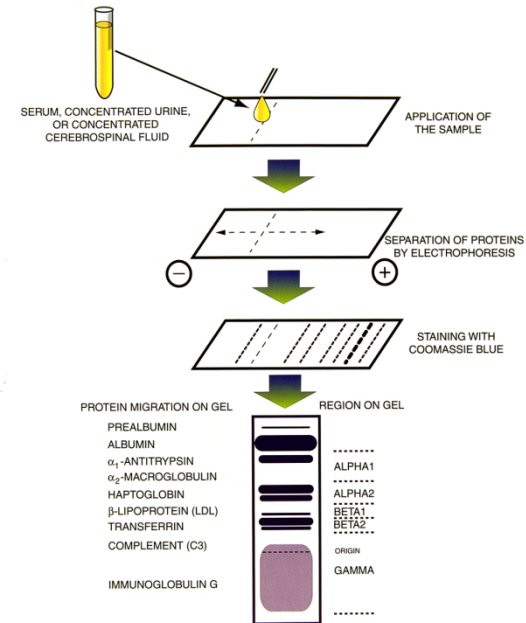
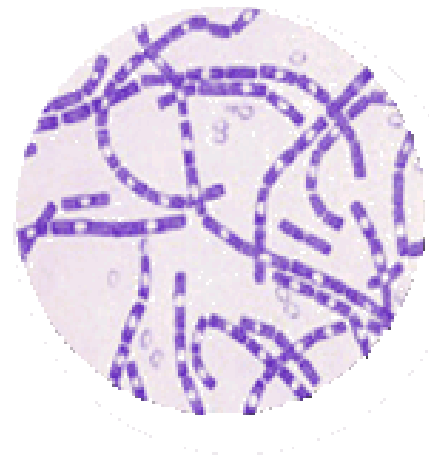


# Introduction to BioMEMS & Medical Microdevices

## *Intro to Clinical Laboratory Medicine*

Prof. Steven S. Saliterman, <http://saliterman.umn.edu/>



# Topics

- Specimen collection.
- Studies by categories include:
  - Chemistries & Immunology.
    - Antibodies
    - ELISA & other techniques.
  - Hematology
    - Hemopoiesis
  - Microbiology
  - Urinalysis
  - Anticoagulation
  - Arterial Blood Gases.

