Introduction to the Rhesus Blood Group

Justin R. Rhees, M.S., MLS(ASCP)^{CM}, SBB^{CM}
University of Utah Department of Pathology
Medical Laboratory Science Program

The University of Utah
Department of Pathology

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Objectives

1. Describe the major Rhesus (Rh) blood group antigens in terms of biochemical structure and inheritance.
2. Describe the characteristics of Rh antibodies.
3. Translate the five major Rh antigens, genotypes, and haplotypes from Fisher-Race to Wiener nomenclature.
4. State the purpose of Fisher-Race, Wiener, Rosenfield, and ISBT nomenclatures.

Background

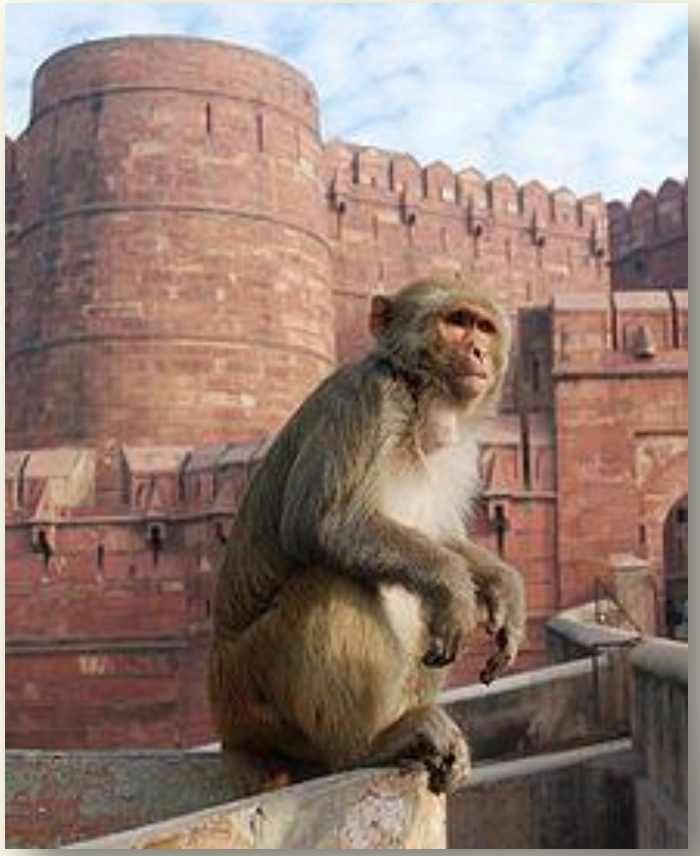
- How did this blood group get its name?
- 1937 Mrs. Seno; Bellevue hospital
- Unknown antibody, unrelated to ABO
- Philip Levine tested her serum against 54 ABO-compatible blood samples: only 13 were compatible.

Rhesus (Rh) blood group

1930s several cases of Hemolytic of the Fetus and Newborn (HDFN) published.

Hemolytic transfusion reactions (HTR) were observed in ABO-compatible transfusions.

In search of more blood groups, Landsteiner and Wiener immunized rabbits with the blood of the Rhesus monkeys.



