

# Preparation of Simulated FMH Positive Samples

## OBJECTIVE

Since true feto-maternal hemorrhage (FMH) samples demonstrating various concentrations of Rh positive fetal red cells in the maternal circulation of Rh negative red cells are relatively rare; laboratories need a method to simulate FMH to allow crossover testing when transitioning from FMH test kit to another test kit employing similar methods of FMH detection.

## PROCEDURE

(Prepare cells on day of use)

Select cells with the appropriate predictive genotypes of  $R_1r$  and  $rr$  from donor units. Segments from these donor units can be used to acquire packed red cells for the procedure as described below.

1. Wash red cells X 3 in saline
2. For final wash, centrifuge @ 900-1000 x g for 5 minutes to obtain packed cell volume of approximately 80%, remove supernatant
3. Prepare 3% cell suspensions in saline by adding 40  $\mu$ L packed cells to 1.0 mL saline
  - Prepare 10 mL 3%  $rr$  by adding 0.4 mL packed cells to 10 mL saline
  - Prepare 1 mL 3%  $R_1r$  cells by adding 0.04 mL packed cells to 1 mL saline
4. Prepare test samples using the 3% cell suspensions
  - a. **0.4%  $R_1r$** : add 0.02 mL of 3%  $R_1r$  to 4.98 mL 3%  $rr$  cells\*  
*\*Remove 20  $\mu$ L from 5mL of 3%  $rr$  cells and then add 20  $\mu$ L of  $R_1r$  cells*
  - b. **0.3%  $R_1r$** : mix 1.5 mL 0.4%  $R_1r$  cell suspension + 0.5 mL 3%  $rr$  cells
  - c. **0.2%  $R_1r$** : mix 1 mL 0.4%  $R_1r$  cell suspension + 1 mL 3%  $rr$  cells

- d. **0.1% R<sub>1</sub>r**: mix 0.5 mL 0.4% R<sub>1</sub>r cell suspension + 1.5 mL 3% rr cells
- e. **0.00% R<sub>1</sub>r** (100% rr): remaining 3% rr cells

**Note:** Use of other methods to prepare cell suspensions of various concentrations, e.g. hematocrit, are acceptable. The important condition of producing the simulated samples is to have the positive and negative cells at the same starting concentration. Use of commercially available panel cells or screening cells could be considered acceptable with the understanding that cell suspensions may vary slightly between the vials selected to prepare the cell admixtures. If using saline, cells should be prepared on day of testing. If access to a commercial red cell diluent like Ortho Resuspension Solution (ORS) is available, cells could be prepared in advance and stored refrigerated.

**Caution:** Simulated samples prepared by this method cannot be used to perform the Kleihauer-Betke (KB) stain method to quantify FMH since red cells used in the simulation do not contain fetal hemoglobin.