# Specimen Collection

## Obtaining a Specimen by Venipuncture



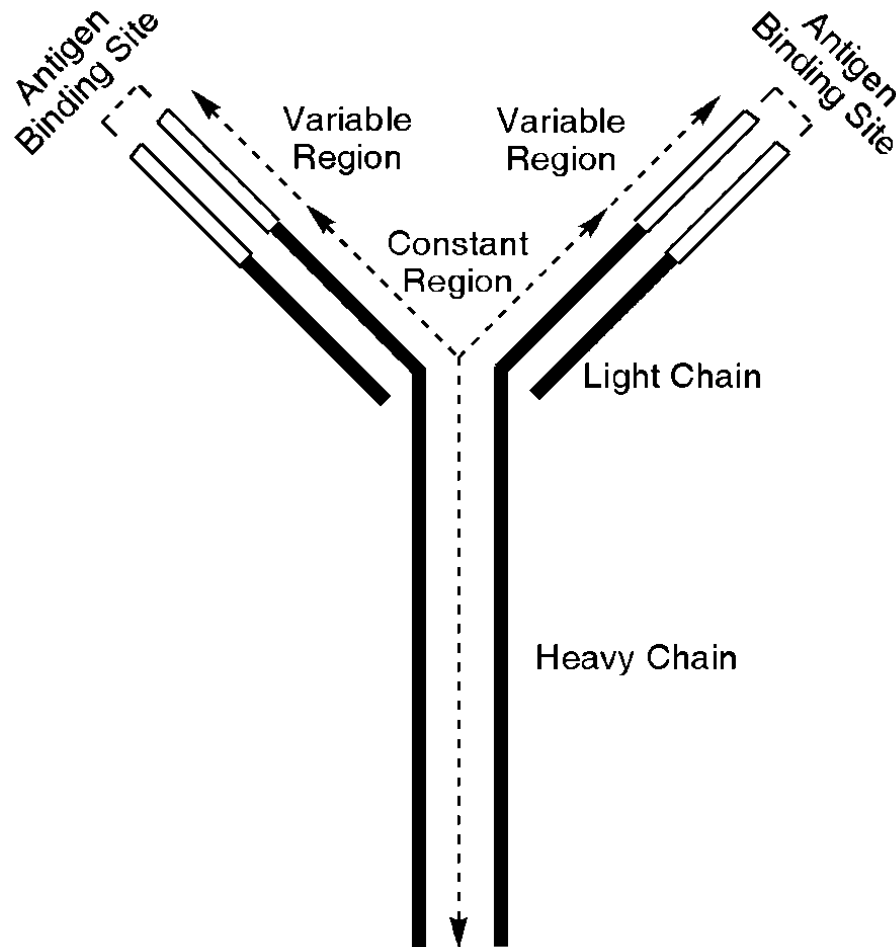
# *Specimen Processing...*





# 1. Chemistries & Immunology

## Antibodies



### Variable Regions

Determine antigen specificity.

Subdivided into the hypervariable (HV1-3) & framework regions (FR).

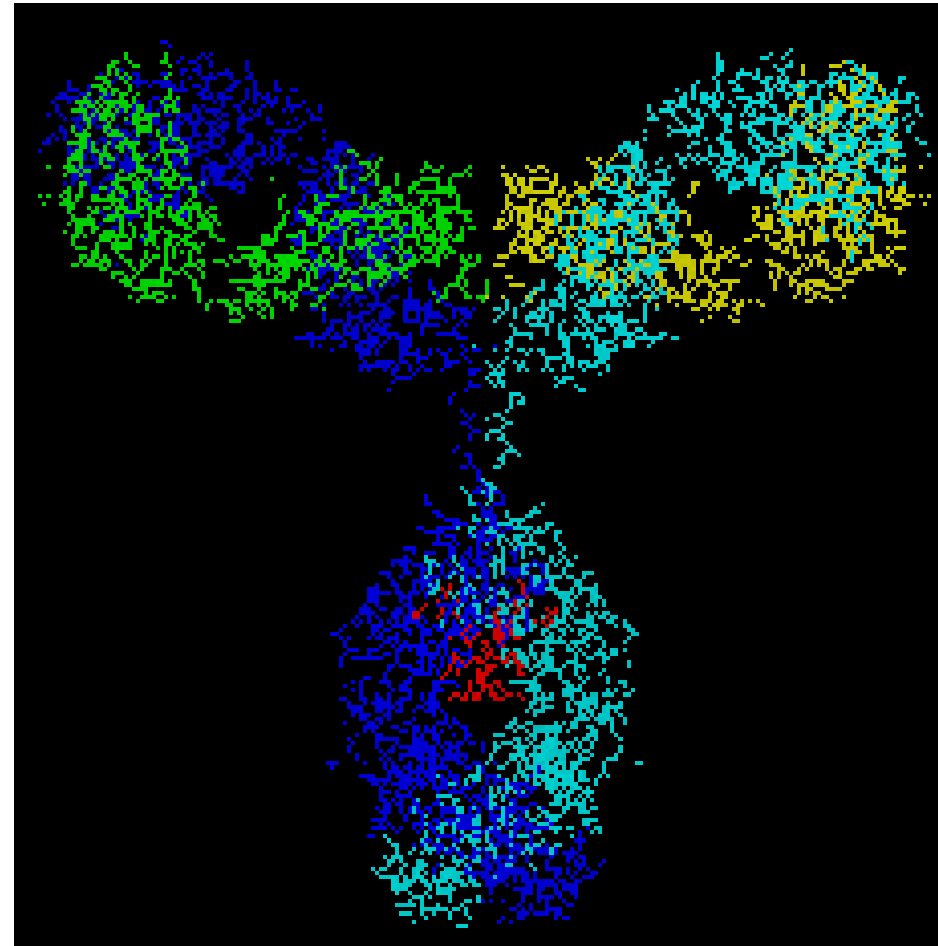
Proteases may cleave this region, leaving the *FAB* (fragment antigen binding).