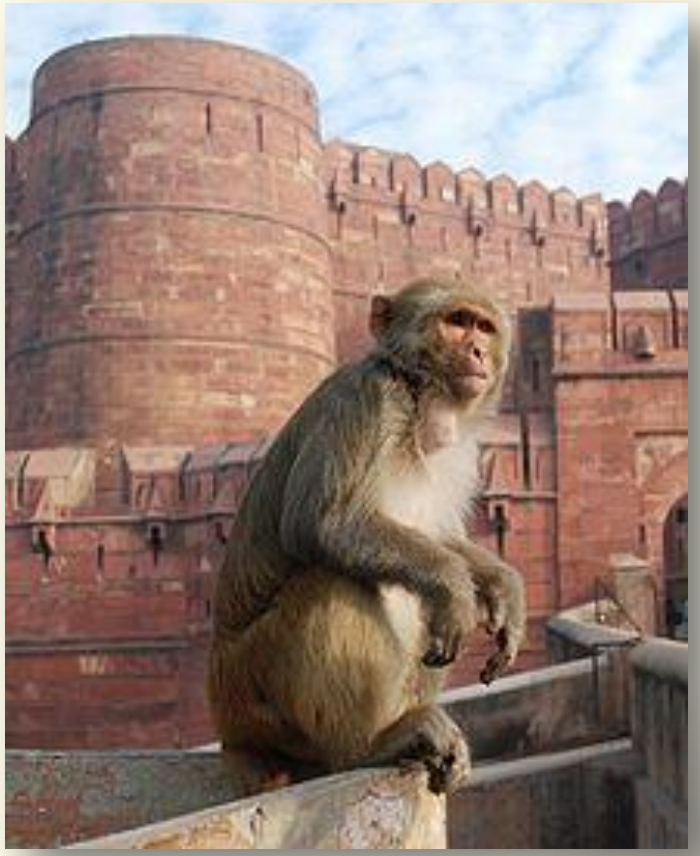
Rhesus macaque

Rhesus (Rh) blood group

1940 Landsteiner and Wiener reported an antibody that reacted with about 85% of human red cell samples.

It was supposed that anti-Rh was the specificity causing the “intragroup” incompatibilities observed.

1941 Levine found in over 90% of *erythroblastosis fetalis* cases, the mother was Rh-negative and the father was Rh-positive.



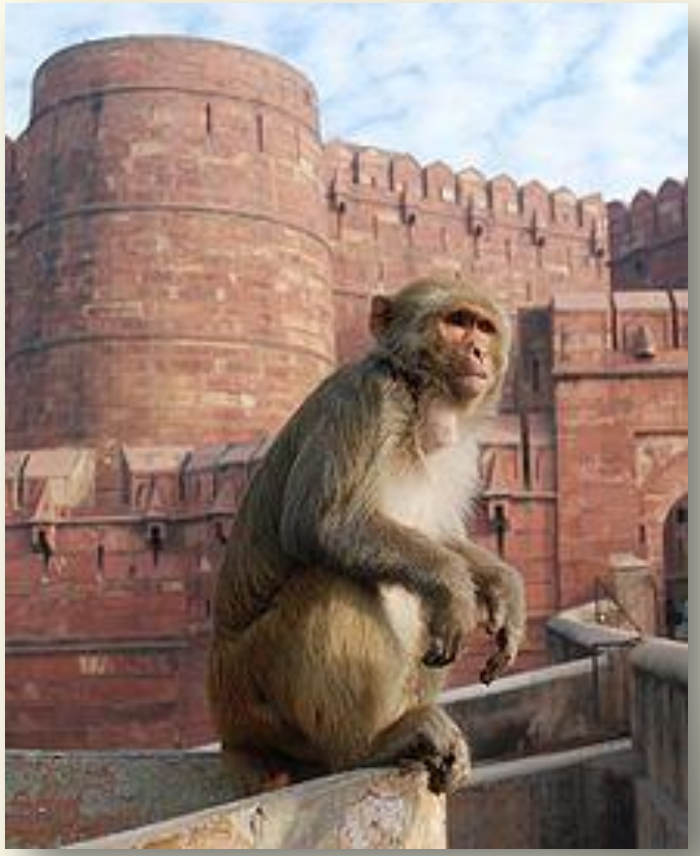
Rhesus macaque

Rhesus (Rh) blood group

Human anti-Rh and animal anti-Rh are not the same.

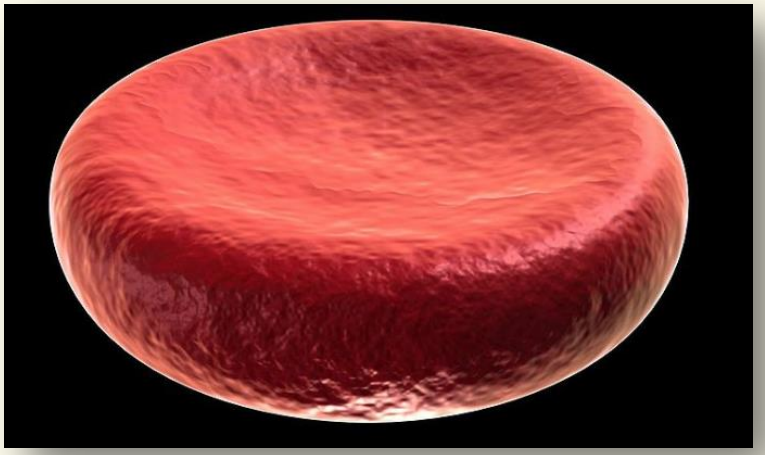
However, “Rh” was embedded into blood group antigen terminology.

The animal anti-Rh antibody was renamed “anti-LW” for Landsteiner and Wiener.



