

Basic Rh – Special Considerations

Objectives:

1. Discuss Rh haplotypes, using both Fisher-Race and Weiner nomenclature, and explain how to determine f status using other Rh typings.
2. Compare and contrast weak D and partial D.
3. List unique circumstances in which an antibody might have Rh specificity.

Quick lesson:

There are >50 antigens in the Rh blood group system. However, 5 are considered “common.” They are... **D, C, E, c, e**

Rh antigens are inherited as “haplotypes,” one from each parent:

	Haplotype Fisher-Race nomenclature	Haplotype Weiner nomenclature
Rh (D) positive	DcE	R ₁
	DcE	R ₂
	Dce	R ₀
	DCE	R _Z
Rh (D) negative	dce	r
	dCe	r'
	dcE	r''
	dCE	r ^y

The three most common haplotypes in Caucasian individuals are... **R₁, R₂, r**

The most common haplotype in individuals of African descent is... **R₀**

Inheritance of haplotypes



So, if you inherit **DCe** from mom, and **DCe** from dad, your genotype (using Fisher-Race nomenclature) would be:

DCe/DCe

In Weiner nomenclature, your genotype would be

R₁R₁

The common Rh antigens expressed on your cells would be... **D, C, & e**

The common Rh antigens **NOT** expressed on your cells would be... **E & c**

Haplotype inherited from mom	Haplotype inherited from dad	Haplotypes (wiener nomenclature)	Common Rh antigens expressed on cells	Common Rh antigens NOT expressed
DcE	dce	R ₂ r	D, E, c, e	C
DcE	DcE	R ₂ R ₂	D, c, E	C, e
dce	dCe	r'r	C, c, e	D, E
DCe	DCe	R ₁ R ₁	D, C, e	E, c
Dce	dce	R ₀ r	D, c, e	C, E
dce	dce	rr	c, e	D, C, E

By testing for the common Rh antigens, we can infer the most probable genotype:

D	C	E	c	e	Probable genotype	Other possible genotypes
+	+	0	0	+	R ₁ R ₁	R ₁ r'
+	+	0	+	+	R ₁ r	R ₀ r' R ₁ R ₀
+	+	+	+	+	R ₁ R ₂ ←	R ₁ r'' R ₂ r' R ₂ R ₀ R ₂ r R ₀ r ^y

We say this is probable, because R₁ and R₂ haplotypes are very common. All the other possibilities include less common haplotypes

f antigen:

- Rh antigen (sometimes listed on antigrams, sometimes not)
- Anti-f is clinically significant antibody made by individuals lacking f antigen
- Anti-f should be “ruled out” in antibody workups (like other clinically significant antibodies to common RBC antigens)

The f antigen is expressed...

...when c and e are present on the same haplotype (in cis)

f expression	
Haplotypes that express f	D <u>ce</u> = R ₀ d <u>ce</u> = r
Haplotypes that don't express f	DCe = R ₁ DcE = R ₂ DCE = R _Z dCe = r' dcE = r'' dCE = r ^y

Interestingly...

- R₀r (Dce/dce) expresses f (double dose!)
- R₁R₂ (DCe/DcE) doesn't express f (c & e are on different haplotypes)

You can tell if a cell expresses f by:

		Rh					Kell		Duffy		Kidd		MNS				
		D	C	E	c	e	K	k	Fy ^a	Fy ^b	Jk ^a	Jk ^b	M	N	S	s	
1	R ₁ R ₁	+	+	0	0	+	0	+	+	+	+	+	+	+	+	+	+
3	R ₂ R ₂	+	0	+	+	0	0	+	0	+	+	+	0	+	+	+	+
4	R ₀ r	+	0	+	+	+	0	+	0	+	0	+	+	+	0	+	+
5	r'r	0	0	+	+	+	0	+	0	+	0	+	+	+	0	0	0
6	r''r	0	0	+	+	+	0	+	0	+	+	+	0	+	0	+	+
7	rr	0	0	0	+	+	+	+	0	+	+	0	+	0	+	+	+