### Constant Regions

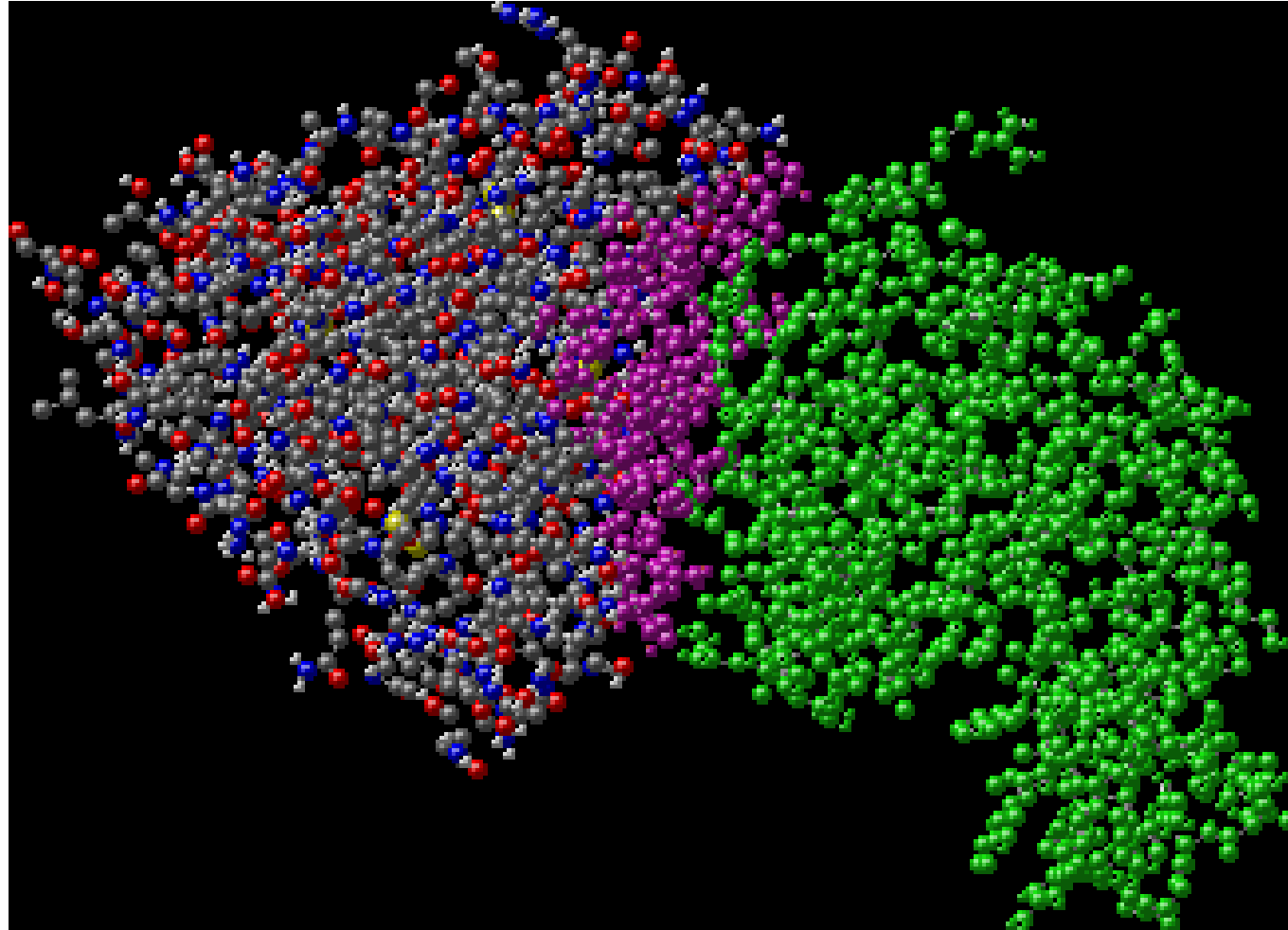
Determines the mechanism to destroy the antigen.