Rhesus macaque

Rh antigens



Rh proteins:

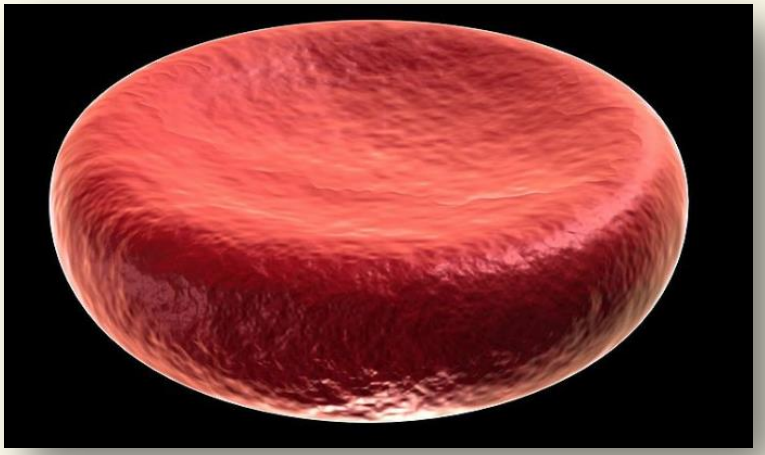
Non-glycosylated

Transmembrane

Maintain structural integrity of RBC membrane

May have a role in ammonia or CO_2 transport

Rh antigens



Rh blood group:

Over 50 known antigens

Highly polymorphic

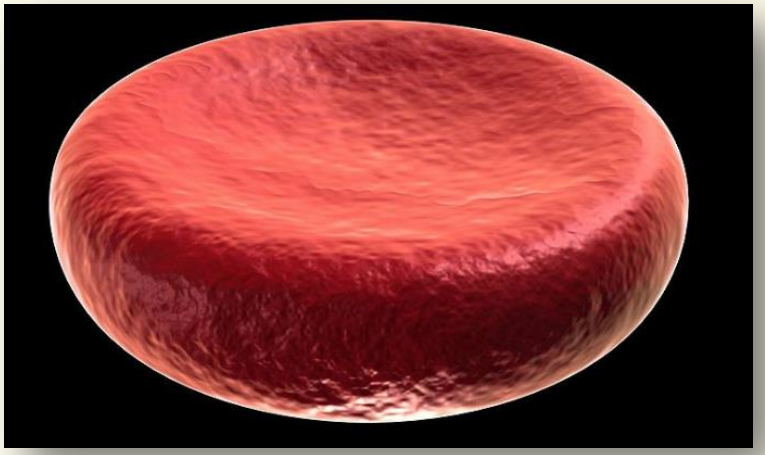
D, C, c, E, e most important

RHD gene codes for presence or absence of D polypeptides

RHCE gene codes for Ce, cE, ce or CE polypeptides

RHAG gene produces an Rh-associated glycoprotein and serves as a coexpressor

Rh antigens



D antigen is highly immunogenic

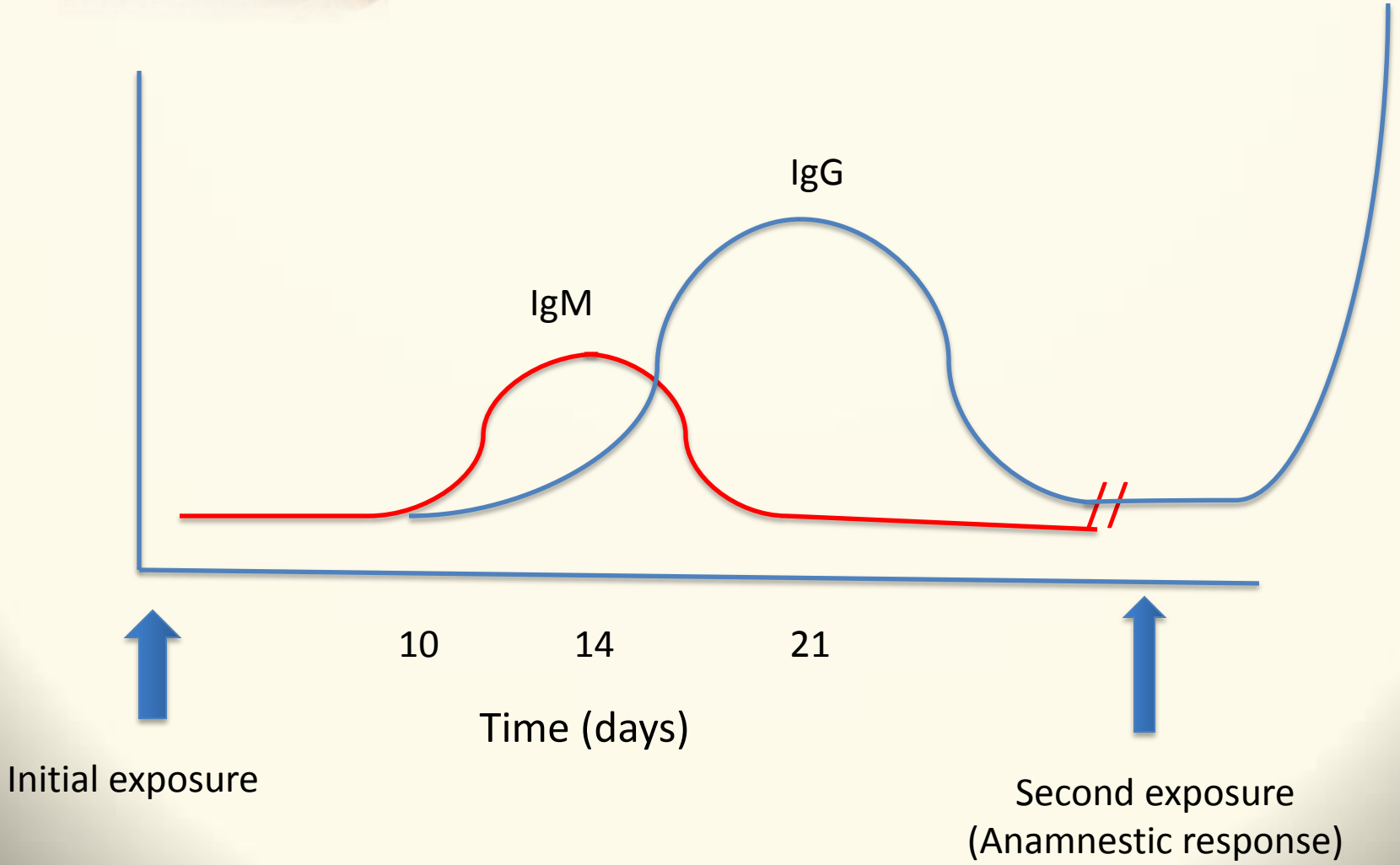
0.1 mL D+ blood

Immunogenicity:
D > c > E > C > e

Anti-D and anti-E most commonly encountered

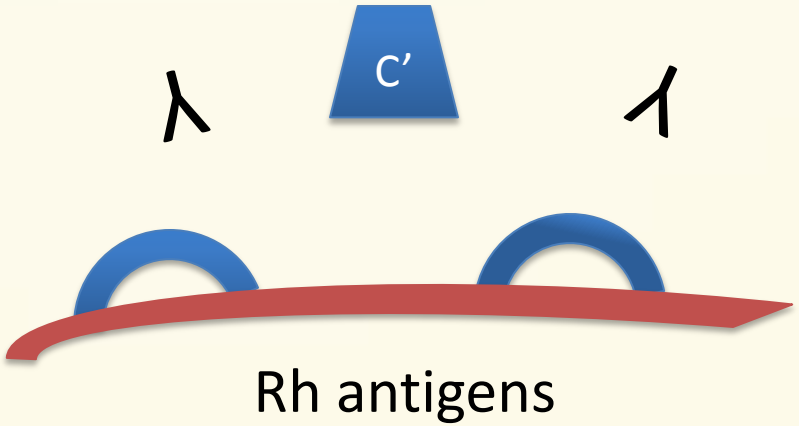
Antigen	Frequency
D	85%
c	80%
C	70%
e	98%
E	30%

Rh antibodies



Rh antibodies

- ✓ Clinically significant
- ✓ Compatibility
- ✓ Complement mediated intravascular hemolysis does not occur



Antibody Characteristics

Antigen	Optimal Temp	IgG	IgM	HTR	HDFN	Dosage	Enzyme
D	37						
C	37						
E	37						
c	37						
e	37						
ce/f	37						
Cw	37						
G	37						
V	37						
VS	37						

Antibody Characteristics

Antigen	Optimal Temp	IgG	IgM	HTR	HDFN	Dosage	Enzyme
D	37	Yes					
C	37	Yes					
E	37	Yes					
c	37	Yes					
e	37	Yes					
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Cw	37	Yes					
G	37	Yes					
V	37	Yes					
VS	37	Yes					

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ce/f	37	Yes	Occ	Yes			
Cw	37	Yes	Occ	Yes			
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V	37	Yes	Occ	Yes			
VS	37	Yes	Occ	Yes			

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D	37	Yes	Occ	Yes	Yes		
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c	37	Yes	Occ	Yes	Yes		
e	37	Yes	Occ	Yes	Yes		
ce/f	37	Yes	Occ	Yes	Yes		
Cw	37	Yes	Occ	Yes	Yes		
G	37	Yes	Occ	Yes	Yes		
V	37	Yes	Occ	Yes	Yes		
VS	37	Yes	Occ	Yes	Yes		

