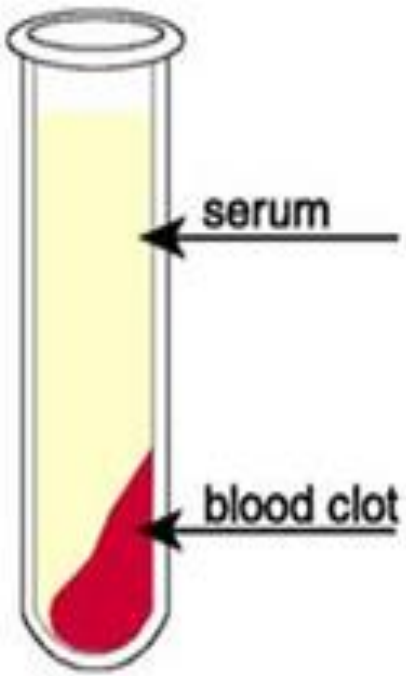
An introduction to sera &vaccines

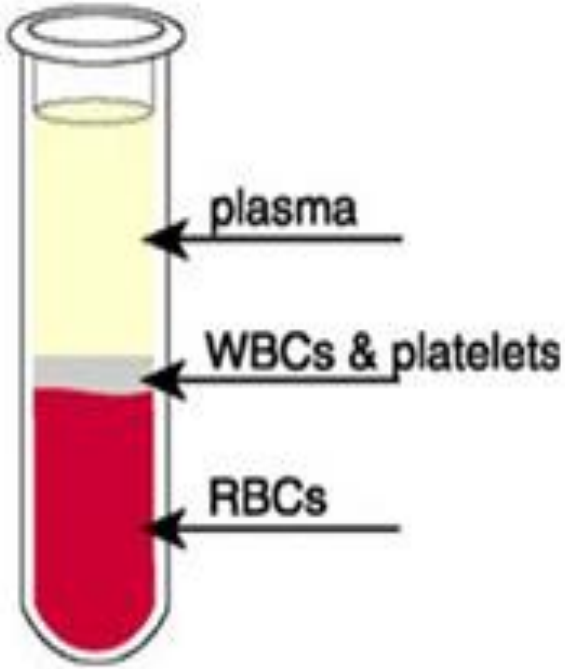
- **Serum** defined as blood plasma without fibrinogens, which includes all proteins not used in blood clotting; all electrolytes, antibodies, antigens, hormones; and any exogenous substances (microorganisms), and Antibodies that have been produced in response to a specific stimulus can be identified easily in the serum**.**
- **Plasma** the liquid part of the blood and lymphatic fluid, which makes up about half of the volume of blood. Plasma is free of cells and, unlike serum, has not clotted, that consists of water and its dissolved constituents including especially proteins (such as albumin, fibrinogen, and globulins).



Serum

vs

Plasma



Serum = Plasma – Clotting Factors

Serology is the scientific study of serum and other body fluids with regard to the response of the immune system to pathogens or introduced substances, study of the antigen-antibody reaction of diagnosis of infectious diseases, autoimmune disorders immune allergies and neoplastic diseases.

- Serological reaction takes different forms, because of variations in the condition of the antigen, the presence of saline and temperature.

- Wide varieties of serologic techniques are available to detect either an antibody or antigen using various materials and reagents.

Collection, preparation and preservation of serological specimens

- Occasionally, serological tests will require plasma, whole blood, urine, spinal fluid and other body fluids.
- Blood specimens should be collected before meal to avoid the presence of chyle, an emulsion of fat globules that often appears in serum during digestion.
- It is ideal and necessary to use sterile dispensable blood collection system using disposable or vactuainers. Blood should be collected by vein puncture.
- If syringes and needles are used care must be taken to allow the blood to run gently into the clean collecting tube to avoid rupturing of cells. The vactuainer system consists of a needle holder and glass vacuum tube instead of the syringe.

- If turbidity or precipitation is present, filtering or centrifugation is recommended.
- Urine specimen containing blood, large amounts of protein, or excessive bacterial contamination should not be used.
- Collect the urine specimen in a clean glass or plastic container. The urine sample may be stored at 2-8^{0}C for up to 72 hours prior to testing.
- Boric acid is also used as preservative for urine sample. For longer storage, the urine sample should be frozen at -20^{0}C.
- Thaw frozen samples by placing the frozen sample in a water bath at 37^{0}C and then mix thoroughly before use.
- If turbidity or precipitation is present after thawing, filtering or centrifugation is recommended. Do not refreeze urine sample.
- Most serological tests should be performed within an hour after sample collection. If this could not be possible, preserve the specimen until the test is done.

