0	+	0	+	0	+	+	0	+	0	+	0	+	0	+	0	0	0	9			
0	0	+	0	+	0	0	+	0	+	0	+	+	0	0	+	0	+	+	0	+	+	+	0	0	+	10			
+	0	+	0	+	0	0	+	0	+	0	+	0	0	+	+	0	0	+	0	+	0	0	0	0	+	TC			

Appears to be Anti-M showing dosage

Does the Antibody Screen Demonstrate an Anti-M?

PANOSCREEN Master List			412-13																																		
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PANOSCREEN VIAL	Donor		Rh - Hr				Kell						Duffy		Kidd		Lewis		P	MN			Lutheran		Xg												
	Barcode #	Add'l Typings	Rh	Donor ID	D	C	c	E	e	C ⁺	K	k	Kp ^a	Kp ^b	Js ^a *	Js ^b	Fy ^a	Fy ^b	Jk ^a	Jk ^b	Le ^a	Le ^b	P ₁	M	N	S	s	Lu ^a	Lu ^b	Xg ^a *							
I	198416		R1R1	B10123	+	+	0	0	+	0	0	+	0	+	0	+	+	0	0	+	0	+	0	+	+	+	0	+	0	+	0	+	+				
II	298416	Yt(b+)	R2R2	C4053	+	0	+	+	0	0	0	+	0	+	0	+	0	+	+	0	0	+	+	0	+	0	+	0	0	+	0	+	0				
III	398416		rr	G1692	0	0	+	0	+	0	+	+	0	+	0	+	0	+	+	+	+	+	0	+	0	+	0	+	0	+	0	+	+				

* Indicates those antigens whose presence or absence may have been determined using only a single example of a specific antibody.
An antigen designated with a 'w' represents a weakened expression of the antigen that may or may not react with all examples of the corresponding antibody.

Screening Cell	Immediate Spin
1	1+
2	2+
3	0



Is the reactivity on the A₁ Cells due to Anti-A₁ or Anti-M?

Anti-A	Anti-B	Anti-D	Anti-A ₁	A ₁ Cells	B Cells	A ₂ Cells
4+	0	3+	0	1+	3+	0



Additional Testing

Cell Tested	Anti-M	Interpretation
Patient Cells	0	M negative
A1 Cells (Reverse Group)	4+	M positive
A2 Cells (Reverse Group)	0	M negative
B Cells (Reverse Group)	0	M negative

Anti-A	Anti-B	Anti-D	Anti-A ₁	A ₁ Cells	B Cells	A ₂ Cells
4+	0	3+	0	1+	3+	0

Test More A₁ Positive Cells with Anti-M

Cell ID	Anti-M	Interpretation
555	0	M negative
777	0	M negative
999	0	M negative

Test Patient Plasma Against M Negative A₁ Positive Cells

Cell ID	Anti-M	Interpretation	Patient Plasma
555	0	M negative	0
777	0	M negative	0
999	0	M negative	0

Anti-A	Anti-B	Anti-D	A ₁ Cells*	B Cells
4+	0	3+	0	3+

* M negative Cells

Interpretation: Patient is an A Positive
Screen: Anti-M reactive at Room Temperature Only

Sherlock says, “Elementary, dear friend, elementary”

Skills you used:

- Critical Thinking
- Investigative Reasoning
- Problem Solving
- Logic
- An many more . . .



