

## Exercise 4

## Rh Phenotyping

**Textbook:** Quinley, Chapter 8

**Skills:** 20 Points

### Objectives:

1. State the antigens of the Rh blood group system.
2. Define the terms “dominant”, “codominant”, “heterozygous”, and “homozygous” as they relate to inheritance of the Rh antigens.
3. Define the terms “phenotype” and “genotype” as they relate to blood group antigens.
4. Briefly state the procedure whereby an individual’s Rh phenotype is determined.
5. Recall each of the 8 Rh genes in both Fisher-Race and Wiener notation.
6. Given an Rh phenotype list the most probable genotypes in both Wiener and Fisher-Race notations.
7. Determine the Rh phenotype and probable genotype of two (2) patient samples.

### Discussion

In this procedure, commonly tested antigens of the Rh system will be studied. Normally the only Rh antigen identified in routine pretransfusion testing is the D antigen. Four additional Rh antigens are: C, E, e, and  $\bar{e}$ . Information obtained through the identification of these antigens may be used in: family studies, resolution of antibody problems, population studies, cases of disputed parentage, and to predict whether the sexual partner of a woman with Rh antibodies is likely to transmit genes that will result in offspring negative for the particular antigen.

The Rh system initially appears simple and straightforward since it involves only five (5) antigens (C, e, E,  $\bar{e}$ , and D), but is in fact one of the more complicated human blood group systems known. The gene complex is inherited and individuals may be analyzed in terms of separate antigens: D or its absence (d), C or  $\bar{c}$ , and E or  $\bar{e}$ . Gene D is **dominant** to its **allele** d, because gene d is an **amorph** which makes no detectable antigenic product. The alleles C and  $\bar{c}$ , and E and  $\bar{e}$  are **codominant** and if both alleles are present, both will be expressed.

Wiener theorized that the Rh antigens displayed by an individual are the result of only a pair of genes, one inherited from each parent. If these are identical, the individual is **homozygous** for that gene and all the products of the gene will be expressed in a double dose on the person's red cell. When the genes inherited from each parent are not identical, the person is **heterozygous** for two different genes and, since the Rh genes are codominant, the products of both will be expressed on the person's red cells. Antigens that are products of both genes will be present in double dose, while those produced by only one of the pair of genes will be present in single dose.

#### **EXAMPLE:**

DCe/DCE (slash separates the gene) transmitted from each parent  
antigen D = homozygous, present on both sides  
antigen C = homozygous, present on both sides  
antigens  $\bar{e}$  and E = heterozygous, present in one side only

A **phenotype** is the assortment of antigens actually detectable on an individual's red cells using selected antisera. In many blood group systems, the phenotype is an exact expression of the **genotype**. Unfortunately, this is not the case in the Rh system. Since any one antigen may derive from any of several genes, identifying antigens does not always allow the genotype to be deduced with certainty. Presumptions regarding the **most probable** genotype rest on knowledge of the frequency with which particular antigenic combinations derive from a single gene complex. To determine the genotype with more certainty, it is necessary to do family studies which are not always possible. The following is an example of a phenotype and the possible genotypes which could be present.

**EXAMPLE:**

Phenotype (antigens detected on the red cells) is: D, C, c, e

Possible Genotypes

(Fisher-Race)

DC $\bar{c}$ /d $\bar{c}$  $\bar{e}$

DC $\bar{c}$ /D $\bar{c}$  $\bar{e}$

D $\bar{c}$ e/dC $\bar{e}$

Possible Genotypes

(Wiener)

R<sup>1</sup>/r

R<sup>1</sup>/R<sub>0</sub>

R<sub>0</sub>/r'

In the general population, the **most common D positive genotype is R<sup>1</sup>/r**. The **most common D negative genotype is rr**.

**Principle**

The Rh phenotype is determined by testing the patient red blood cells with the five standard antiserums: anti-D, anti-C, anti-c̄, anti-E, and anti-ē. If the antigen to which the antiserum is directed is present, agglutination of the rbc's will occur. No agglutination of the rbc's indicates the absence of the antigen. Based on the reactions with these serums, certain statistical assumptions are made, not always correctly. For instance, if a cell reacts with anti-C but not with anti-c̄, the antigen C is assumed to be present in a double dose (homozygous for C). The exceptions to this are rare, but may be significant in paternity testing.

