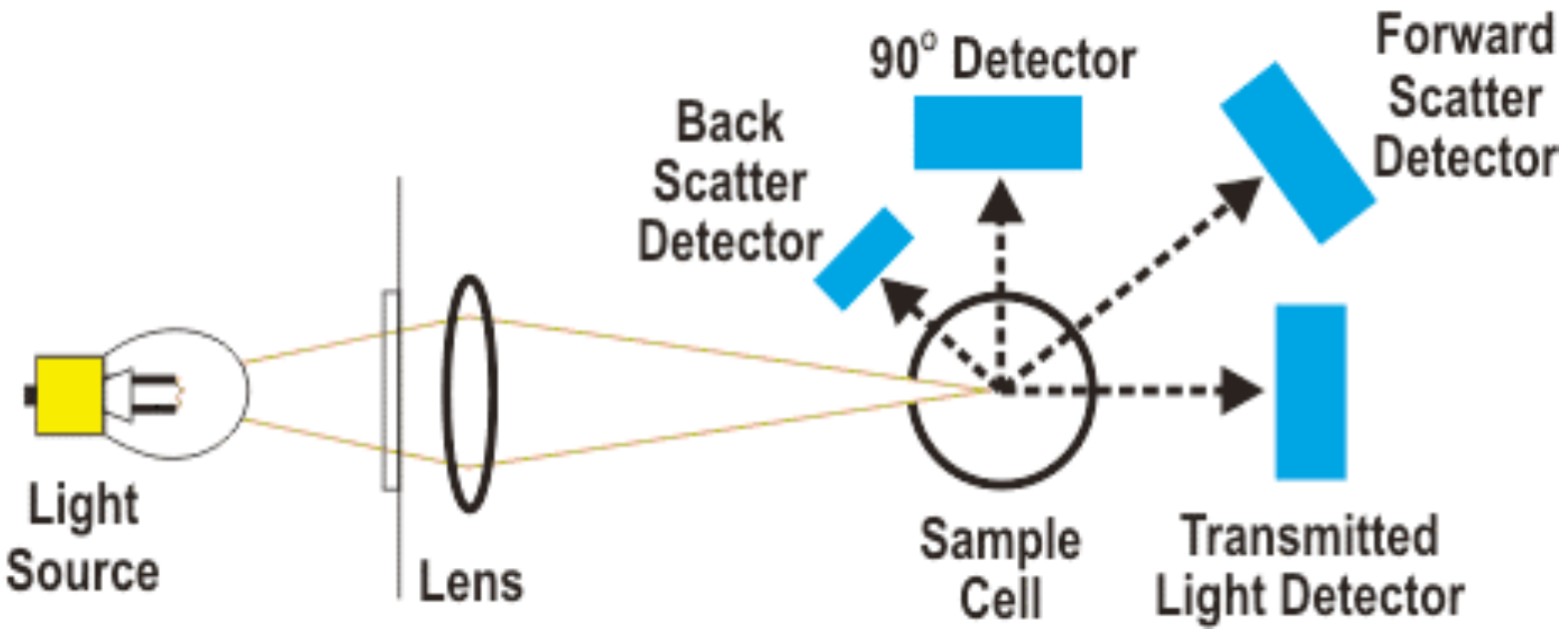
TURBIDIMETRY & NEPHELOMETRY



Mohsin Al-Saleh, Senior Biomedical Scientist
(BSc. MLS-2004/MSc Clini. Chem. 2013)
ID#6837

Lecture Objectives:

- Introduction (An Overview about laboratory techniques)
- Definition of Turbidimetry and Nephelometry
- Type of Nephelometry
- Tyndall effect
- NTU (Nephelometric Turbidity Unit)
- Factors affecting light scattering
- Advantages
- Disadvantages
- Clinical application
- Limitation of light scattering techniques
- Hook effects
- Conclusion

Laboratory techniques & methods:

- Laboratory techniques and methods are performed on patient specimens such blood, urine, tissues, …etc.

- To detect biomarkers, enzymes activity, and concentration

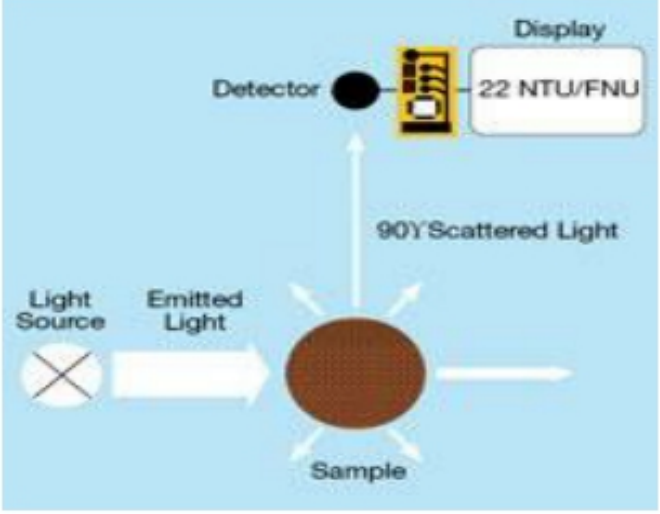
- Techniques could be basic to advance level:

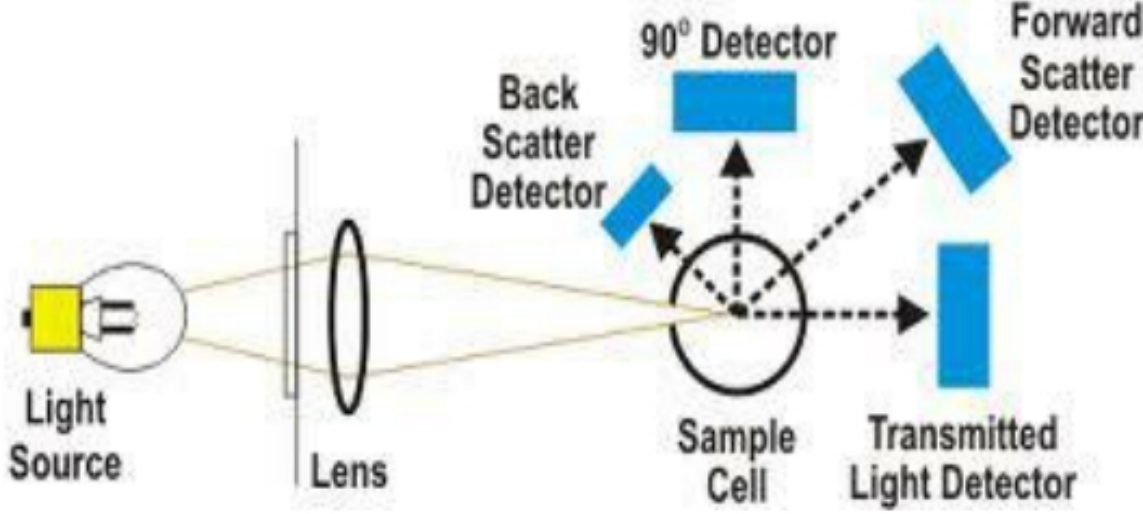
(Screening tests, Definitive test, Confirmatory test)

- These techniques yield results in the form of data:

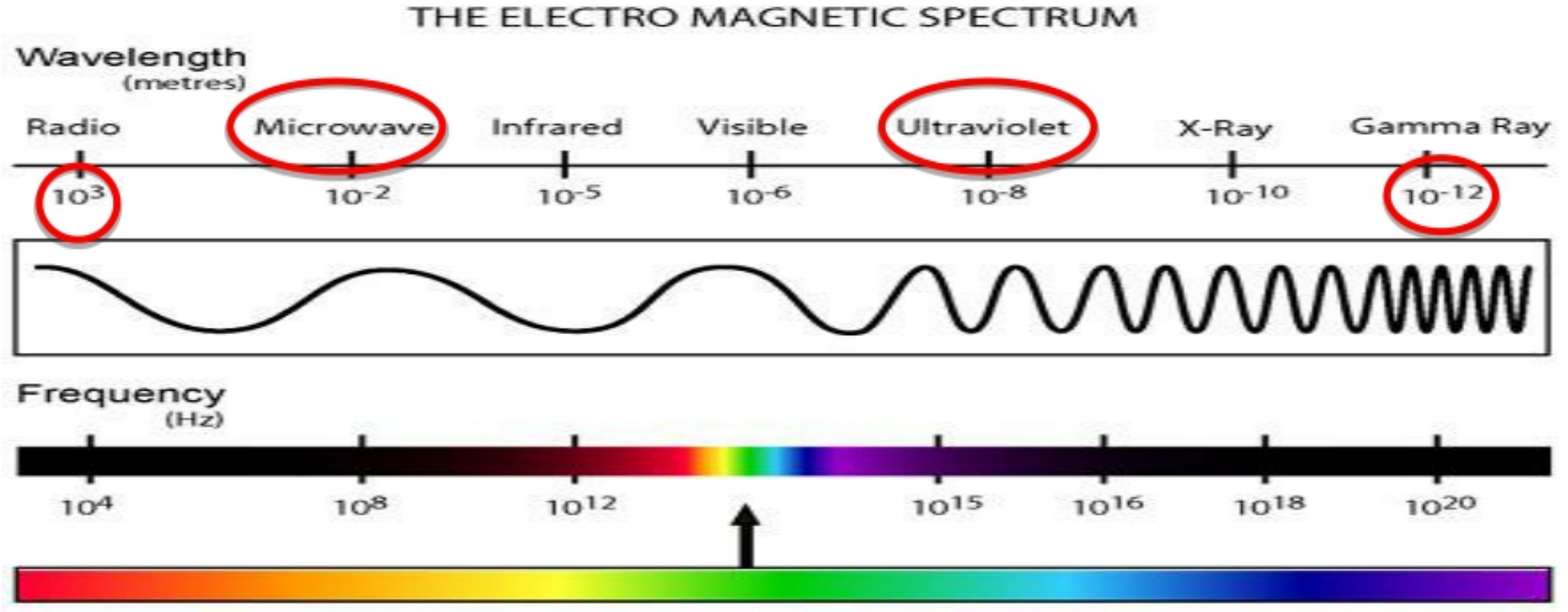
(Qualitative, Semi-quantitative, and Quantitative)