Antibody Characteristics

Antigen	Optimal Temp	IgG	IgM	HTR	HDFN	Dosage	Enzyme
D	37	Yes	Occ	Yes	Yes	**No**	
C	37	Yes	Occ	Yes	Yes	Yes	
E	37	Yes	Occ	Yes	Yes	Yes	
c	37	Yes	Occ	Yes	Yes	Yes	
e	37	Yes	Occ	Yes	Yes	Yes	
ce/f	37	Yes	Occ	Yes	Yes	**No**	
Cw	37	Yes	Occ	Yes	Yes	Yes	
G	37	Yes	Occ	Yes	Yes	**No**	
V	37	Yes	Occ	Yes	Yes	**No**	
VS	37	Yes	Occ	Yes	Yes	**No**	

Antibody Characteristics

Antigen	Optimal Temp	IgG	IgM	HTR	HDFN	Dosage	Enzyme
D	37	Yes	Occ	Yes	Yes	**No**	Enhanced
C	37	Yes	Occ	Yes	Yes	Yes	Enhanced
E	37	Yes	Occ	Yes	Yes	Yes	Enhanced
c	37	Yes	Occ	Yes	Yes	Yes	Enhanced
e	37	Yes	Occ	Yes	Yes	Yes	Enhanced
ce/f	37	Yes	Occ	Yes	Yes	**No**	Enhanced
Cw	37	Yes	Occ	Yes	Yes	Yes	Enhanced
G	37	Yes	Occ	Yes	Yes	**No**	Enhanced
V	37	Yes	Occ	Yes	Yes	**No**	Enhanced
VS	37	Yes	Occ	Yes	Yes	**No**	Enhanced

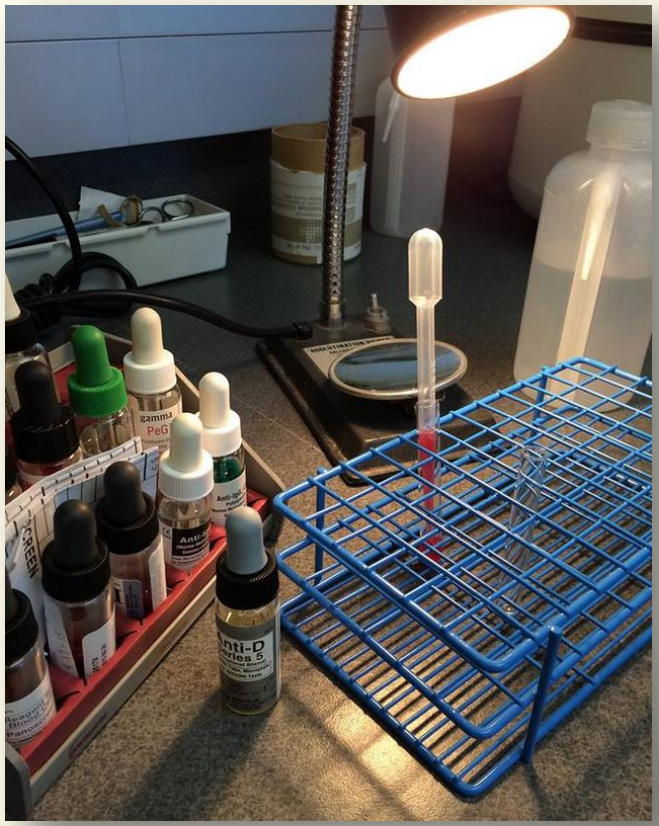
The Language of Rh

- ✓ **Fisher-Race:** genetics and serology
- ✓ **Wiener:** shorthand
- ✓ **Rosenfield:** presence or absence of a given antigen
- ✓ **ISBT:** catalogues each antigen within a blood group system

Different nomenclatures serve different purposes

Fisher-Race Terminology

- Based on closely linked alleles D, C/c, and E/e
- d is an ***amorph*** and does not produce a phenotypic product
- d= absence of D antigen



	Gene Combination	*Caucasian*	*African American*
Common	**DCe**	**42**	17
	dce	37	26
	DcE	14	11
	Dce	4	**44**
Rare	**dCe**	2	2
	dcE	1	<1
	DCE	<1	<1
	dCE	<1	<1

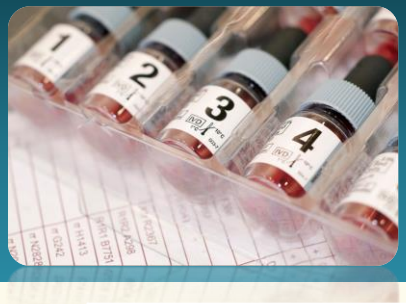
Most common haplotypes

Approximate percentages in the U.S.[2]

