OBJECTIVES

Upon completion of this exercise, appropriate discussion, and related reading, the student will be able to:

1. Perform an agarose electrophoresis procedure for lipoprotein.
2. Discuss the lipid profile illustrated by the results of the unknown serum sample used in this exercise.

PRINCIPLE

Using the Corning agarose electrophoresis system, four lipoprotein bands may be identified. They are in order of increasing electrophoretic mobility, chylomicrons, beta-lipoproteins (LDL), pre-beta lipoproteins (VLDL), and alpha-lipoproteins (HDL). Following electrophoresis, the separated fractions are stained with Fat Red 7B. Fat Red 7B stains unsaturated fatty acids in the lipoprotein complexes.

Plasma separated from blood anticoagulated with EDTA or serum are the recommended samples for this procedure.

MATERIALS

- Universal Buffer pH 8.6
- Methanol-water
- Agarose Gel
- Control Serum
- Fat Red 7B stain
- Staining trays
- Electrophoresis Chamber
- 55°C drying oven

PROCEDURE

1. Fill the electrophoresis cell base with 190 mL of Universal PHAB Buffer (95 mL in each chamber). Do not reuse buffer.
2. Gently peel the Agarose Universal Electrophoresis Film from its plastic cover, being careful to handle the agarose film only by it edges.
3. Fill the sample wells of the agarose film with 1.0 mL of plasma or serum.
4. Insert the loaded agarose film into the cassette holder of the Electrophoresis Cell Cover, agarose side facing out, matching the anode (+) side of the agarose film with the anode (+) side of the cell cover.
5. Place the cell cover on the electrophoresis cell base. (The power supply will automatically switch on.)
6. Allow the sample to migrate for 35 minutes at 90 volts.
7. Following migration, remove the cell cover from the electrophoresis cell base. Drain the excess buffer from the cell cover without inverting the cover. Grasp the agarose film by its edges and remove it from the cassette holder.

8. Wipe the moisture from the back of the agarose film, then place the film on a shelf of a drying chamber or appropriate incubator/oven. Dry at 55° C ± 5° for 15-20 minutes or until dry.

9. Remove the agarose film from the oven and allow to cool to room temperature.

10. Place the dried agarose film, agarose side up, on moistened filter-paper placed in the bottom of a staining dish.

11. Using a clean 10 mL glass pipet, dispense 10 mL of Fat Red 7B working stain solution evenly over the surface of the agarose film. Do not touch the pipet to the agarose surface.

12. Stain for four minutes. When the stain turns dark blue and begins to precipitate, the staining is complete.

13. Transfer the agarose film to the Methanol-Water clearing solution. Gently agitate for approximately 60 seconds or until the background is clear.

14. Wipe moisture from back of agarose film and dry at 55°C ± 5° for 15—20 minutes or until dry.

15. Interpret visually or quantitate using the appropriate densitometric equipment at 520 nm.

DATA SHEET, EXERCISE #18
NAME: ___________
DATE: ___________

Place electrophoretogram or a copy in the space below.

INTERPRETATION

Check the most appropriate interpretation for each control or unknown analyzed.
Sample #  Normal  Type I  Type II  Type III  Type IV  Type V

Discussion Questions

1. Why is it important that the buffer solution in the chamber not be reused?
2. In what way is interpretation of an electrophoretic pattern with a densitometer better than a visual interpretation?
3. What is the most common cause of poor results when performing electrophoresis procedures?