- **Quantification** is important for disease diagnosis, classification and monitoring





Nephlometry and Turbidimetry

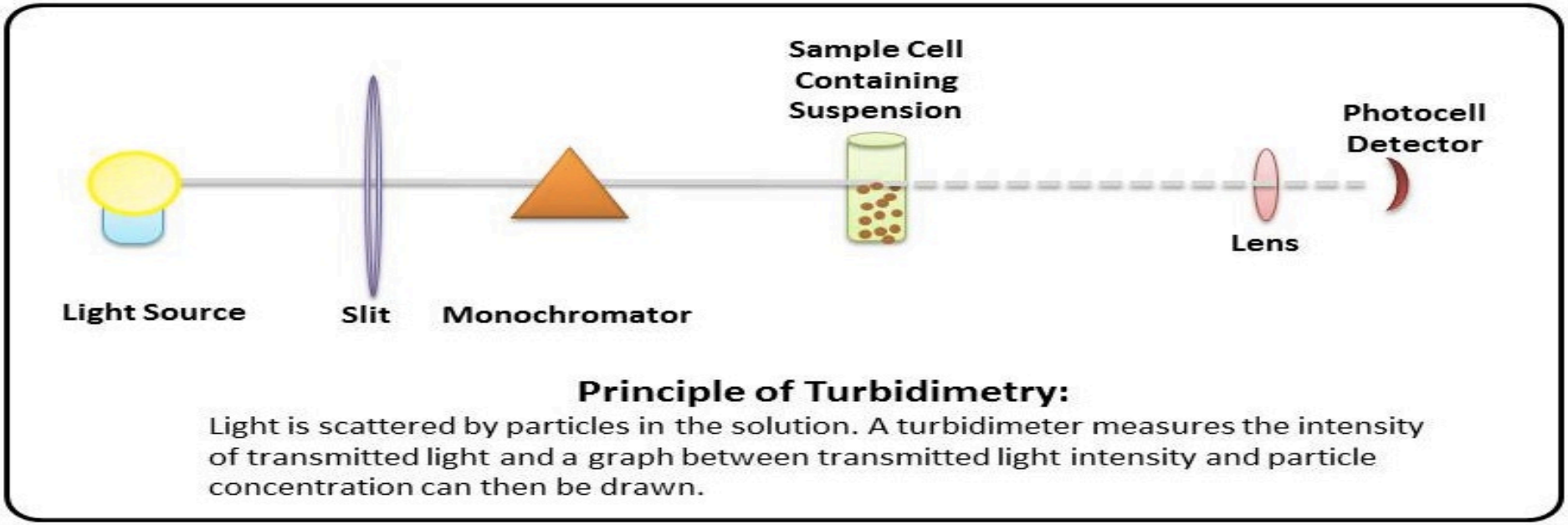


Turbidimetry & Nephelometry:

- These techniques used in the laboratory to determine the levels of several blood plasma/serum **proteins**
- For example the total levels of antibodies isotypes or classes: Immunoglobulin M, Immunoglobulin G, and Immunoglobulin A
- This technique is widely used in clinical laboratories because it is relatively easily automated
- Antibody and the antigen are mixed in concentrations such that only small aggregates are formed that do **not quickly settle to the bottom**

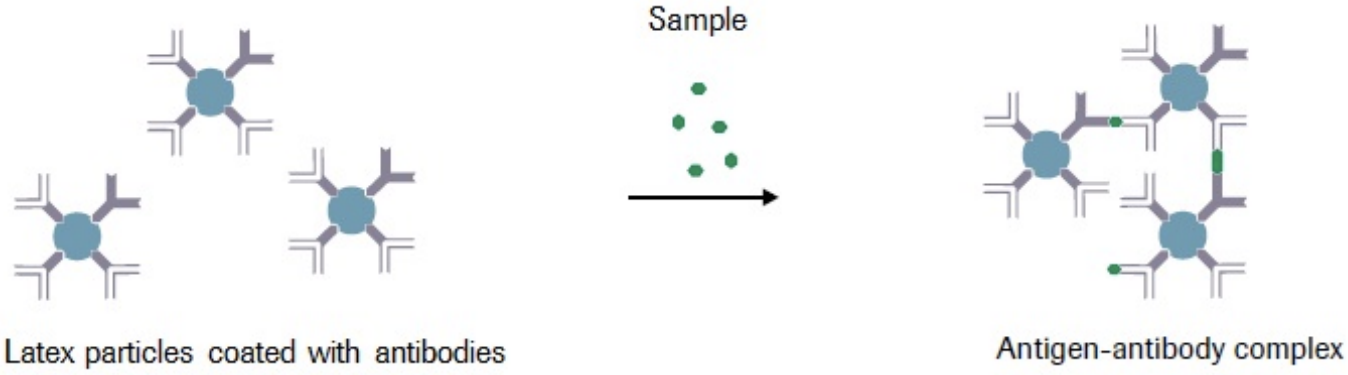
Turbidimetry:

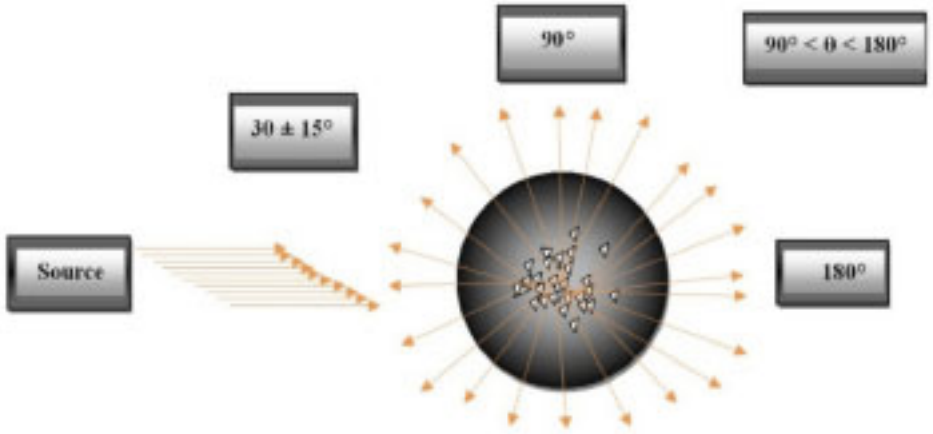
- Measurement of the **decrease** in intensity of **incident light** as it passes through a solution of particles , that is caused by **scattering**, **reflectance** and **absorbance** of the incident light. In turbidimetry the detector is located **at 180°** from the incident beam



Turbidimetry:

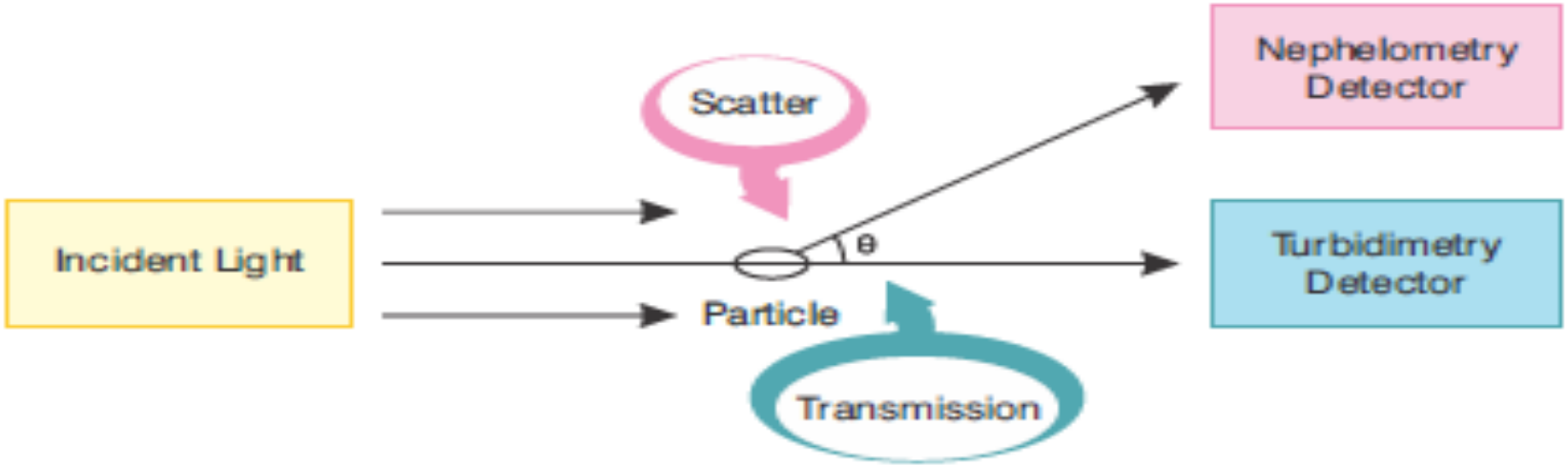
- Turbidimetry is very similar to spectrophotometry





Nephelometry:

- Detection of light energy scattered or reflected towards a detector that is not in the direct path of the transmitted light **at 90° or 30°**. It measures increase in energy of light scattered by sample in a cuvette.



Nephelometry:

- It is based on the principle that a diluted colloidal suspension of small particles will scatter light (**usually a laser beam**) passed through it rather than simply absorbing it.
- Particle density is then a function of the light scattered into the detector from the particles.
- Nephelometry can be used to detect either antigen or antibody, but it is usually run with **antibody as the reagent** and the patient **antigen as the unknown**
- A **nephelometer** is an instrument for measuring concentration of suspended particulates in a liquid

Types of Nephelometry:

- In the Lab, two types of tests can be run:

1. End point nephelometry
2. Kinetic (rate) nephelometry

- **End point nephelometry** tests are run by allowing the antibody/antigen reaction to run through to completion (until all of the present reagent antibodies and the present patient sample antigens that can aggregate have done so and no more complexes can form).

