Coagulation Methods

Mechanical method

- Electro mechanical detection method incorporates a change in electrical conductivity between two metal probes emerged in a solution. Two probes move in and out of the solution at constant intervals. The electrical circuit between the two probes is not maintained as the moving probe rises in and out of the solution. When the fibrin clot is formed in the solution, the timer stops.

- The second method of mechanical clot detection involves monitoring the movement of a steel ball within a test solution by magnetic sensors. A change in the movement of a steel ball may be detected when there is increased viscosity of the test solution, changing its range of motion or by a break in a constant with the magnetic sensors when the steel ball becomes incorporated into a fibrin clot as the cuvette rotates.

- Photo-optical end point detection**,** involves detection of a clot formation being measured by a change in optical density (OD) of a test sample. This is known as turbidimetric methodology. A specified wavelength is passed through a test plasma, and a certain amount of light is detected by the photodetector. The amount of light detected is dependent on the color and clarity of the plasma sample. When a fibrin clot is formed, formation of fibrin strands causes lights to scatter allowing less light to fall on the photo detector

Chromogenic endpoint detection

- It uses a specific color producing substance known as a chromophore which has an optical absorbance peak at 405 mm on a spectrophotometer. The principal of this assay is based on the attachment of pNA to a synthetic chromogenic substrate. The substrate is made up of a series of amino acids, the composition of which is dependent on the structure of the enzymatic target of the coagulation protein being measured. The goal for the coagulation protein to attach the chromogenic substrate at a specific site between the defined amino acid sequence and the pNA, thereby cleaving pNA from the substrate. Free pNA has a yellow color, it is measured by a photo detector at a wavelength of 405 nm. As free pNA is cleaved, the amount of light absorbance is increased, leading to a greater change in OD of the solution.

Immunologic end point detection

- Immunologic assays are based on antigen-antibody reactions. Microlatex particles are coated with a specific antibody directed against the antigen to be measured. A beam of monochromatic light is then passed through the suspension of microlatex particles. When the antibody coated micro particles come in contact with antigen in a solution, the antigen and antibody attach and form bridges between the particles causing them to agglutinate. As the size of agglutinates becomes larger and closer to the wavelength of monochromatic light beam, the larger amount of light is absorbed. The increase in light absorption is proportional to the size of agglutinates, which in turn, is proportional to the antigen level present in the sample, which is read from a standard curve.

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Measurement principle:

- Modes of analysis: chronometry and photometry.
- Chronometry: modification in viscosity of the medium, detection by electromagnetic sensors.
- Photometry: measurement of optical density to 405nm and to 540nm.
  - colorimetry
  - turbidimetry (immunology)

The principle consists of measuring the variation in amplitude of the ball's oscillation. A reduction in amplitude corresponds to an increase in the medium's viscosity, in other words, in the coagulation phenomenon.

At constant viscosity, the amplitude of the ball's oscillation is constant. When the viscosity increases (the phenomenon of coagulation), the amplitude of the ball's oscillation decreases. An algorithm uses this variation in amplitude to determine coagulation time.