Most common genotypes

	Fisher-Race	Approximate prevalence in Caucasian population
Common Genotypes	DCe/dce	33
	DCe/DCe	18
	dce/dce	15
	DCe/DcE	11
	DcE/dce	9
	DcE/DcE	2

Antigen	Frequency
D	**85%**
c	80%
C	70%
e	98%
E	30%



Less common genotypes

	Fisher-Race	Approximate prevalence in Caucasian population
Less Common Genotypes	dCe/dce	<1
	dCe/dCe	0.01
	dcE/dce	<1
	dcE/dcE	0.03
Found in about 20% of African Americans	Dce/dce	2
	Dce/Dce	0.1
	dCE/dce	rare

Wiener terminology

- Wiener is the "shorthand" version of Fisher-Race
  - R= presence of D
  - r= d, or absence of D antigen
  - 1 or single prime= presence of C
  - 2 or double prime= presence of E

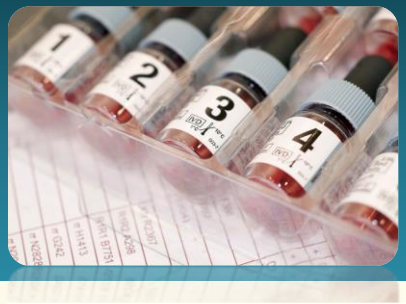
Antigen	Wiener
D	R
d	r
C	1 or ‘
E	2 or “

	Fisher-Race	Wiener
Rh positive	Dce	R0
	DCe	R1
	DcE	R2
	DCE	RZ
Rh negative	dce	r
	dCe	r’
	dcE	r”
	dCE	ry

Why…

Common Genotypes

	Wiener	Fisher-Race	Approximate prevalence in Caucasian population
Common Genotypes	**R1r**	DCe/dce	33
	R1R1	DCe/DCe	18
	rr	dce/dce	15
	R1R2	DCe/DcE	11
	R2r	DcE/dce	9
	R2R2	DcE/DcE	2



Less Common Genotypes

	Wiener	Fisher-Race	Approximate prevalence in Caucasian population
Less Common	**r'r**	dCe/dce	<1
	r'r'	dCe/dCe	0.01
	r"r	dcE/dce	<1
	r"r"	dcE/dcE	0.03
	R0r	Dce/dce	2
	R0R0	Dce/Dce	0.1
	ryr	dCE/dce	rare

Rosenfield and ISBT

Is anything missing?

?

Rosenfield

- This system simply describes the presence or absence of the antigen on the RBC. There is no genetic basis.
  - D=1, C=2, E=3, c=4, e=5
  - Example R1r (DCe/dce): Rh:1,2,-3,4,5
  - E is number 3; E antigen is not present and is therefore designated with -3

ISBT

- International Society of Blood Transfusion Numeric Terminology.
  - Rh blood group is assigned the prefix 004
  - Each antigen assigned to the Rh blood group is given a unique number to complete the six digit number.
    - Example: E antigen 004003
- Advantage over Rosenfield is that it is a purely numeric system, which is easier for data processing.

Question 1

- A patient's red blood cells are tested for the following Rh antigens:

Anti-D	Anti-C	Anti-E	anti-c	anti-e
+	+	0	+	+

Antigens present: D, C, c, e

Most likely genotype:	DCe/dce	R1r
Other possibilities:	Dce/dCe	R0r'
	DCe/Dce	R1R0

Question 2

- A patient's red blood cells are tested for the following Rh antigens:

Anti-D	Anti-C	Anti-E	anti-c	anti-e
0	0	0	+	+

Antigens present: c, e
Most likely genotype: dce/dce rr
Other possibilities: None

Question 3

- A patient's red blood cells are tested for the following Rh antigens:

Anti-D	Anti-C	Anti-E	anti-c	anti-e
+	+	0	0	+

Antigens present: D, C, e

Most likely genotype: DCe/DCe R1R1

Other possibilities: DCe/dCe R1r'