## *e.g. IgG Antibody...*

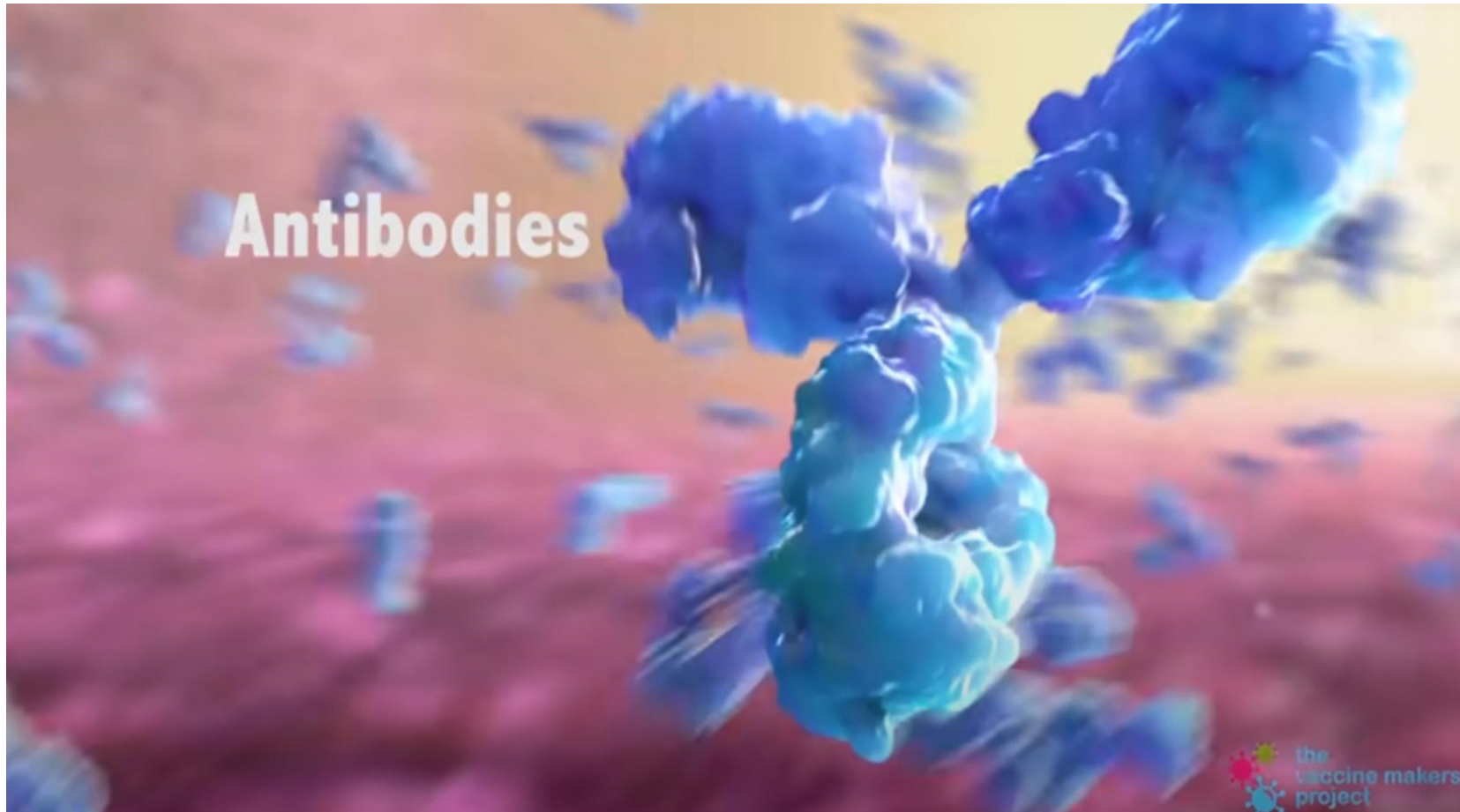
- Heavy chains in **blue** and **blue-green**.
- Light chains in **green** and **yellow**.
- Carbohydrate in **red**.



# *Antibody-Antigen Interaction...*



# *Antibodies at Work...*



# ELISA

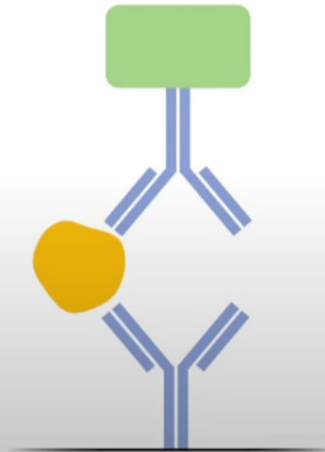


- The Enzyme-linked Immunosorbent Assay (ELISA)
- A biomolecular technique that utilizes the specificity of an antibody, as well as the sensitivity of enzyme assays, to detect and quantify molecules such as hormones, peptides, antibodies, and proteins.
- Uses:
  - Identification of cancer biomarkers for early detection of cancer.
  - Drug screening (urine) and concentrations in patients undergoing treatment.
  - Pregnancy screening.
  - Detection of platelet antibodies – e.g. idiopathic thrombocytopenic purpura (ITP) and systemic lupus erythematosus.
  - Virus detection e.g. HIV (human serum cystatin C), and West Nile virus.