- Serum specimen should be refrigerated at 4 – 6°C and if not done with in 72 hours or longer it should be stored at –20°C (frozen).

- Serum which has been frozen may show microclots or fibrin when thawed. This should be removed by centrifugation before specimen is used.

- Sodium azide (1g/L) also used as preservative for blood sample. Specimen to be frozen must be properly sealed and labeled with full patient identification.

- Specimen for cold agglutination must be drawn into warmed syringe & not stored in refrigerator. Care should be taken to transfer the serum to a fresh clean container.

- The sample should be free from hemolyzed blood as this may interfere with serological tests.

- Plasma samples are obtained by treating fresh blood with an anticoagulant, then centrifuging and separating the supernatant.

- Cerebrospinal fluid (CSF) is collected by lumbar puncture. It is usually performed at L3 – L4 or lower to avoid damage to the spinal cord.

- It is usually collected by medical doctors or trained medical officers. Urine collected in clean containers at any time of the day may be used for serological tests.

- Morning urine generally contains the highest concentration of analyte (hormone). It should have a specific gravity of at least 1.015.

Determination of end point and titer

- Serum may need to be diluted in a single or as a serial dilution if it contains a concentrated amount of antibody.
- After serially diluting the patient serum, equal amount of an antigen is added to each dilution to observe the immunologic reaction.
- The last tube that shows a visible immunologic reaction is known as the end point of the test, the dilution of antiserum (antibody) at the end point is known as the titer.

- The reciprocal of the greatest reacting dilution of the serum is considered as the measure of titer or the concentration of antibody.

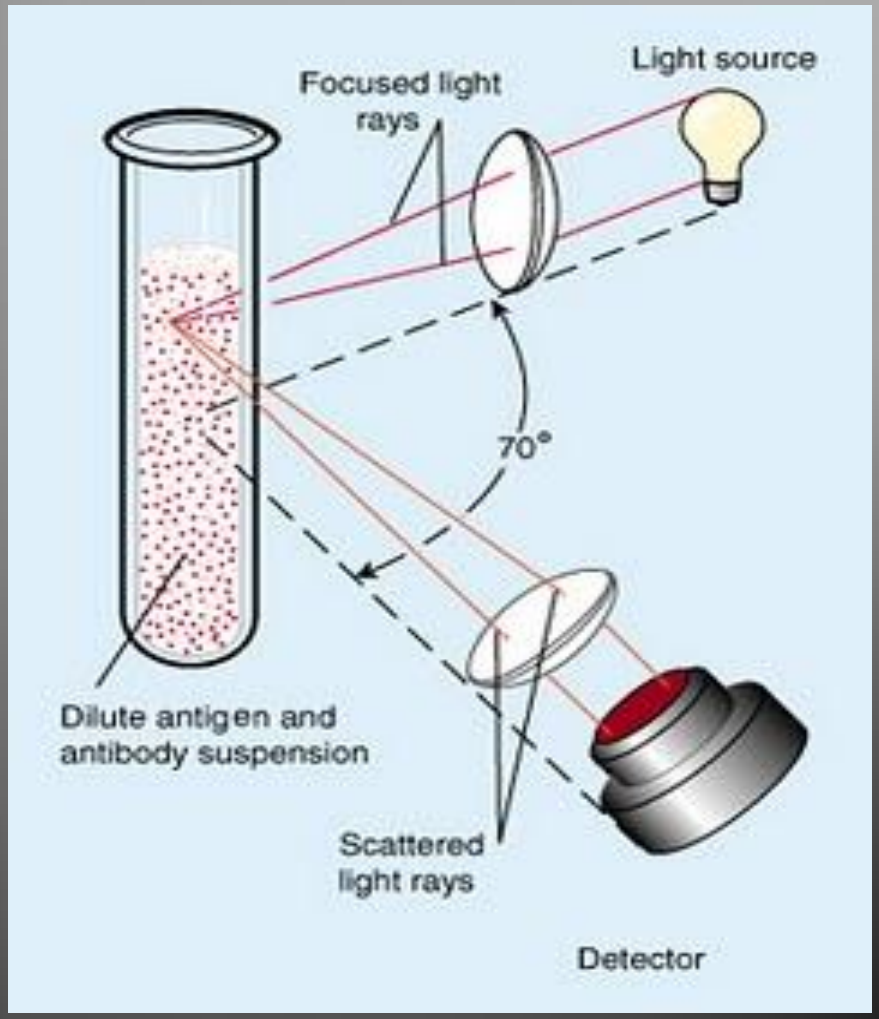
- For example, if the highest dilution of the serum that shows a visible reaction is a 1:32 dilution, the titer of the test is expressed as 32