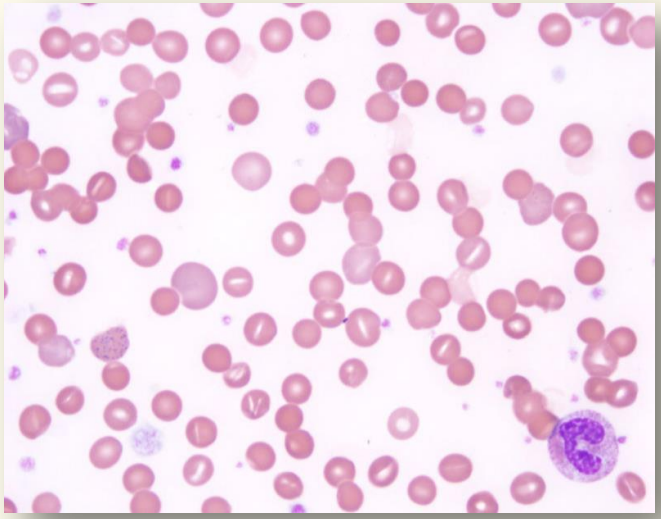
Objectives

1. Describe the D--, Rh_{null}, and Rh_{mod} phenotypes.
2. Compare and contrast the three mechanisms resulting in Weak D phenotype.
3. List three instances in which the Weak D status of an individual may be determined, and one instance in which Weak D status *must* be determined.
4. Describe the following: G, f(ce), C^w, V and VS.

Deletions

- Rare, D--
  - Person lacks Cc and Ee
  - Often has unusually strong D antigen expression.
  - “Exalted D”
  - Normal RHD genes, and a hybrid RHCE-RHD-RHCE gene in which the Cc Ee proteins are replaced with D
  - Antibody produced is called ***anti-RH17*** or ***anti-Hr_0***
- Variation: D● ● Evans + Rh:37

Rh_{null}



stomatocytes and spherocytes

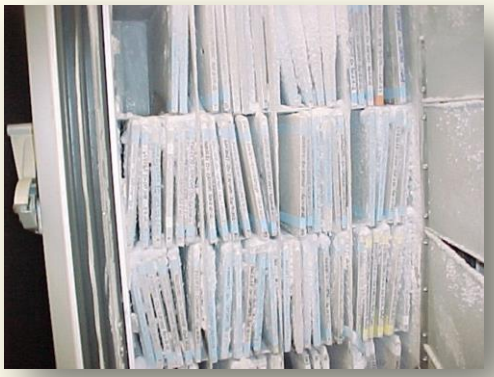
- "Rh deficiency syndrome"
- ---/---
- Lack all Rh proteins
- 2 types: Regulator & Amorphic
  - **Regulator**: mutation in the *RHAG* gene.
    - *RHD* and *RHCE* genes are usually normal.
  - **Amorphic**: *RHAG* gene is normal.
    - Mutations in *RHCE* and common deletion of *RHD* gene.

Rh_{mod}

- ***Partial*** suppression of RH gene expressions due to mutations in the *RHAG* gene.
- Exhibit similar features to Rh_{null}, but symptoms are less severe.

Rh_{null}

- First described in 1961 Aboriginal Australian woman
- By 2010, 43 people with Rh_{null} phenotype have been reported worldwide
- Difficult to transport rare blood across country borders
- Not all countries have frozen blood banks



Frozen Donor Blood

Geneva, Switzerland

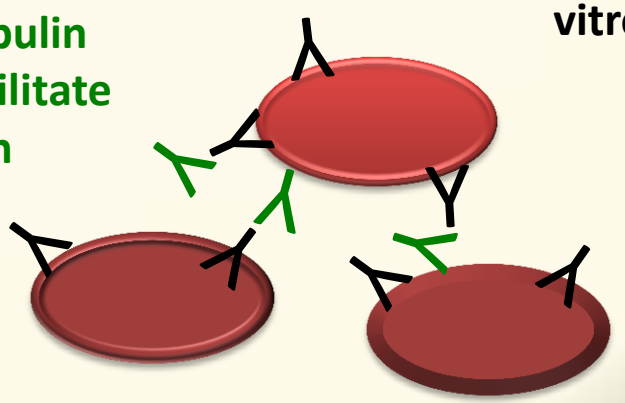
"The Most Precious Blood on Earth" P Bailey. The Atlantic. Oct 27, 2014.

Weak D

- Three mechanisms are responsible for the Weak D phenotype:
  - Genetic Weak D
  - C Trans
  - Partial D (D Mosaic)

The indirect antiglobulin test is required to facilitate a visible reaction

Reagent anti-D sensitizing to RBCs in vitro



Genetic Weak D

- Inheritance of weak D genes
- <2% of Caucasians, higher in African Americans
- D antigens complete, few in number

C Trans

Position effect

The allele carrying D is ***trans*** (in the opposite haplotype) to the allele carrying C:

Dce/d**C**e

Partial D (Mosaic)

Determination of D status

- No differentiation of Weak D causes
- Policies regarding testing of Weak D
- Regulatory requirements