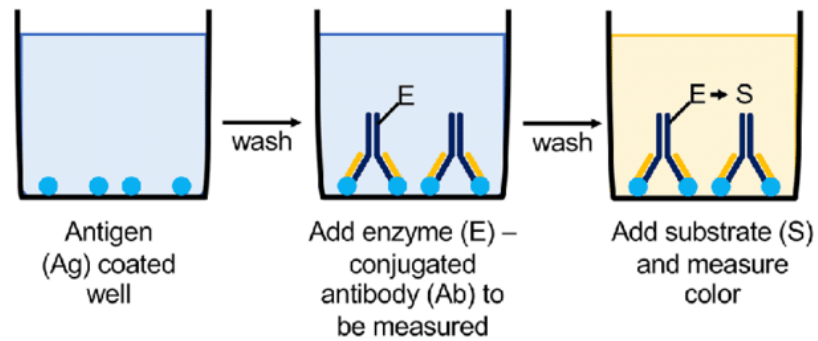
*ELISA...*

# ELISA

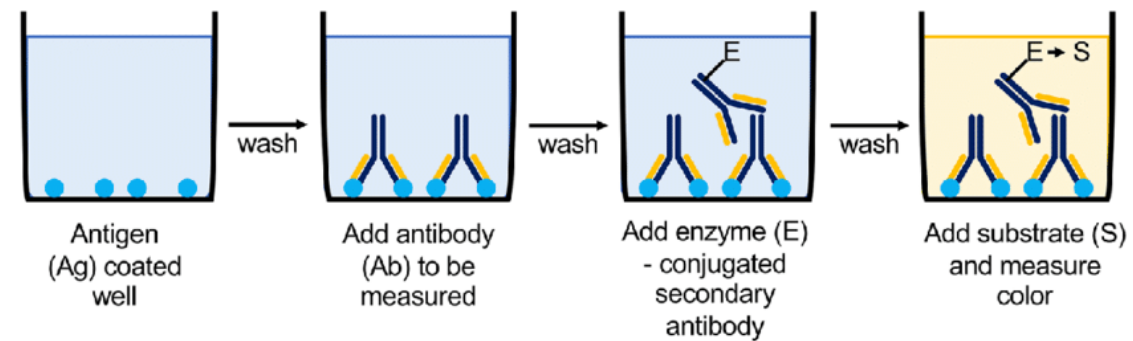


# Methodologies...

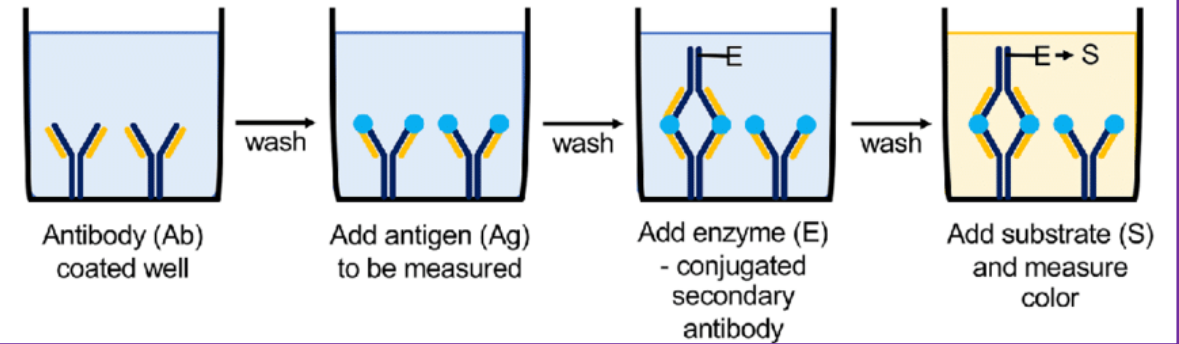
**(a) Direct ELISA**



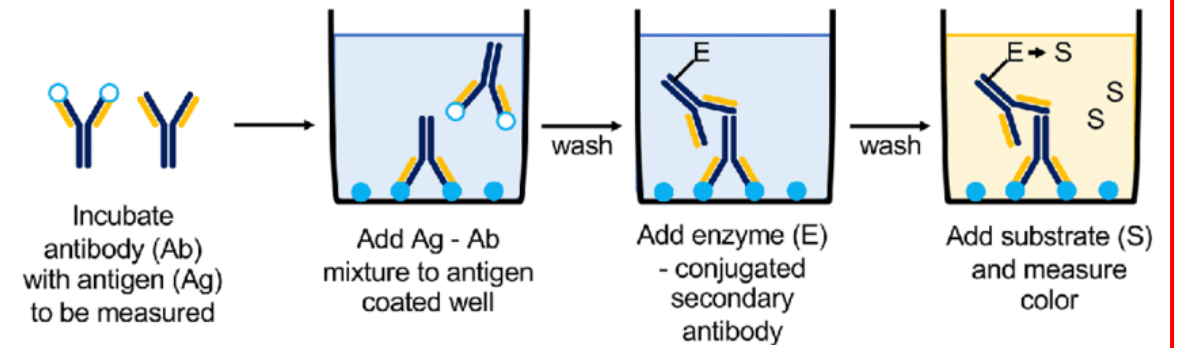
**(b) Indirect ELISA**



**(c) Sandwich ELISA**



**(d) Competitive ELISA**



# Chemiluminescence Immunoassays



- Rapid and accurate diagnosis of autoimmune disease.
  - SLE, Rheumatoid Arthritis, Sjogrens syndrome, systemic sclerosis, antiphospholipid syndrome, celiac disease, autoimmune thyroid diseases, primary biliary cirrhosis, and autoimmune disease.
- The *label* is a luminescent molecule. (luminescence is the emission of visible or near-visible 300–800 nm).
- Chemiluminescent methods
  - *Direct*—using luminophore markers (acridinium and ruthenium esters).
  - *Indirect*—using enzyme markers (alkaline phosphatase with adamantyl 1, 2-dioxetane aryl phosphate (AMPPD) substrate and horseradish peroxidase with luminol or its derivatives as substrate).
  - Either method may be competitive or non-competitive.

