Lecture 12 Outline
Instrumentation

A. Introduction

Traditionally measure by functional assays

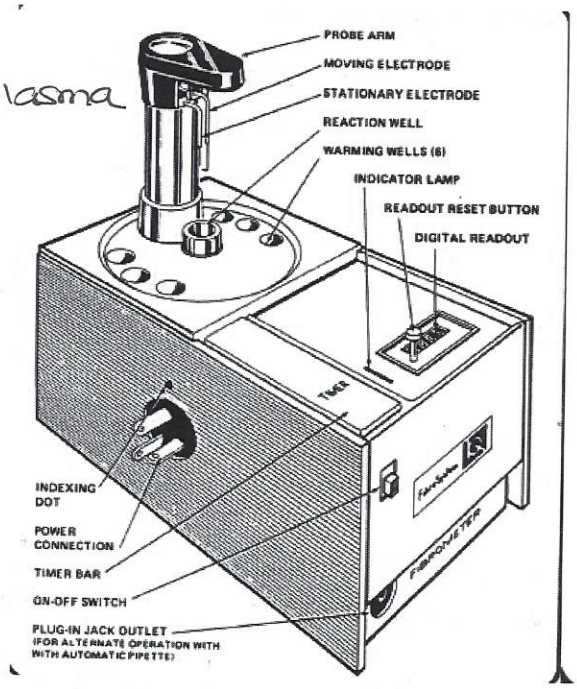
B. Manual (tilt tube)

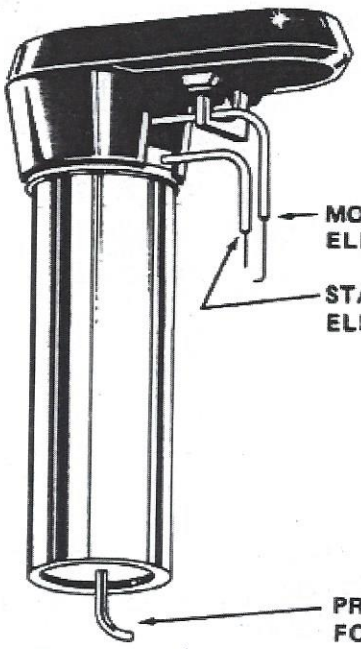
Do everything yourself
- Timing
- Checking clot

C. Semi-Automated

Tech required to still detect some things
- time
- detect clot
- Fibrometer

Fibrometer
Pipette pt. plasma
Manually





→ Stops when resistance gets too great due to clot & this stops the timer

Drops into sample

D. Fully Automated

centrifuge tube
sample & reagents added automatically
Dont need duplicates anymore
Reproducibility, accuracy, precision all greater

E. Methods of Endpoint Detection

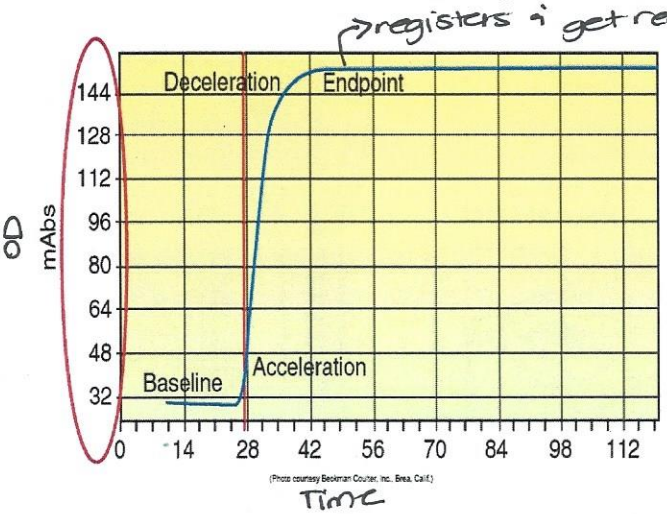
5 different methods

F. Photooptical

- Light source
- ↓ light when clot forms
- Measure change in optical density as the clot forms
- Measurement station - where spectrophotometer is
- When OD changes enough, it registers & you get your clotting time
- Problem: lipemic sample (turbid)
  - centrifuge out lipids

  Icteric sample (yellow)
  - Hard to detect change b/c of color

Reading at multiple wave lengths can help



Hemolyzed sample
- color impacts reading
- factor activation

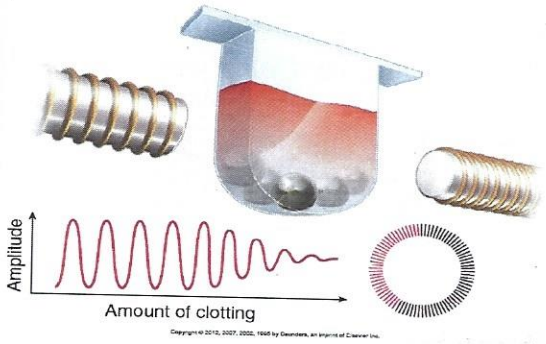
G. Mechanical

① Electromechanical - As resistance of probes get greater, electrical conductivity between 2 probes changes as sample clot changes
ex. Fibrometer

② Electromagnetic field applied to cuvette w/ pt. sample & steel b in it. When clot forms, ball stops moving.

* Mechanical is gold standard b/c color does not affect testing.

disadvantage - can't generate / can't see clot formation on a visual graph w/ mechanical.



H. Chemical/Chromogenic

- uses synthetic substrate that mimics something physiologic & color producing substance (chromophore) is added to the synthetic substrate
  - ↳ pNA (para nitroaniline)
- * Measure OD
- NO clot formed, but producing color to measure how much is working
- can be directly or indirectly proportional
- Problem: lipemic → dilute out

I. Immunologic

1. Antigenic Antibodies Reactions
2. Hook antibodies to micro latex particles

looking at ΔOD, not clotting

3. In test, patient sample (which may have the antigen) is mixed with latex particles and allowed to react for a specified period of time. Beam of light is passed through the suspension of particles. Initially, when the wavelength of light is greater than the diameter of the particles in suspension, only a small amount to light will be absorbed by the particles. When these particles come in contact with any patient antigen present, the antigen attaches to the antibody and forms bridges between the particles, causing them to agglutinate. As the diameter of the agglutinates becomes larger and closer to the wavelength of the beam, the greater the amount of light that is absorbed. The increase in light absorbance is proportional to the size of the agglutinates, which, in turn, is proportional to the antigen level present in the sample, which is read from a standard curve.

Stago Instruments measure vWF antigens

ex) D-Dimer test
ex) vWF antigen test
} on Stago instrument
↳ mechanical
chromagenic
Immunologic

J. Nephelometric –

Modification of photoptical

measure forward angle scatters of light or 90° angles

K. Other Instrumentation

1. Other instruments for specialty testing, especially with increased knowledge about hemostasis
2. Not all instruments in coag; some may be in other areas (e.g. molecular diagnostics, chemistry (CRP))
3. PFA, platelet aggregometer

L. Advantages of Newer instruments

1. Faster
2. More accurate
3. More precise Same results
4. Have QC programs on instrument
5. Can measure multiple samples at one time
6. Can do multiple different tests (analytes) at one time
7. Can read barcode → positive patient ID
8. Can transmit results to LIS
9. Can ID reagents with bar codes, so don't mix up
10. Can be adapted to robotics [STA-R]
11. Expanded computer capabilities (store patient data, QC, standard curves, more than one lot number at a time, programmable)