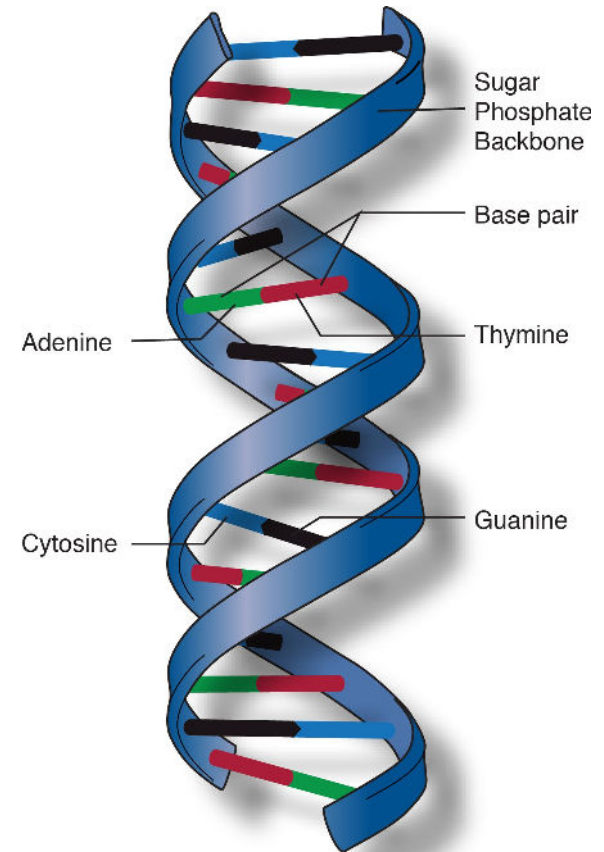
DNA and Transfusion Medicine



Phenotype

Observable expression of a gene

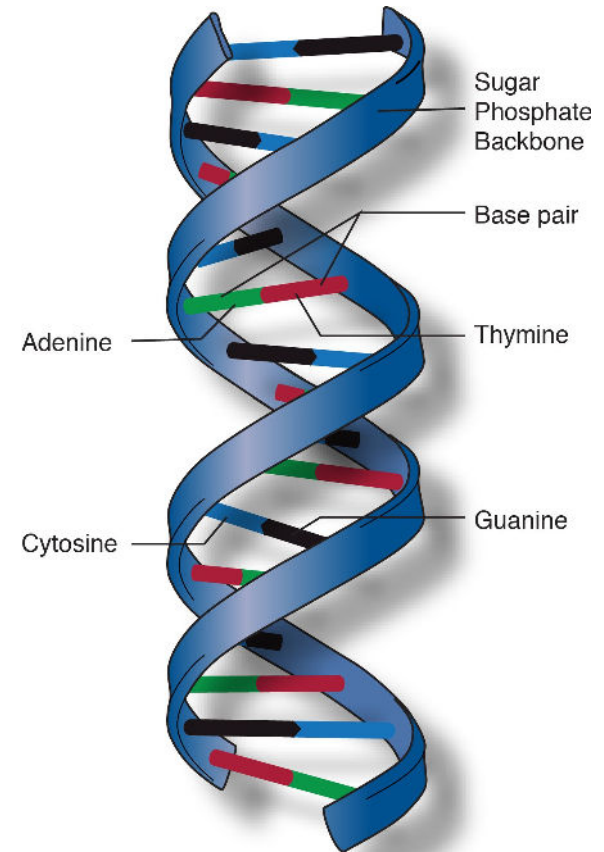
Presence or absence of antigens on RBCs as determined by serologic testing represents a person's phenotype



Genotype

The set of genes inherited from a person's parents

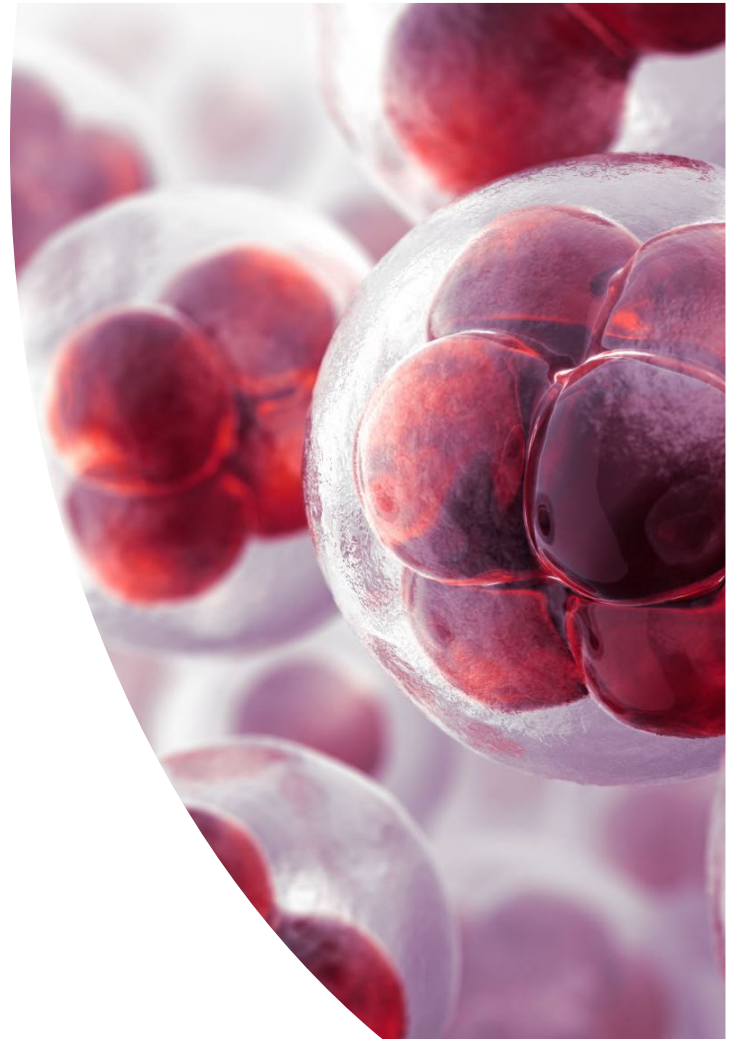
Presence or absence of antigens on RBCs predicted by DNA-based testing



Phenotype vs Genotypes of Blood Groups

A person may phenotypically “type” as a group A individual. This would be their phenotype.