# Chemiluminescence...

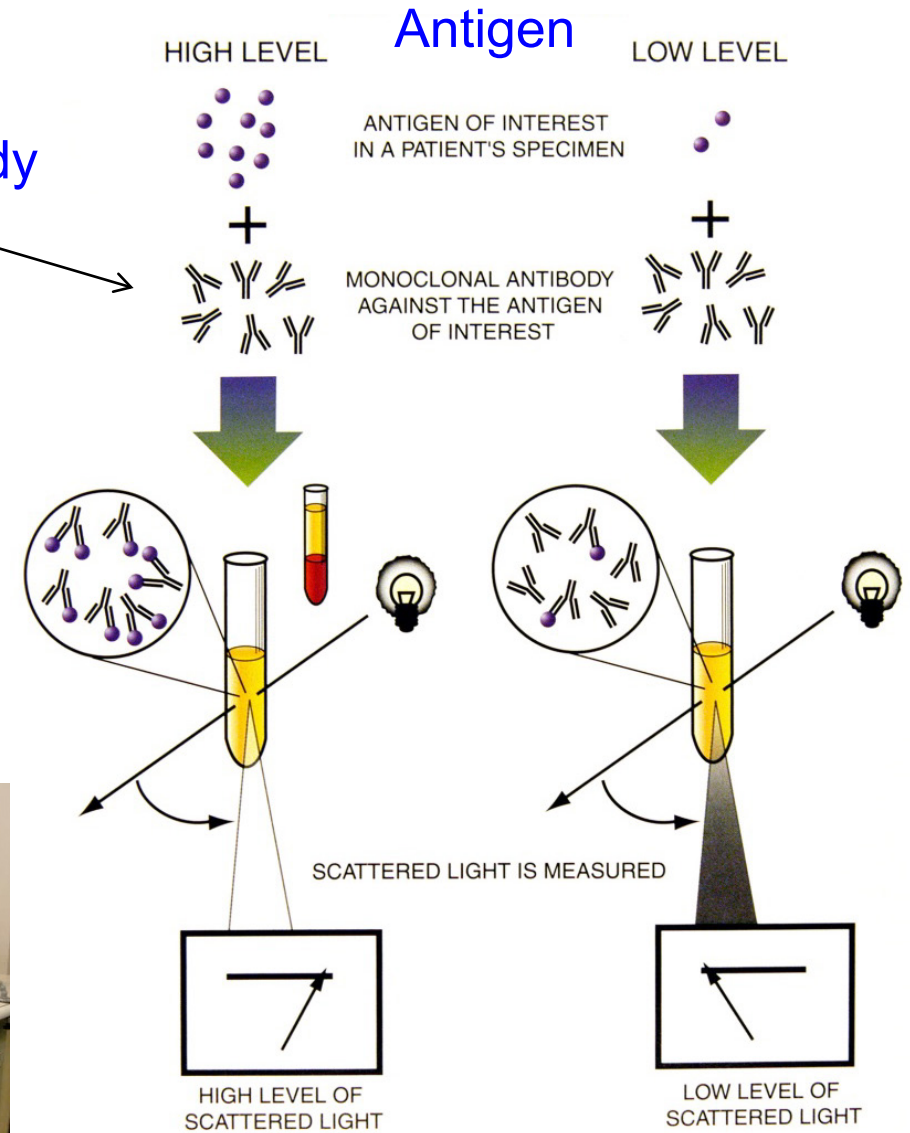


# Nephelometry

- Nephelometry/ turbidimetry is based on the scattering or absorption of light by solid or colloidal particles suspended in solution.
- Used in immunology to determine the levels of several blood plasma proteins.



