Lesson 4

Electrophoresis

Electrophoresis is a commonly used laboratory method for the separation, identification, and quantification of molecules of large molecular size. Common applications of electrophoresis include serum proteins, isoenzyme analysis, lipoprotein analysis, and so on.
Electrophoresis means the movement of charged molecules or ions in an electric field with the practical addition that subsequent detection of molecules is possible.
The most common form of electrophoresis performed in the clinical laboratory uses buffered agarose or cellulose acetate as the media.
Most molecules separated by electrophoresis contain both positive and negative charges. These molecules are called zwitterions.
The direction of migration of a protein in an electric field depends on the pH of the buffer and the isoelectric point of the protein. The isoelectric point is defined as the pH at which the sum of all positive and negative charges on the molecule adds up to zero. A protein at its isoelectric point has no charge and will not migrate in an electric field. At a pH below the isoelectric point, the protein has a positive charge and will therefore migrate toward the cathode. At a pH above the isoelectric point, the protein will migrate toward the anode.
Other factors that influence the migration of proteins in an electric field are the size and shape of the molecules, the strength of the electric field, temperature, and pore properties of the electrophoresis medium.

Detection method
Different stains for serum proteins are available, for example Coomasie blue. Gold or silver are 100 times more sensitive than Coomasie stain.
The equipment of the electrophoretic separation system comprises a power source, an electrophoretic chamber and, in some cases, a densitometer. The power source may regulate the potential difference between the cathode and the anode by constant voltage or constant current.

Quantitation of the electrophoretogram by densitometry
A densitometer is a comparator. It compares the amount of light that passes through a sample to the amount that passes through in the absence of a sample. Densitometry has much in common with spectrophotometry or filter photometry except that in densitometry, a material in a solid phase is detected as opposed to a liquid sample in a cuvette.

Read the text and decide if the statements are true or false.

1 Electrophoresis is used for all molecules sizes.
2 Electrophoresis consists in movement of charged molecules in an electric field.
3 Buffered agarose is more common as the medium than cellulose acetate.
4 Zwitterions have no electric charge.
5 Protein movement depends only on the pH of the buffer.
6 The isoelectric point is a sum of all electric charges on the molecule.
7 A protein can move either to the cathode or the anode.
8 Coomasie stain are less sensitive than gold.
9 Densitometry is completely different from spectrophotometry.
10 Densitometry is used for solid materials.