Looking at wiener nomenclature:
remember, R₀ (Dce) and r (dce) express f

You can also tell if a cell expresses f by observing which of the common Rh antigens are expressed and inferring the most probable genotype:

	Rh					K	Jk ^b	MNS			
	D	C	E	c	e			M	N	S	s
1	+	+	0	0	+	0		+	+	+	+
2	+	+	0	0	+	+		0	+	0	+
3	+	0	+	+	0	0		+	0	+	+
4	+	0	0	+	+	0		+	+	0	+
5	0	+	0	+	+	0		+	+	0	0
6	0	0	+	+	+	0		0	+	0	+
7	0	0	0	+	+	+		+	0	+	+
8	0	0	0	+	+	0		0	+	+	+

Probable genotype: R₁R₁
This cell is f-negative

Probable genotype: R₀r
This cell is f-positive (double dose)

Probable genotype: r''r
This cell is f-positive (single dose)

Probable genotype: rr
This cell is f-positive (double dose)

Practice: does this cell express f? (Fill in the blanks)




D	C	E	c	e	Probable genotype	Does this cell express f?	If so, double or single dose?
+	0	+	+	0	R ₂ R ₂	No	NA
+	0	+	+	+			
0	+	0	+	+			
0	0	0	+	+			
+	+	0	0	+			

Answers:

D	C	E	c	e	Probable genotype	Does this cell express f?	If so, double or single dose?
+	0	+	+	0	R ₂ R ₂	No	NA
+	0	+	+	+	R ₀ r	Yes	Single
0	+	0	+	+	r''r	Yes	Single
0	0	0	+	+	rr	Yes	Double
+	+	0	0	+	R ₁ R ₁	No	NA

Rh Variant Alleles:

- Mutations in *RHD* or *RHCE* gene
- May code for antigens different than conventional Rh antigens (weak expression or partial, missing some antigen epitopes)
- More common in individuals of African descent
 - Antigen positive individual makes corresponding alloantibody when exposed to conventional antigen (often occurs in sickle cell patients)

Difference between Weak D and Partial D			
	Conventional D	Weak D	Partial D
Explanation	D antigen is complete and numerous on RBC surface	Quantitative difference: number of D antigens on RBCs is much lower than usual.	Qualitative difference: D antigens expressed on RBCs are not complete; they are missing epitopes
Representation of RBCs			
Do they make <u>alloanti-D</u>?	No	NO: Weak D types 1, 2 & 3 do NOT make anti-D when exposed to RBCs expressing conventional D antigen. Weak D types 4.0 and 4.1 can also be treated as D+.	Yes: may make anti-D (antibody to missing epitopes of D) when exposed to RBCs expressing conventional D antigen
How is testing affected?	RBCs react 3-4+ with anti-D reagents	Some reagents may not detect weak D (Weak D = ≤2+ reactivity at immediate spin, but moderately or strongly reactive at IAT)	RBCs may react with some anti-D reagents (monoclonal) strongly because the reagent antibody is directed against epitope(s) the patient's cells express.

More on Weak vs Partial D

- Serologically, can't distinguish weak or partial D types
 - Serologically, may result in discordant D typing (D+ one time, D- the next)
 - D+ individual makes alloanti-D
- *RHD* genotyping can differentiate and guide transfusion (and Rh immune globulin) recommendations
- Variant alleles also occur in the *RHCE* gene, leading to partial or weak expression of other Rh antigens

Practice:

	Anti-D	Anti-D
Patient cells	+	0
Positive control	+	+
Negative control	0	0

1. List possible explanations for these results:

-
-
-

2. What is the best way to guide transfusion recommendation in this case? _____

Answers: 1. Variant D expression (weak and/or partial), Anti-D antibodies to different epitopes of D (different clones), Tested by different methods (IS vs IAT). 2. *RHD* genotyping

Antibodies with Rh specificity?