Basic Immunologic Procedures

- Measurement by Light
- Precipitation
- Electrophoretic Techniques
- Agglutination Reactions – still popular
- Labeled reactions – very popular
- Molecular techniques – gaining in popularity especially for pathogens.

Nephelometry

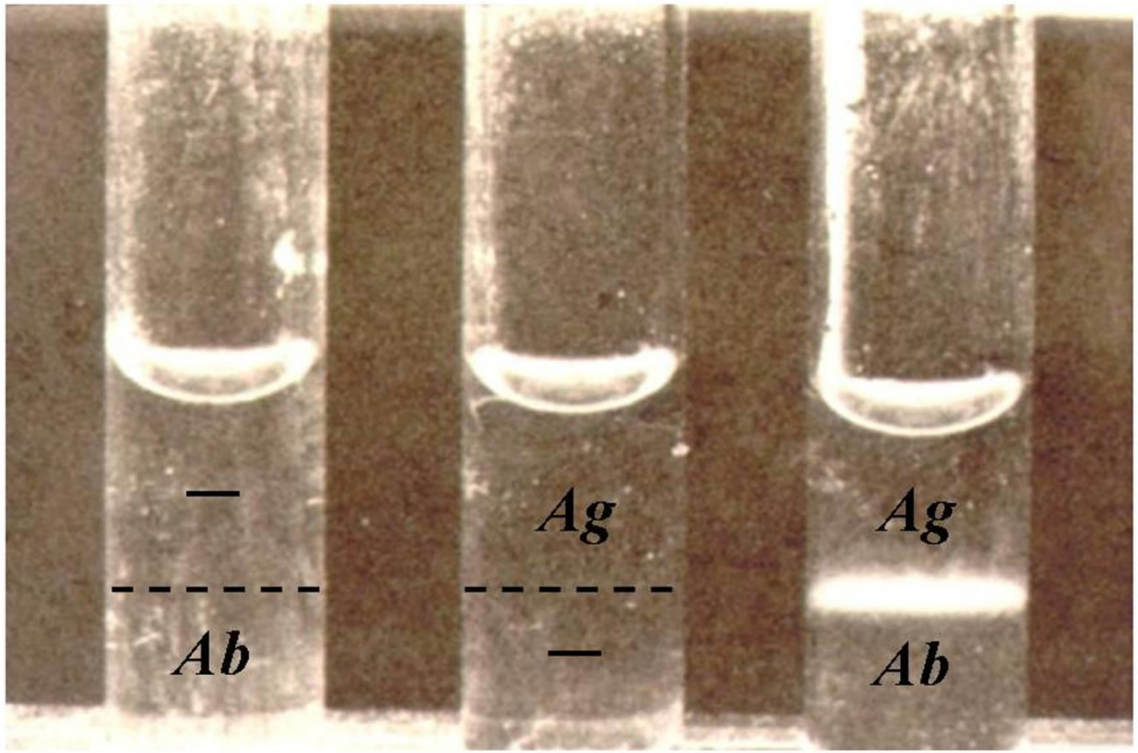
- Measures turbidity of sample by passing light thru it, amount of light scatter is measured.
- Two types:
  - Endpoint – reaction goes to completion
  - Kinetic – light scatter measured at specific time. Reaction occurs at a steady rate and the timing of measurement can be done.