Monoclonal Antibody



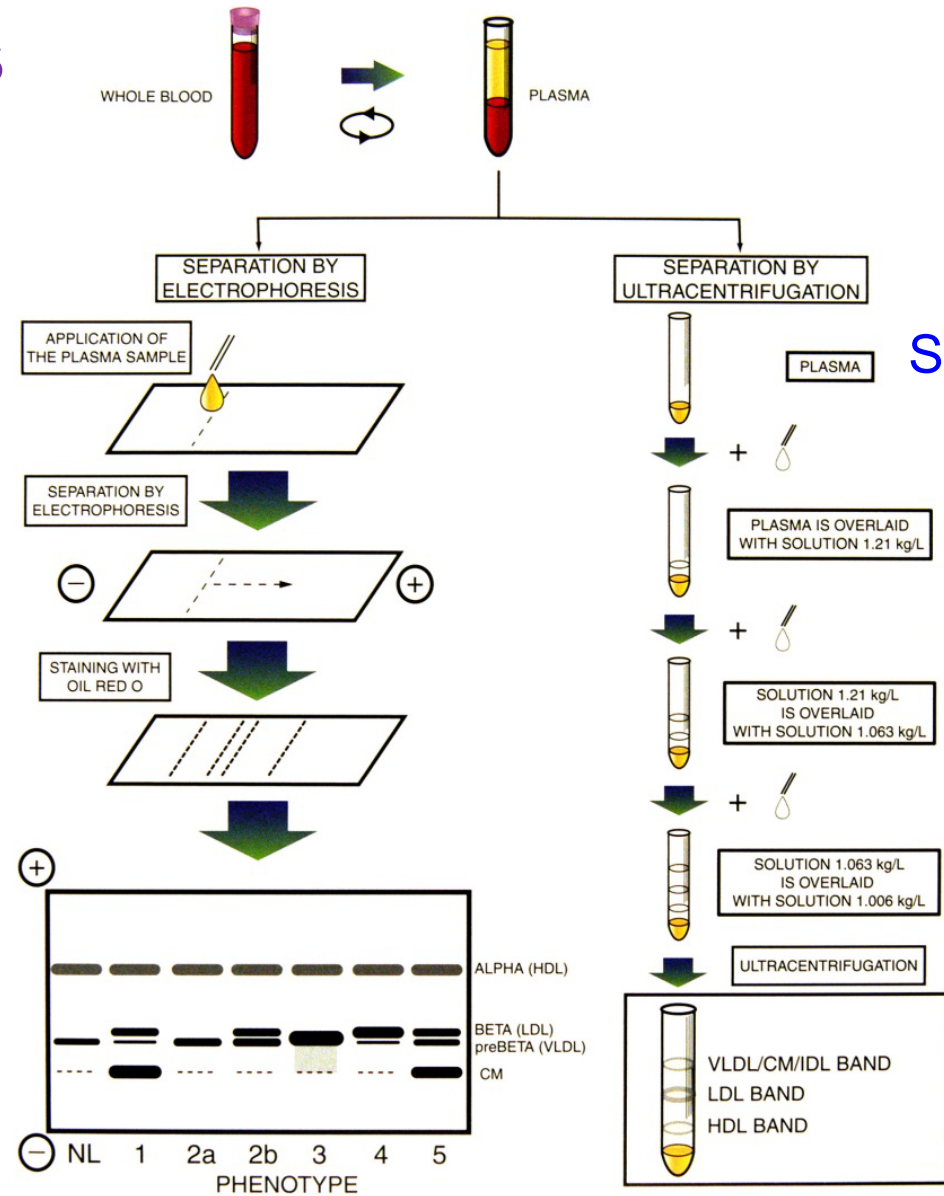
Measurement of Scattered Light



# Lipoprotein Analysis

## Separation by