In the past the reagent antiserums are made from individuals who have developed an antibody against a specific blood group antigen. Although human source anti-serums are still available, the move is to provide these reagents from clones. Agglutination of an individual's red cells by a specific antiserum indicates the presence of the corresponding antigen. No agglutination indicates its absence.

## Reagents

1. Blood Bank Reagent Rack (See Procedure 3)
2. Anti-C, Anti-E, Anti- $\bar{e}$ , Anti- $\bar{c}$ , Rh control
3. Can of Blood Bank plastic pipets
4. Blotting squares (biowipes)
5. 12x75 test tubes
6. Serofuge
7. Heat Block
8. Agglutination viewer
9. Squirt bottle of 0.85% saline
10. Sharpie

## Procedure

1. Place serum in a properly labeled tube (if not already done) and prepare a 4-6% red cell suspension for each patient in an appropriately labeled tube (refer to procedure 3).
2. Set up tubes for ABO and D type and an additional tube for each Rh typing sera (*remember, anti-D is part of the forward type*).
3. Label a tube for the other Rh antigens with the *patient's first and last initial and the Rh antigen* to be tested.
4. Add 1 drop of each reagent forward typing sera to each appropriate tube.
5. Place two (2) drops of the appropriate Rh anti-serum into its appropriately labeled tube.
6. Add 3 drops of patient serum to the A<sup>1</sup> and B reverse cell typing tubes.
7. Place 1 drop appropriate reagent reverse cell into the properly labeled reverse type tube.
8. Place one (1) drop of the 4-6% patient cell suspension to each forward type tube.
9. Centrifuge for 15-20 seconds.
10. Gently resuspend cell button and examine for macroscopic agglutination.
11. Grade the agglutination reaction and immediately record the results.
12. Incubate all negative Rh typing tests and their controls for 15 minutes at 37°C.
13. Repeat steps 5-7 except for D. If patient appears D negative, perform the D<sup>u</sup> test (refer to ABO/D tube test).
14. During incubation record the results of the positive and negative control tubes for the “other” Rh typing seras.

## Recording Results

Record the reactions of the individual's cells with each appropriate antiserum. Then determine the individual's phenotype and **most probable** genotype in the appropriate spaces. See example provided. Use the attached chart for assistance.

## Interpretation of Results

Once the results of antigen typings have been obtained, the most probable genotype must be determined. At first this will be confusing and difficult, but interpretation will get easier with practice. The following example may help.

Results obtained:

anti-D	anti-C	anti-c	anti-E	anti-e
4+	4+	4+	0	4+

Positive reactions indicate the presence of the antigen, negative reactions indicate the absence of the antigen. The *phenotype* of this patient (antigens detectable on the rbc) is: D, C, c, e.

An Rh gene complex consists of a D or d, C or c, E or e antigens is inherited from each parent. When a patient is D positive the phenotype of the person is either D/d (heterozygous for D) or D/D (homozygous for D). Make a separate column for each possible genotype, the other Rh antigens will be added to this “base” type.

Heterozygous D	Homozygous D
D /d	D /D

The results from the Rh phenotype indicates that the patient is C<sup>+</sup> and c<sup>+</sup>, the individual is *heterozygous* for the C antigen, so place a C or c on either side of the Dd as follows. It is critical to remember to list ALL possible combinations.

DC /dc	DC /Dc (only combination possible)
Dc /dC	

The next antigen to “plug in” is E. The patient is E=e<sup>+</sup> indicating that this individual is *homozygous* for e, so e will be present on both sides.

DCe /dce	DCe /Dce
Dce /dCe	

You must now convert the Wiener notations to Fisher-Race using the chart supplied, also indicate the frequencies in which these genotypes occur:

DCe /dce	R <sup>1</sup> r	33%
Dce /dCe	R <sup>0</sup> r'	<0.1%
DCe /Dce	R <sup>1</sup> R <sup>0</sup>	2%

One thing to remember is that frequencies given are for the white population. In the black population the R<sup>0</sup> genotype is very common. It is critical to know the race of the individual when determining most probable genotypes.

## Interpreting Results

Memorize this chart!