Precipitation

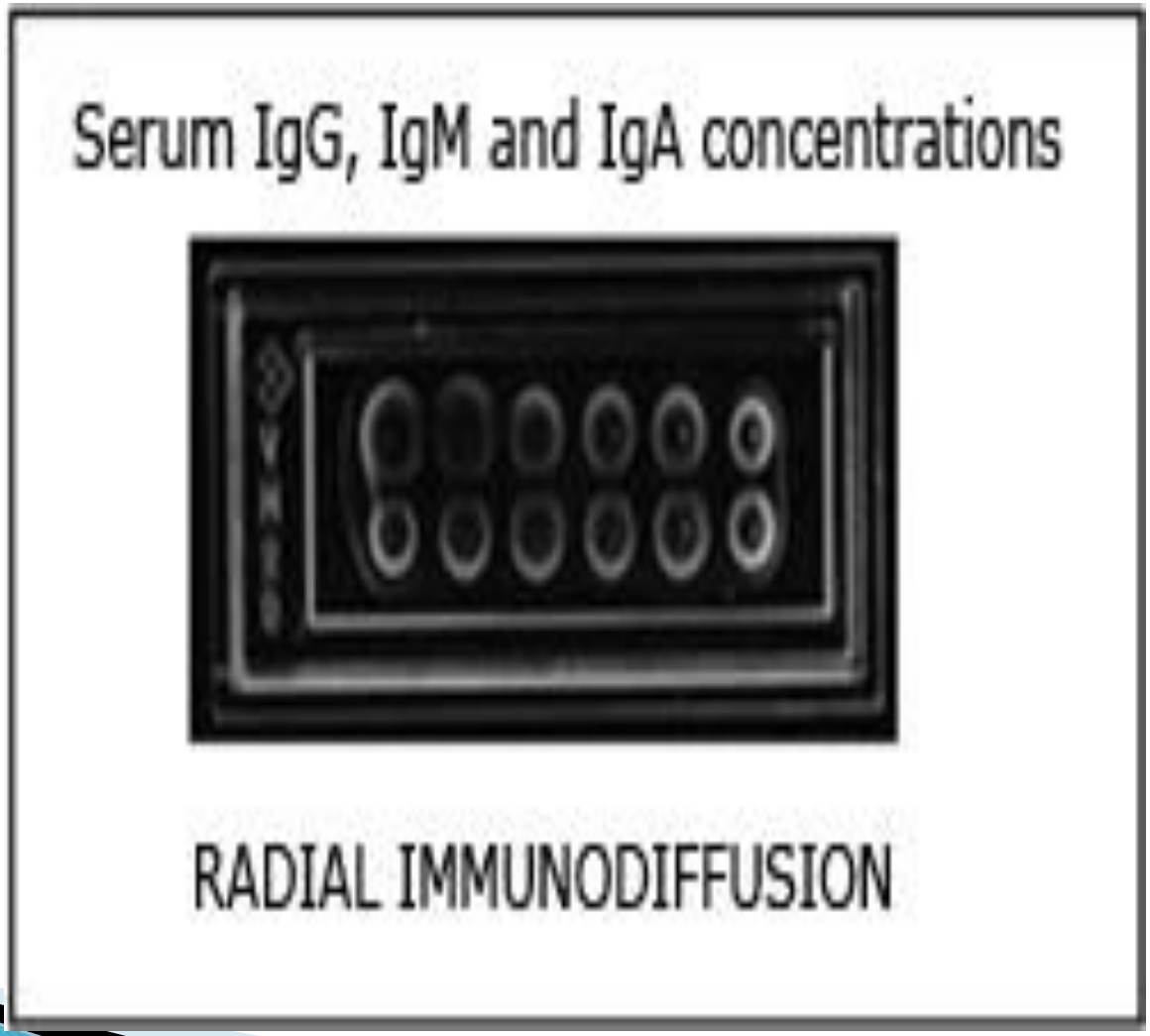
- Involves combining soluble antigen with soluble antibody to produce visible, INSOLUBLE complexes.
- Relative concentrations of antigen and antibody must be equal