However, that A individual may genetically be a Group AA or a Group AO where the O blood group antigen is not detected by serological methods. This is an example of a genotype.

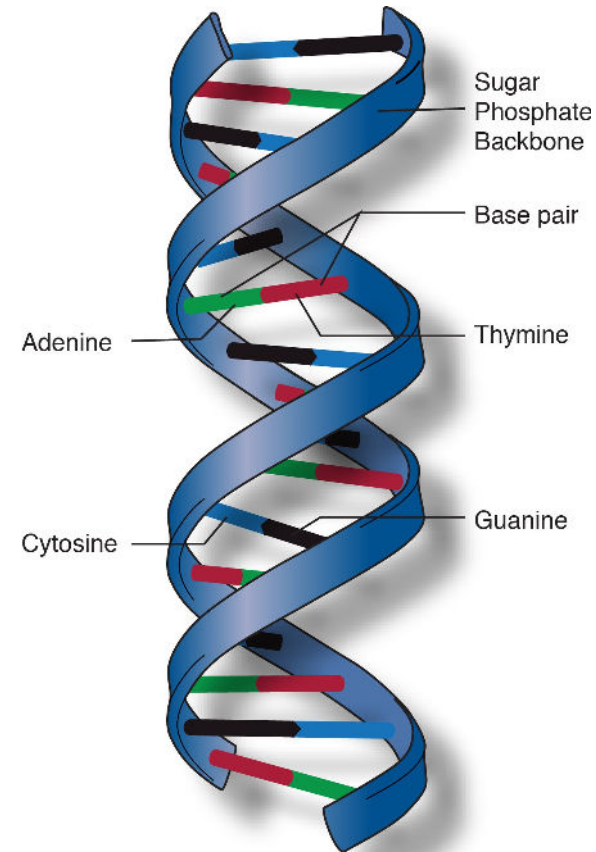


Molecular Methods for Predicting RBC Antigen Phenotypes

- Genomic DNA is isolated from any nucleated cell source
- Most DNA-based assays involve amplification of a target gene sequence through PCR
- PreciseType™
 - BioArray/Immucor
 - FDA Approval 21MAY2014
- ID CORE XT™™
 - Progenika/Grifols
 - FDA Approval 11OCT2018

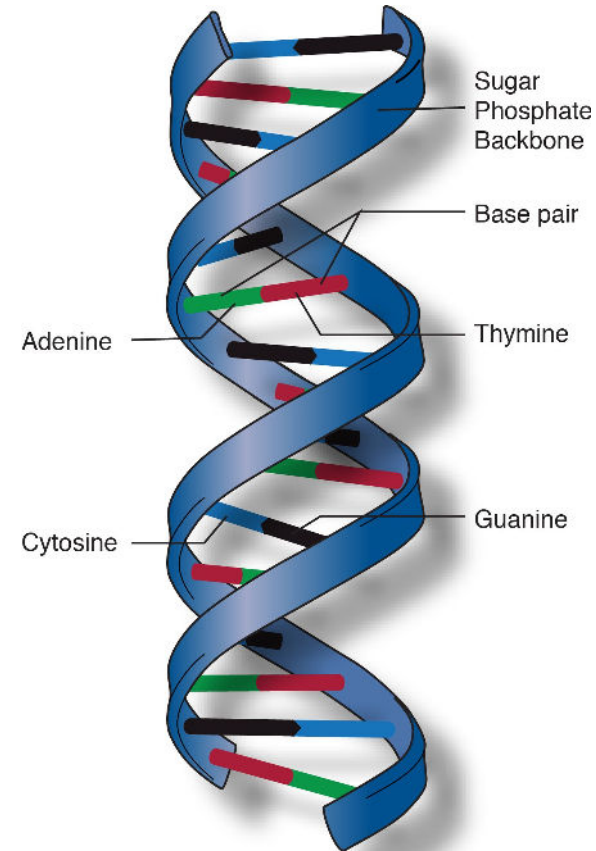
Clinical Application of DNA-Based Molecular Testing

- Predict RBC phenotypes of a fetus or a transfusion recipient whose RBCs are coated with IgG
- Reliable Blood Group Determination in Recently Transfused Patients