- However, the large particles will fall out of the solution and cause a false scatter reading, thus **kinetic nephelometry was devised**.

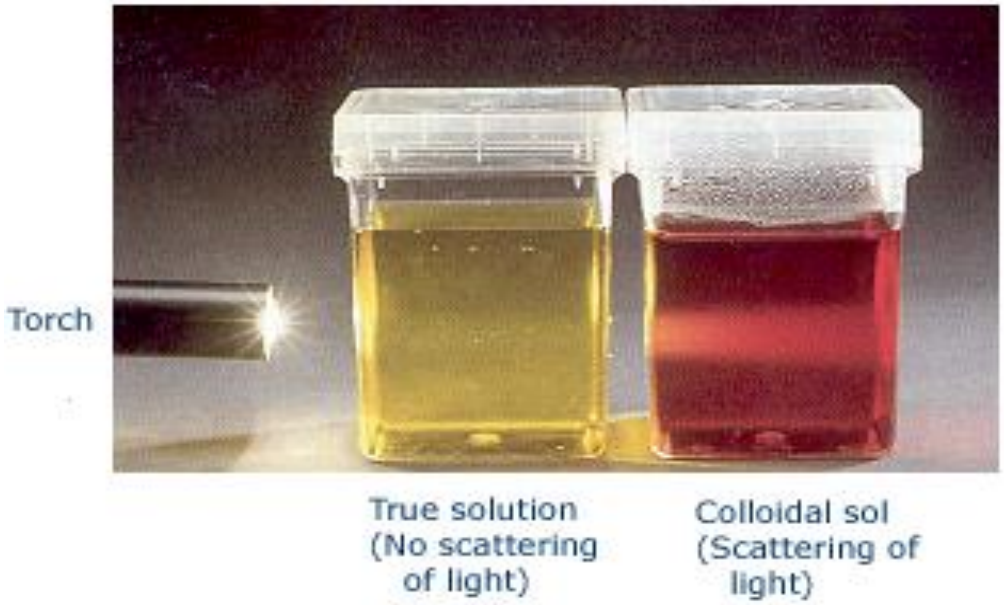
Types of Nephelometry:

- **In kinetic nephelometry,** the rate of scatter is measured right after the reagent is added. As long as the reagent is constant the rate of change can be seen as directly related to the amount of antigen present.

Kinetic is better than End point

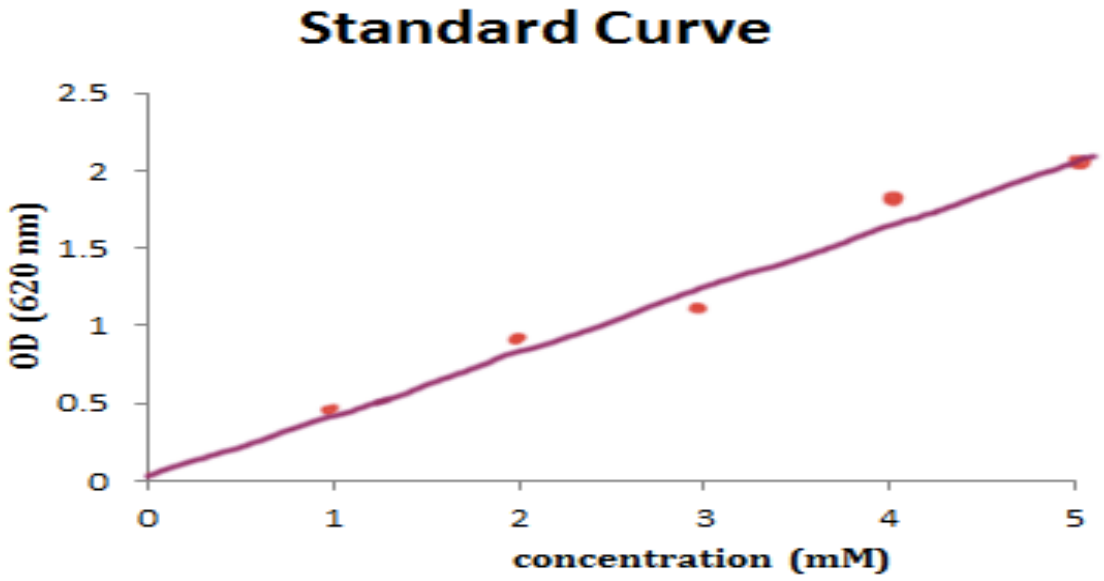
Tyndall Effect:

The Tyndall Effect is the effect of light scattering in many directions in colloidal dispersion, while showing no light in a true solution. This effect is used to determine whether a mixture is a true solution or a colloid. Under the Tyndall effect, the longer-wavelength light is more transmitted while the shorter-wavelength light is more scattered.



