A laboratory is introducing serum separator (“barrier”) phlebotomy tubes. The 10 mL tubes containing the gel will be centrifuged for 10 minutes in a 52° angle rotor in a bench-top centrifuge. The centrifuge has a listed top speed of 2200 rpm.

The medical technologists in this laboratory soon note that often the tubes have clots that are incompletely covered by the barrier gel. This usually occurs when the centrifuge is filled to capacity. This problem is causing significant contamination of the serum with blood cells as well as reducing the amount of serum that can be poured off free of cellular contamination.

A technologist looks up a reference (p. 23, 24) on the use of these barrier tubes and notes that a force of 1000 x g for 10 minutes is recommended for proper use of the tubes. The technologist finds out that the fixed angle rotor in use in the laboratory has a radius of 5.75 inches from the bottom of the tube holder to the drive shaft.

**QUESTIONS**

1. What is the most likely cause of the problem?
2. How can the technologist use these data to allow the laboratory to use the barrier tubes properly?

**Questions to Consider**

1. What is the principle of action of the “barrier” phlebotomy tubes?
2. Is the centrifugal force being employed sufficient to use the barrier tubes properly?
3. What other factors affecting centrifuge performance can result in a relative centrifugal force that is insufficient for proper use of the barrier tubes?

**Answer:**

1. The most likely problem is insufficient centrifugal force. By calculating the centrifugal force, one can establish if it is adequate to meet the manufacturer’s specifications.
2. The laboratory can take the following steps to solve the problem: One can centrifuge these samples longer. However, this could result in an inordinate delay in sample processing and cause heating and deterioration of the sample as well (p. 75-76). One can change rotors or centrifuges to have a larger radius during the centrifugation procedure, or increase the rpm, or both. One can also use a swinging-bucket rotor to allow the gel to be spread as a thinner, horizontal layer over the separated clot. Optimally, the laboratory might do all of these, that is, use a swinging-bucket rotor with a larger effective radius that can be spun at higher speeds.

**Answers to Question to Consider**

1. The barrier tubes contain a silicone-type gel that works on the following principle. The gel has a specific gravity (approximately 1.04 g/cc) which is less than that of the clot and greater than that of the serum. During centrifugation, the applied relative centrifugal force (RCF) pellets the clot beneath the less dense gel while the serum floats on top of the gel. To use the integrated gel-tubes, sufficient centrifugal force must be applied to pellet the clot beneath the gel (p. 24, 71-72).

2. Using the formula on page 25: $$RCF = 1.12 \times 10^{-5}; \quad (R) \quad (rpm)^{2}; \quad g \quad 9.8 \quad m/s^2; \quad RCF = \text{relative centrifugal force}$$
(15\ cm) (2200\ rpm)^2 \ &= 813 \times g \ \text{(Using the nomogram on p. 26, one can estimate the RCF as 825 x g.) This RCF is the maximum centrifugal force that can be generated at the bottom portion of the tube, i.e. at the point furthest from the center of the rotor. Towards the center of the tube, the applied g force will be less. This centrifugal force is not within the manufacturer’s suggested limits and is probably the reason for the separation problems.}

3. The RCF calculated above is a theoretical determination of the maximum g force that can be applied. As the number of tubes being centrifuged increases, the actual g force at a given speed setting will decrease. This happens because the speed setting on the centrifuge only adjusts the amperage (current) to the motor driving the rotor. The actual rpm generated varies with total load in the rotor. That is why the problem of insufficient sample separation occurred more often with full rotors; under these conditions, the g force will be even less than the maximum calculated. The actual rotor speed (rpm) under working conditions should be determined by use of a suitable tachometer (p. 23-26). Another factor to consider is the angle of the tube during centrifugation (p. 24, 71-72). Because of the 52° angle, the gel layer is dispersed over a larger, slanted area. This slant increases the chance that a portion of the clot will remain uncovered by the barrier gel, especially when the centrifugation is being performed under less than optimal conditions, as was the case in this laboratory.