Antigens	Fisher-Race	Wiener
D,c,e	De $\bar{e}$	R <sup>o</sup>
D,C,e	DC $\bar{e}$	R <sup>1</sup>
D $\bar{c}$ E	D $\bar{c}$ E	R <sup>2</sup>
D,C,E	DCE	R <sup>z</sup>
e, $\bar{e}$	d <sup>e</sup> $\bar{e}$	r
C, $\bar{e}$	dC $\bar{e}$	r'
$\bar{c}$ ,E	d $\bar{c}$ E	r''
C,E	dCE	r <sup>y</sup>

In interpreting reactions for genotypes, always remember that if the D antigen is present it does **not** mean that it is homozygous. Figure the genotype with D on one side and d on the other (heterozygous for D). Use the chart on the following page and select the genotype with the highest frequency. For example, if the D, C,  $\bar{c}$  and  $\bar{e}$  antigens are present, there are three (3) possible genotypes:

	Fisher-Race	Wiener	% frequency from following table
1.	DC $\bar{e}$ /d $\bar{c}$ $\bar{e}$	R <sup>1</sup> /r	33%
2.	DC $\bar{e}$ /D $\bar{c}$ $\bar{e}$	R <sup>1</sup> /R <sub>o</sub>	2%
3.	D $\bar{c}$ e/dC $\bar{e}$	R <sub>o</sub> /r'	0.01-0.1%

The *most probable genotype* is R<sup>1</sup>r since this has the highest statistical probability.

## Rh Genotypes and Their Serological Reactions

Genotypes		Reactions with anti-					Caucasian Frequency (%)
Fisher-Race	Wiener	D	C	E	c	e	
DCe/dce	R <sup>1</sup> /r	+	+	0	+	+	33
DCe/Dce	R <sup>1</sup> /R <sup>0</sup>	+	+	0	+	+	2
Dce/dCe	R <sup>0</sup> /r'	+	+	0	+	+	†
DCe/DCe	R <sup>1</sup> /R <sup>1</sup>	+	+	0	0	+	18
DCe/dCe	R <sup>1</sup> /r'	+	+	0	0	+	*
DcE/dce	R <sup>2</sup> /r	+	0	+	+	+	11
Dce/dcE	R <sup>0</sup> /r''	+	0	+	+	+	†
DcE/Dce	R <sup>2</sup> /R <sup>0</sup>	+	0	+	+	+	*
DcE/DcE	R <sup>2</sup> /R <sup>2</sup>	+	0	+	+	0	2
DcE/dcE	R <sup>2</sup> /r''	+	0	+	+	0	*
DCe/DcE	R <sup>1</sup> /R <sup>2</sup>	+	+	+	+	+	12
DCe/dCE	R <sup>1</sup> /r <sup>y</sup>	+	+	+	0	+	‡
DCe/dcE	R <sup>1</sup> /r''	+	+	+	+	+	1
DcE/dCE	R <sup>2</sup> /r <sup>y</sup>	+	+	+	+	0	‡
DcE/dCe	R <sup>2</sup> /r'	+	+	+	+	+	*
DCE/DCE	R <sup>z</sup> /R <sup>z</sup>	+	+	+	0	0	‡
DCE/DCe	R <sup>z</sup> /R <sup>1</sup>	+	+	+	0	+	*
DCE/DcE	R <sup>z</sup> /R <sup>2</sup>	+	+	+	+	0	†
DCE/dCe	R <sup>z</sup> /r'	+	+	+	0	+	‡
DCE/Dce	R <sup>z</sup> /R <sup>0</sup>	+	+	+	+	+	†
DCE/dcE	R <sup>z</sup> /r''	+	+	+	+	0	‡
DCE/dce	R <sup>z</sup> /r	+	+	+	+	+	*
DCE/dCE	R <sup>z</sup> /r <sup>y</sup>	+	+	+	0	0	‡
Dce/dCE	R <sup>0</sup> /r <sup>y</sup>	+	+	+	+	+	‡
Dce/dce	R <sup>0</sup> /r	+	0	0	+	+	2
Dce/Dce	R <sup>0</sup> /R <sup>0</sup>	+	0	0	+	+	†
dCe/dce	r'/r	0	+	0	+	+	*
dCe/dCe	r'/r'	0	+	0	0	+	‡
dcE/dce	r''/r	0	0	+	+	+	*
dcE/dcE	r''/r''	0	0	+	+	0	†
dCe/dcE	r'/r''	0	+	+	+	+	†
dCE/dce	r <sup>y</sup> /r	0	+	+	+	+	‡
dCE/dcE	r <sup>y</sup> /r''	0	+	+	+	0	‡
dCE/dCe	r <sup>y</sup> /r'	0	+	+	0	+	‡
dCE/dCE	r <sup>y</sup> /r <sup>y</sup>	0	+	+	0	0	‡
dce/dce	r/r	0	0	0	+	+	15