Light scattering measurement:

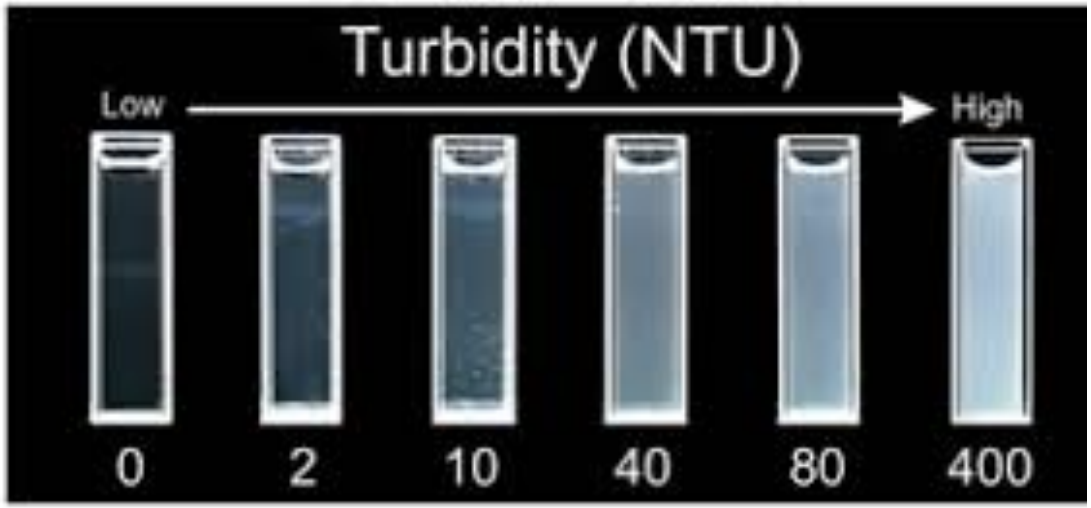
- The amount of light scatter is measured and compared to the amount of scatter from known mixtures (Standards).

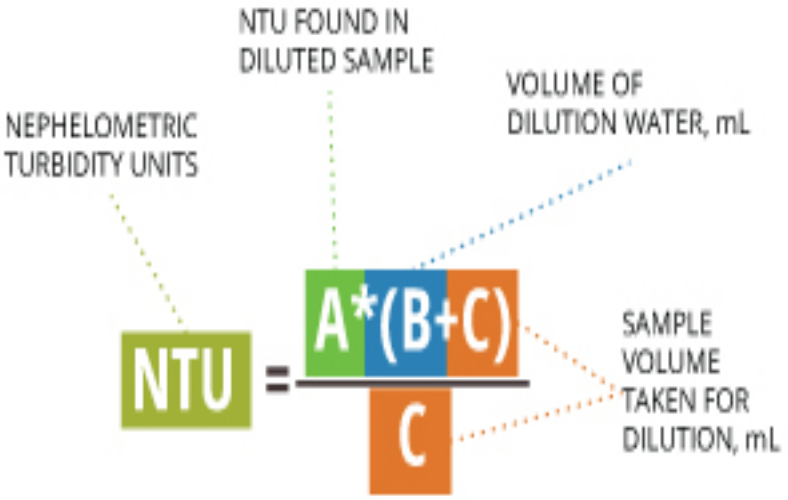


- The amount of the **unknown** is determined from a standard curve.

What is NTU?

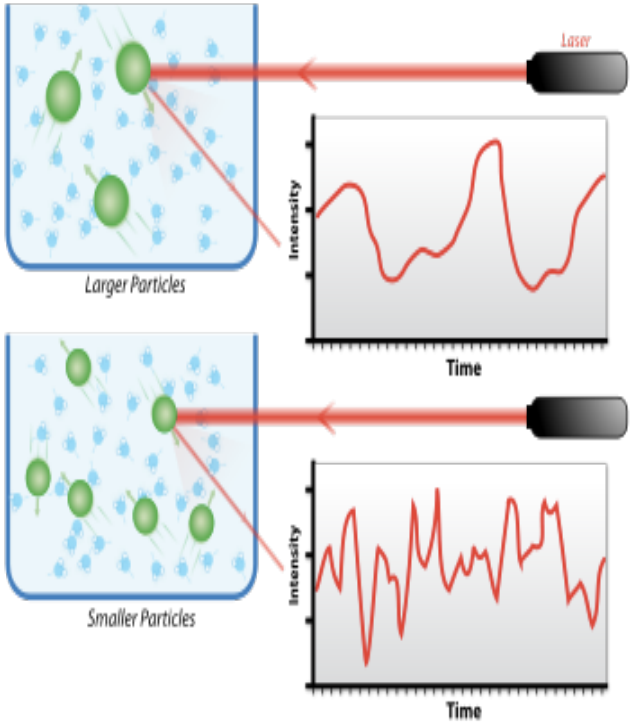
- The units of turbidity from a calibrated nephelometer are called Nephelometric Turbidity Units (NTU). To some extent, how much light reflects for a given amount of particulates is dependent upon properties of the particles like their shape, color, and reflectivity.