RhIg and Weak D status of neonates

Mother: D-negative

Baby: Weak D-positive

Need for RhIg prophylaxis

Weak D Testing Policies

- 3 situations in which Weak D testing may be determined:
  - Intended recipients of blood transfusion
  - Expectant mothers
  - Neonates
- When Weak D testing must be performed:
  - Blood Donors

Updates

- CAP and AABB have recently recommended *RHD* genotyping to determine the cause of Weak D phenotype in patients.[5]
- Workgroup: *RHD* genotyping could potentially prevent
  - 24,700 unnecessary RhIG injections
  - 47,700 Rh-negative RBC units being transfused

Objectives

1. ~~Describe the D--, Rh_{null}, and Rh_{mod} phenotypes.~~
2. ~~Compare and contrast the three mechanisms resulting in Weak D phenotype.~~
3. ~~List three instances in which the Weak D status of an individual may be determined, and one instance in which Weak D status *must* be determined.~~
4. Describe the following: G, f(ce), C^w, V and VS.

The G antigen and anti-G

- G is unlike D/C/c/E/e
- Present on ANY cell that carries either the D or C antigen. (With very rare exceptions)
- G is absent when a person's red cells lack both D and C

The G antigen and anti-G

G is present	G is absent
D+C+	D-C-
D-C+	
D+C-	

A person will have G if they carry one of the following three alleles: *RHD, RHCe, or RHCE*

Case 1

	Rh-Hr					Kell		Duffy		Kidd		P	MNSs				Results		
	D	C	c	E	e	K	k	Fya	Fyb	Jka	Jkb	P1	M	N	S	s	37	AHG	CC
1	+	0	+	+	+	0	+	0	0	+	+	+	+	+	0	+	0	3+	
2	+	0	+	0	+	0	+	0	0	+	0	+	+	0	0	+	0	3+	
3	+	0	+	+	0	0	+	0	0	+	0	+	0	+	+	+	0	3+	
4	0	+	0	+	+	0	+	0	0	+	+	0	+	0	+	+	0	3+	
5	0	0	+	+	+	0	+	0	0	0	+	+	0	+	0	+	0	0	✓
6	0	0	+	0	+	0	+	0	0	+	0	0	+	0	0	+	0	0	✓
7	0	0	+	0	+	+	+	0	+	0	+	0	0	+	+	0	0	0	✓
8	+	+	+	+	+	0	+	+	+	+	+	0	+	+	0	+	0	3+	
AC																	0	0	✓

Case 1

	Rh-Hr					Kell		Duffy		Kidd		P	MNSs				Results		
	D	C	c	E	e	K	k	Fya	Fyb	Jka	Jkb	P1	M	N	S	s	37	AHG	CC
1	+	0	+	+	+	0	+	0	0	+	+	+	+	+	0	+	0	3+	
2	+	0	+	0	+	0	+	0	0	+	0	+	+	0	0	+	0	3+	
3	+	0	+	+	0	0	+	0	0	+	0	+	0	+	+	+	0	3+	
4	0	+	0	+	+	0	+	0	0	+	+	0	+	0	+	+	0	3+	
5	0	0	+	+	+	0	+	0	0	0	+	+	0	+	0	+	0	0	✓
6	0	0	+	0	+	0	+	0	0	+	0	0	+	0	0	+	0	0	✓
7	0	0	+	0	+	+	+	0	+	0	+	0	0	+	+	0	0	0	✓
8	+	+	+	+	+	0	+	+	+	+	+	0	+	+	0	+	0	3+	
AC																	0	0	✓

The f antigen and anti-f

- f(ce)
- expressed when c and e are on the same haplotype (cis position)

Dce/DCE (R0Rz)						DCe/DcE (R1R2)				
D	C	E	c	e		D	C	E	c	e
+	+	+	+	+		+	+	+	+	+

D**ce** anti-f will react DCe/DcE no reaction

- not a compound antigen; f is a single entity
- Anti-f can cause HDFN and TXRN

C^w

- Low incidence antigen
- Antithetical to the high-incidence antigen MAR
  - Found in about 2% of Caucasians and very rare in African Americans.
- Examples of both RBC Immune and non-RBC Immune

V, VS

- Antigens are found in about 30% of African Americans, rare in Caucasians.

Summary

- ✓ The Rh blood group system is clinically important in transfusion medicine.
- ✓ Different nomenclature systems can be used to help describe phenotypes and genotypes.
- ✓ Because the Rh system is so polymorphic, antigens may be expressed weakly.
- ✓ Although uncommon in routine blood banking, several other antibody specificities within the Rh blood group are of clinical importance, including G, f(ce), C^w, V and VS.

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