\* = less than 1 percent but greater than 0.1 percent

† = less than 0.1 percent but greater than 0.01 percent

‡ = less than 0.01 percent

## Comparison of Wiener and Fisher-Race Concepts of the Rh Blood Group System

Wiener Concept			Fisher-Race Concept		Approximate Frequency in Caucasoids of New York City (%)
Gene	Agglutinin	Blood Factors	Gene Complex	Blood Factors	
R <sup>0</sup>	Rh <sub>0</sub>	Rh <sub>0</sub> , hr', hr''	Dce	D, c, e	2.5
R <sup>1</sup>	Rh <sub>1</sub>	Rh <sub>0</sub> , rh', hr''	DCe	D, C, e	51.2
R <sup>2</sup>	Rh <sub>2</sub>	Rh <sub>0</sub> , rh'', hr'	DcE	D, c, E	16.5
R <sup>Z</sup>	Rh <sub>Z</sub>	Rh <sub>0</sub> , rh', rh''	DCE	D, C, E	14.9
r	rh	hr', hr''	dce	c, e	13.4
r'	rh'	rh', hr''	dCe	C, e	1.1
r''	rh''	rh'', hr'	dcE	c, E	0.4
r <sup>y</sup>	rh <sub>y</sub>	hr', hr''	dCE	C, E	0.02

## Rh Phenotyping Recording Results

Note: The #3 patient will be where you will record your results if you have decided to test your own sample.

### A. Type and Rh

Patient Name	Forward Type							Reverse Type		Interpretation
	Anti-A	Anti-B	Anti-AB	Anti-D	*D Ctrl	D <sup>u</sup>	*D <sup>u</sup> Ctrl	A Cells	B Cells	
1. #										
2. #										
3. #										

\*This is performed only if a high protein anti-D reagent is used **or** the patient appears to be AB positive or for D negative patients.

### B. Rh Phenotype - circle the most probable genotype

Name	Reactions with anti-						Phenotype	Most probable genotype	
	D	C	E	$\bar{c}$	$\bar{e}$	Rh ctrl		Fisher-Race	Wiener
Example	3+	3+	O	3+	3+	O	D,C, $\bar{c}\bar{e}$	DC $\bar{c}$ /d $\bar{c}e$	R <sub>1</sub> r
1. #									
2. #									
3. #									

Name \_\_\_\_\_

## **Rh Phenotyping Study Questions**

1. List the antigens of the Rh system. (2.5 points)
2. State three situations in which testing for the Rh antigens provides useful information. (1.5 points).
  
3. Is the allele to D actually detected? Explain. (1 point)
  
4. What does it mean to say that the Rh antigens C and c or E and e are “codominant”. (1 point)
  
5. What is meant by the terms homozygous and heterozygous? (2 pts)
  
6. Define phenotype. (1 point)

**EXERCISE 4**

7. Define genotype. (1 point)

8. State the most common D positive genotype in both Fisher-Race and Wiener notations. Provide the same for the most common D negative genotype. (2 points)

	<b>Fisher-Race</b>	<b>Wiener</b>
D positive		
D negative		

9. How are presumptions regarding the most probable genotype determined? (1 point)

10. Fill in the following chart and memorize!

<b>Fisher Race</b>	<b>Wiener</b>
a.	R <sup>1</sup>
DcE	b.
c.	R <sup>z</sup>
dce	d.
e.	R <sup>2</sup>
dcE	f.
g.	r'

**EXERCISE 4**

For the following phenotypes, list **all** possible genotypes. State each one's statistical probability as given for Caucasians. (Use Fisher-Race and Wiener.) **Circle** the most probable genotype. Point values vary.

11. D, C, E,  $\bar{e}$

12. d,  $e$ ,  $\bar{e}$

13. D, E,  $e$ ,  $\bar{e}$

14. State 2 situations in which a D control *must* be run. (1 point.)