Factors affecting light scattering:

1. Particle size & shape
2. Wavelength dependence
3. Distance of observation (Set-up)
4. Polarization of incident light
5. Concentration of particles
6. Molecular weight of particles



Advantage of Nephelometry over Turbidimetry:

1. Higher signal to noise ratio (Uniform scattering)
2. Higher sensitivity (lower detection limit)
3. High precision over turbidimetry

Disadvantages of light scattering techniques:

- High cost
- Easily damaged
- They require high power supply

Clinical applications:

- **Proteins:** albumin in serum, CSF and urine
- **Complements:** alpha-antitrypsin, beta-2 microglobulin
- **Immunoglobulins:** (IgG, IgA, & IgM), free light chains
- **Complement protein:** C3 and C4 , CRP, hs-CRP transferrin, haptoglobin etc.
- **Haptens:** free drugs
- Turbidimetry can be used in biology to find the **number of cells** in a solution

Limitations of light scattering techniques:

- **Matrix effects:** Lipaemic, haemolyzed, and high protein content of the sample

→Trouble shoot by using double distilled water and proper blanking methods.

- **Antigen excess (Hook effect):** When there is high analyte and we get lower concentrations (lower signals)!

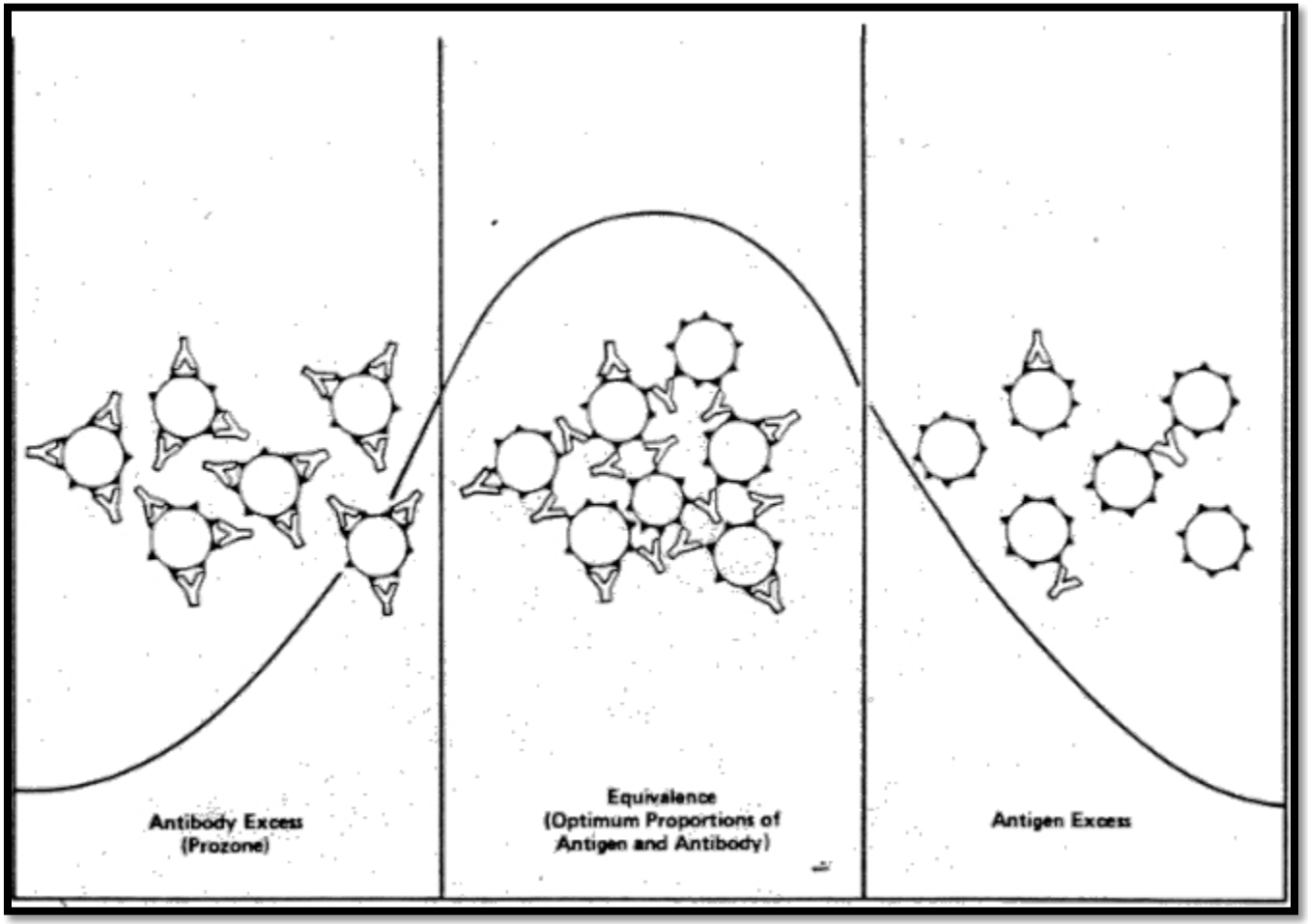
→Causes false negative results in nephelometry analysis