- See chart below
- Differentiated by:
 - Patient history (transfusion history, drug history)
 - Serology (DAT/autocontrol result, eluate testing)
 - *RHD/RHCE* genotyping (to identify variant alleles indicating variant antigen expression)

	Explanation	Example	Further information
Alloantibody	Individual makes antibody to antigen his/her cells lack	e-negative patient makes anti-e	
Alloantibody	Individual makes antibody to antigen present on his/her cells, autocontrol (if not recently transfused) negative	e-positive patient makes anti-e	<ul style="list-style-type: none"> • Patient's cells express partial antigen, and antibody is to epitope(s) patient is missing • Genotyping can help characterize antibody as alloantibody
Autoantibody	Individual makes antibody to antigen present on his/her cells, autocontrol positive	e-positive patient makes autoanti-e	<ul style="list-style-type: none"> • Genotyping can help characterize antibody as autoantibody
Drug antibody	Individual makes antibody to drug that reacts with Rh specificity (often e-specificity)	e-positive patient makes drug antibody with e-specificity	<ul style="list-style-type: none"> • Transfusion history and drug history can infer that antibody is due to drug • One example drug: Zosyn (piperacillin/tazobactam)

Practice:

		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	LISS IAT	
1	R ₁ R ₁	+	+	0	0	+	0	+	+	+	+	+	+	+	+	+	+	3+
2	R ₁ R ₁	+	+	0	0	+	+	+	0	+	0	+	0	+	0	+	+	3+
3	R ₂ R ₂	+	0	+	+	0	0	+	+	0	0	+	+	0	+	0	+	0
4	R ₂ R ₂	+	0	+	+	0	+	+	0	+	+	0	0	+	0	+	+	0
5	r'r	0	+	0	+	+	0	+	+	0	+	0	+	+	0	0	+	3+
6	r''r	0	0	+	+	+	0	+	0	+	+	+	0	+	0	+	+	3+
7	rr	0	0	0	+	+	+	+	0	+	+	0	+	0	+	+	+	3+
8	rr	0	0	0	+	+	0	+	+	+	0	+	0	+	+	+	+	3+
Auto		+	+	0	0	+	0	+	+	+	0	+	+	+	0	+	+	3+

Assuming the patient has NOT been recently transfused, which of the following are possible explanations of the above results? (choose all that apply)

- a) alloantibody- patient has no variant Rh alleles
- b) alloantibody – patient has variant Rh alleles
- c) autoantibody – patient has no variant Rh alleles
- d) drug antibody – patient has taken a drug, and made antibody with Rh specificity

Answers: c,d

Assessing Understanding

1. Fill in the following chart:

Serologic Typings					Probable genotype (Wiener nomenclature)	f antigen expressed? (double/single dose)
D	C	E	c	e		
0	+	0	+	+		
+	+	0	0	+		
+	0	+	+	+		
0	0	0	+	+		

2. Which of the following is true regarding differences between weak and partial D?

- Weak D generally refers to expression of antigen missing epitope(s).
- Individuals with partial D antigens are best treated as D-negative.
- Partial D refers to fewer D antigens expressed (quantitative difference).
- Patients with Weak D Types 1, 2, 3, 4.0 & 4.1 should be treated at D-negative.

3. What is the easiest way to differentiate Rh alloantibody from autoantibody with Rh specificity in a patient who has NOT been recently transfused?

- Review the patient’s drug list
- RHD* and *RHCE* gene sequencing
- Serologic result of the DAT/autocontrol
- Determine patient’s f status based on serologic typings

Answers: 1.

Serologic Typings					Probable genotype (Wiener nomenclature)	f antigen expressed? (double/single dose)
D	C	E	c	e		
0	+	0	+	+	r'r	Yes, single
+	+	0	0	+	R ₁ R ₁	No
+	0	+	+	+	R ₂ r	Yes, single
0	0	0	+	+	rr	Yes, double

2. b

3. c