The Rh System
Determining the Rh Phenotype and Probable Genotype

The material in this handout supplements the handout discussed during CLS 422 Student Lab “The Rh System”. Be certain to review the Student Lab Rh System handout (particularly the section on Rh typing reagents) in addition to this new material.

I. Determining the Phenotype

A. Typically test for D, C, c, E, and e using patient’s red blood cells (RBCs) and antisera (reagent containing antibody directed against a specific antigen).

1. Reagents may be high protein, low protein or monoclonal

2. Follow manufacturer’s directions regarding amount of reagent and RBCs to add, incubation time and temperature, and phase(s) at which test is read.

3. If patient tests negative with D at immediate spin, perform the weak D test.

B. For each antiserum used, test positive and negative control cells each day of use.

1. Positive control should be an RBC with heterozygous antigen expression (i.e. C+c+ or E+e+).

2. Negative control cell must lack the target antigen.

C. Should the patient test positive with all antisera, a negative control should be tested to validate the positive results observed. Consult the manufacturer’s package insert for selection of an appropriate negative control, which may include:

1. Testing the patient’s RBCs against Rh-hr high protein control.

2. Testing the patient’s RBCs against 6% Bovine Serum Albumin (BSA).

3. Performing a DAT on the patient’s RBCs to prove they were not originally coated with antibodies in vivo.
II. Determining the Most Probable Genotype

A. Find all possible genotypes from the phenotype (See example below)

1. If D is positive, the number of possible genotypes is one less than the number of positive reactions (except if all 5 are positive, in which case there are 6 possible genotypes).

2. If D is negative, the number of possible genotypes is two less than the number of positive reactions (unless there are only 2 positive reactions, in which case there is 1 possible genotype).

3. Remember that one haplotype is inherited from each parent (for example, DCe/dce is one genotype having both the DCe and the dce haplotypes).

B. Determine the most common haplotypes:

   Most common → Least common

   1. Caucasians: R₁, r, R₂, R₀, r’, r”, R₇, r

   2. Blacks: R₀, r, R₁, R₂, r’, r”, R₇, r

C. Record haplotypes in proper order:

   R₇, R₁, R₂, R₀, ry, r’, r”, r

Example 1

The patient’s phenotype is D+, C+, E 0, c+, e+.

Since we have 4 positive reactions and the D is positive, we should have 3 possible genotypes. When D is positive, we don’t know if the patient inherited D from 1 parent or both parents. So we immediately have 2 possibilities:

A. One genotype where D was inherited from both parents: D??/D??
B. One genotype where D was inherited from only 1 parent: D??/d??

Next look at alleles C and c. If only one is present, it must be in both haplotypes. If both are present assign one allele to one haplotype, and the other allele to the remaining haplotype.

From our example, we have both C and c present, so C will be assigned to one haplotype and c to the other.

A. DC?/Dc?          B. DC?/dc?
Repeat this process for E and e.
From our example, only e is present, so it must be in both haplotypes.

A. DCe/Dce = R₁R₀      B. DCe/dce = R₁r

These are only 2 of the 3 possibilities. Remember in genotype “B”, we randomly assigned C to the haplotype with D and c to the haplotype without D. To get our third genotype, reverse the positions of C and c.

C. Dce/dCe = R₀r’

To determine which of the 3 choices is most likely, look at the haplotype frequencies for the patient’s race.
In this example, if the patient is White, then “B” R₁r is the most likely; if the patient is Black, then “A” R₁R₀ is more likely.

Example 2

<table>
<thead>
<tr>
<th>Reagent</th>
<th>Anti-D</th>
<th>Anti-C</th>
<th>Anti-E</th>
<th>Anti-c</th>
<th>Anti-e</th>
</tr>
</thead>
<tbody>
<tr>
<td>Results</td>
<td>0</td>
<td>0</td>
<td>2+</td>
<td>2+</td>
<td>2+</td>
</tr>
</tbody>
</table>

Interpreting these results, we have a phenotype of D 0, C 0, E+, c+, e+. We have 3 positive reactions, and no D, so we must have only 1 possible genotype.

Since the patient is Rh negative, we know there is no D in either haplotype: d??/d??

Looking at C and c, only c is present: dc?/dc?

Looking at E and e, both are present, so we will assign one to the first haplotype and the other to the second haplotype: dcE/dce = r’r; the only possibility.

But what if we flipped E and e around? We would get dce/dcE = rr” which is written in the incorrect order. If we write these haplotypes in the correct order, we get r”r again!