Clinical Application of DNA-Based Molecular Testing - Continued

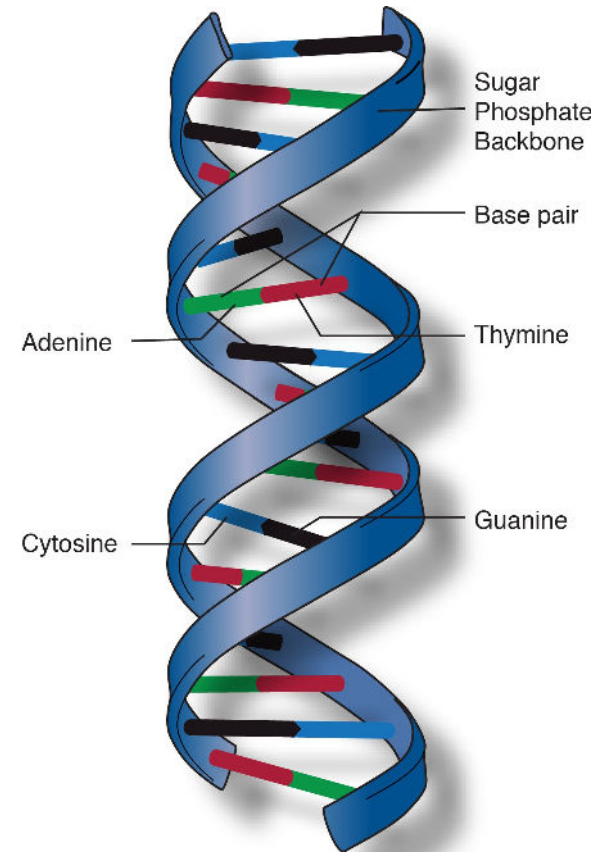
- Aids in differentiation between alloantibodies and autoantibodies
- Predicts phenotypes in patients where monoclonal antibodies are used as therapeutic treatments



Additional Applications of Molecular Testing in Transfusion Medicine

Research Use Only
(RUO)

- Platelet Genotypes
- Rh Variants
- ABO subgroups and variants



Career Opportunities in Transfusion Medicine



Beyond the Test Tube Today . . .

- Transfusion Service Technologists
- Transfusion Service Managers
- Quality Improvement Managers
- Blood Bank Information Systems (Computer Systems)
- Donor Center Blood Collection and Processing
- Immunohematology Reference Lab Technologists

Beyond the Test Tube Today . . .

- Instrumentation
 - Viral Testing in Donor Centers
 - Automated Blood Analyzers – Field Service Engineers and Application Specialists
 - Immucor
 - Ortho
 - Grifols
- Microbiology
 - Sterility testing for Blood Bank Manufacturing for reagents and test kits
 - Cultures of Room Temperature Platelet Products



Beyond the Test Tube Today . . .

Molecular Testing

- PCR Testing
- DNA Extraction
- Assay Development
- Research and Development Opportunities

Technologist in Molecular Biology (MB) by
ASCP

Beyond the Test Tube Today . . .

Regulatory Environments



Clinical
Laboratory
Improvements
Amedments



Seeing Beyond Limits



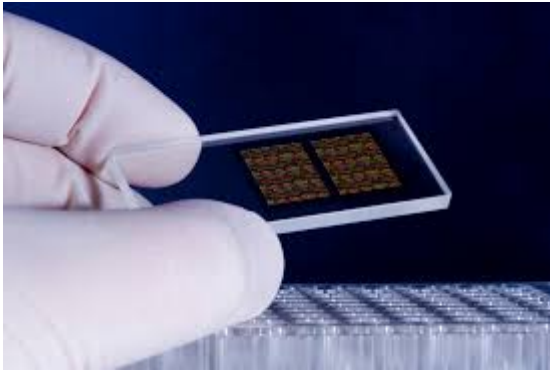
Beyond the Test Tube - Tomorrow

The skills you just demonstrated while resolving our ABO blood typing discrepancy will be the ones that you will use as you head toward the future:

- Critical Thinking
- Investigative Reasoning
- Problem Solving
- Logic

Beyond the Test Tube - Tomorrow

Microarrays



- Blood grouping
- Extended phenotyping
- Antibody detection
- Exclusion of other clinically significant antibodies
- Donor disease screening

Beyond the Test Tube - Tomorrow

The potential, your potential, is not bound to a test tube. You have the tools necessary to take us to the next steps in Immunohematology!



References

- AABB Technical Manual, 20th edition
- Standards for Blood Banks and Transfusion Services, 32nd edition