Liquid Precipitation

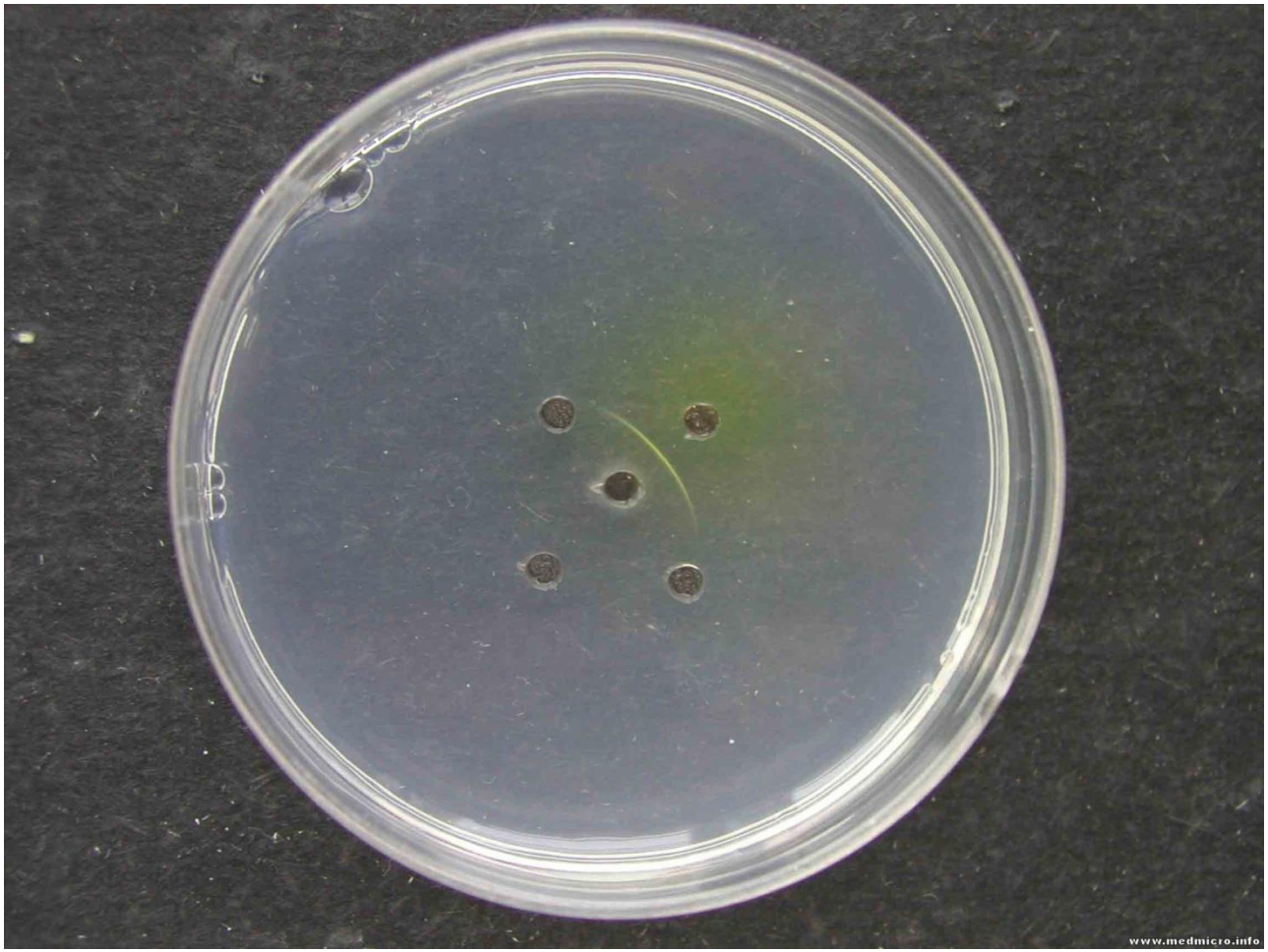


Precipitation in tubes

Gel Precipitation: Radial Immunodiffusion (RID)

