RELATED READING: Chapter 55. See Methods on CD-ROM for Creatine kinase isoenzymes.

OBJECTIVES

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

1. Perform an electrophoresis and develop the electrophoretogram with an enzymic stain.
2. Identify each CK isoenzyme on the electrophoretogram.
3. Interpret the CK isoenzyme pattern, relating it to a possible disease state.

METHOD

Corning electrophoresis with P-L Biochemicals colorimetric development or other appropriate reagent.

PRINCIPLE

CK isoenzymes are separated electrophoretically on agarose plates at 90 V, pH 8.6. A substrate that can be converted to a fluorescent product by CK can then be applied and the isoenzyme bands observed under UV light.

GLOSSARY

Application - a term used to describe the process of placing a small volume of solution onto a stationary phase (thin layer plate, gel, paper, or column) for chromatographic or electrophoretic separation.

MATERIALS

- Barbital buffer (pH 8.6)
- Agarose gel
- CK substrate (Corning #0
- 39°C incubator
- Electrophoresis chamber
- Drying oven
- Sample applicator
- UV light source
- Serum samples
- CK control
PROCEDURE

1. Fill each chamber of electrophoresis cell base with 95 mL barbital buffer, pH 8.6 (good for one use only).
2. Remove plastic backing from agarose film.
3. Apply 1.0 μL aliquots of each serum to a sample well, allowing sample to absorb between applications. Place a control in position 3 or 4 (use 2 applications for control).
4. Load the agarose film into the top cassette so that negative side corresponds to negative side of the bottom cassette.
5. Electrophorese at 90 V for 20 minutes.
6. Near the end of electrophoresis, reconstitute one vial of Cardiotrac CK Fluorometric reagent with 1.0 mL buffer solution. Swirl to dissolve.
7. Prepare Incubator tray with moistened blotter pad and preincubate tray at 39°C.
8. Immediately following electrophoresis, remove film from cassette. Place film on flat surface with positive (+) side near you and blot edges. Pour entire contents of substrate vial along one edge. With a 5 mL serological pipet, gently spread the liquid from positive to negative side by lying pipet on its side in the solution and using it as a “spreader”.
9. Place film gel side up on prewarmed incubator tray, dry and replace cover, and incubate for 20-30 minutes at 39°C. (Increased incubation enhances sensitivity.)
10. Remove the agarose film from the incubation tray and dry at 55°C. Dry in oven for 15-20 minutes or until dry.
11. Inspect with UV light or scan on a fluorescent densitometer.
12. Attach a copy of densitometric tracing or part of the agarose gel to bottom of the data sheet.

DATA SHEET, EXERCISE #22

NAME: ___________

DATE: __________

RESULTS

Observe bands as follows: (cathode to anode)

• CK (BB) - migrates closely with albumin.
• CK (MB) - migrates between CK-BB and CK-MM
• CK (MM) - remains near the origin.

CLINICAL INTERPRETATION

1. Normal Normal CK total with small amounts of enzymatic activity in CK (MM) fraction. No detectable MB or BB.
2. Abnormal CK (MB) present. Indicative of heart damage or necrosis. Usually accompanied by elevated MM and elevated total CK.
3. Abnormal Increased CK (MM). Reflects injury to skeletal muscle due to intramuscular (IM) injection, surgery, etc. Occurs in muscular dystrophy; may be accompanied by elevated MB.
4. *Abnormal* CK (BB) present. Reflects the release of brain tissue due to cerebrovascular accident, necrosis, or other brain trauma or damage to large bowel or pulmonary tissues.

Attach copy of densitometric scan or part of agarose gel below.

**DISCUSSION QUESTIONS**

1. What bands were visible on your sample? What does this suggest?
2. Compare the findings from this exercise with the total CK enzymatic activity on the same sample from the CK exercise. Do the results support each other?
3. How might it be possible to convert the fluorescent stain into a colored stain visible in normal room lighting?