→Prevented by sequential addition of Ag/Ab

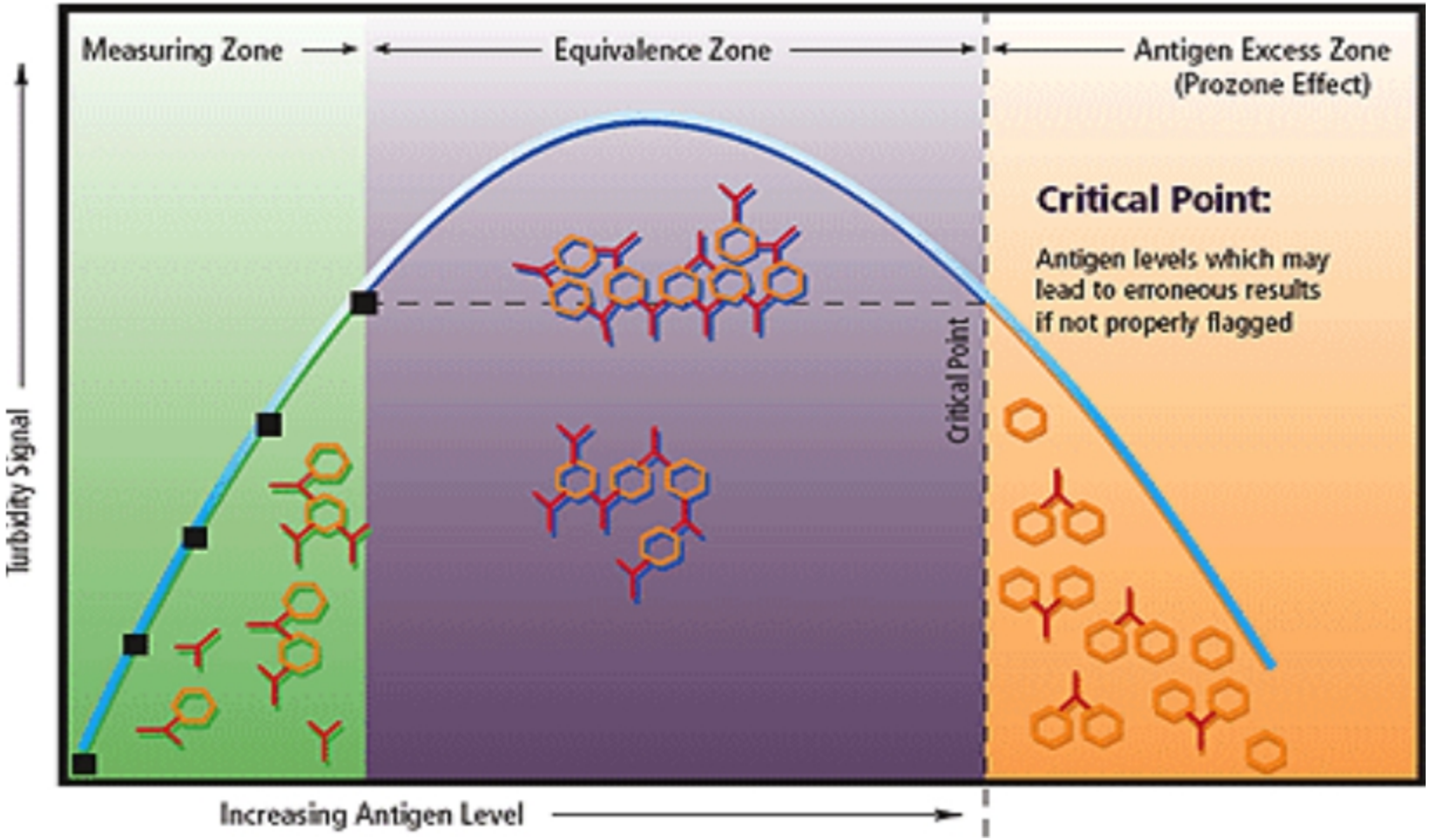
→Addition of washing steps

→Serial dilution for sample

Hook effects:



Hook effects: Causes false Negative



Choice of the method ?

- **TURBIDIMETRY:** - high concentrated suspensions

- **NEPHELOMETRY** - low concentrated suspensions
  - more accurate results

Conclusion:

Nephelometry and Turbidimetry are Analytical methods which have wide range applications from the simple detection of the pollution to determination body constituents hence these play a vital role not only in the analysis of the compounds but also in the clinical analysis.

	Nephelometer	Turbidimeter
Definition	the measurement of the intensity of scattered light at right angles to the direction of the incident light as a function of the concentration of the dispersed phase ,It is most sensitive for very dilute suspensions (100 mg/ L).	Light passing through a medium with dispersed particles, so the intensity of light transmitted is measured.
Instrument used	Nephlometery machine	spectrophotometer
Type of light measured	Scattered light	Transmitted light
Arrangement of photometer	measure the light scattered at right angle to the direction of the propagation of light from the source. It could be movable detectors which allow operator to vary the angle of detection	made in the same direction as the propagation of the light from the source.
Clinical uses	Ag-Ab rxn, immunocomplex rxn,ppts, lipoprotein	Ag-Ab rxn, immunocomplex rxn,ppts, liver dis, protein in urine or CSF

The End