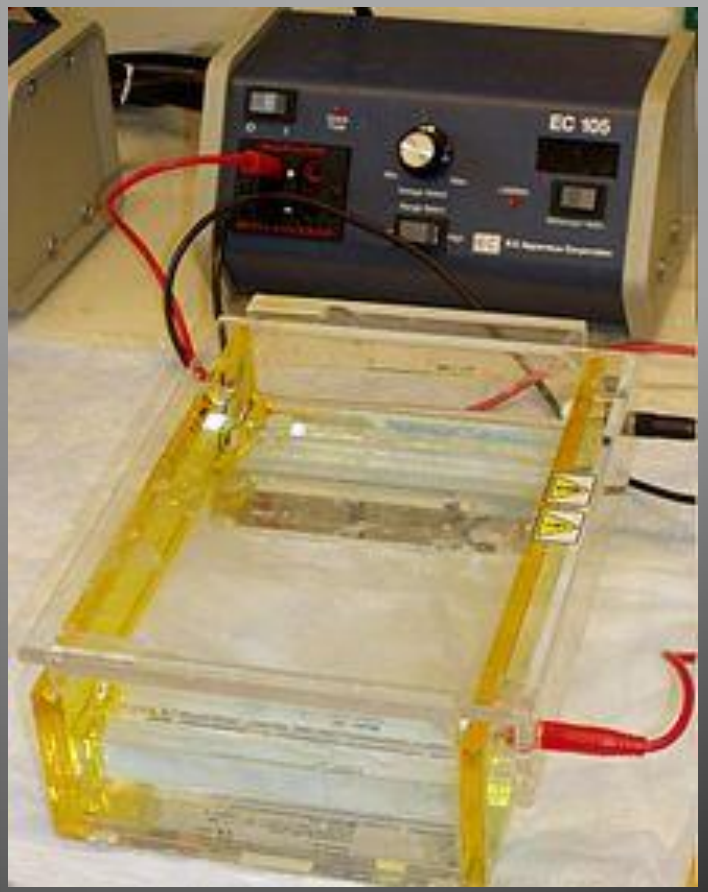


Double Gel Diffusion : Ouchterlony



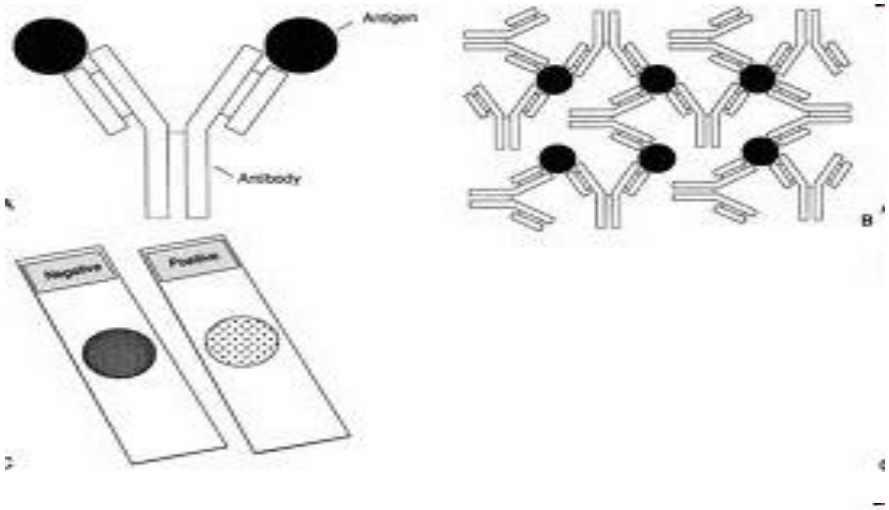
Electrophoresis

- Process of separating proteins in a mixture utilizing their different net electrical charges
- Size and shape can cause frictional drag
- Types
  - Moving Boundary
  - Disc
  - Capillary zone
  - Immunoelectrophoresis



Agglutination

- Occurs in two stages:
  - Sensitization – cannot be seen
  - Lattice formation – visible
- Antigen or antibody can be coated onto or an integral part of a carrier particle:
  - Latex particles
  - Red blood cells-- HEMAGGLUTINATION
  - Charcoal
  - Bacteria
- Agglutination indicates presence of substance being tested for.



Labeled Reactions

- One of the reactants labeled with a tag:
  - I^{125} – measure radioactivity
  - Enzyme – color or intensity of color measured.
  - Measure intensity of light emitted as a result of reaction.
  - Fluorescence

Molecular

- Rapidly exploding field.
- Polymerase Chain Reaction (PCR) allows replication of genetic material specific to an infectious agent or malignancy.
- Probe is prepared which has target sequence.
- Use thermocycler to cause DNA to denature (separate) then cool to cause annealing to probe.
- Amplify the specific target.