Electrophoresis

Staining

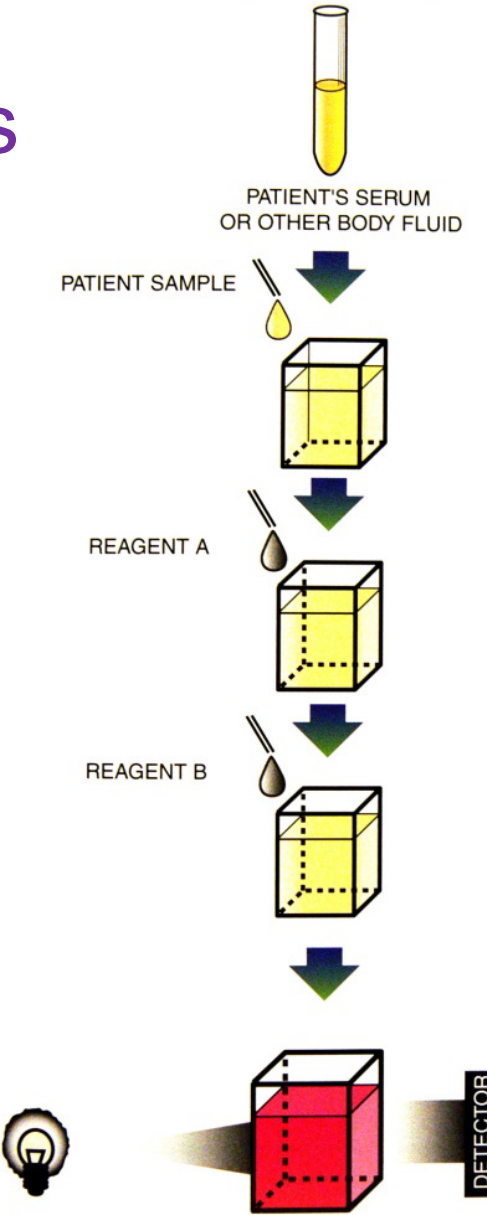
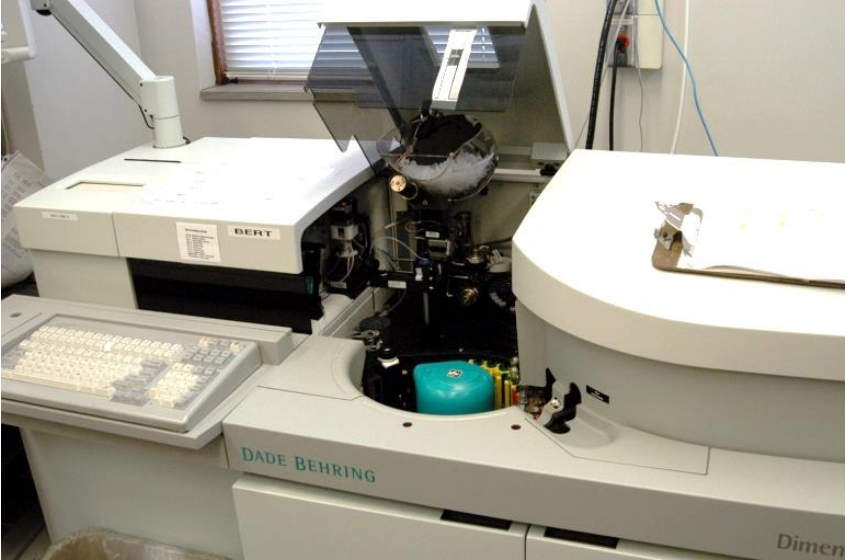


## Separation by Ultracentrifugation

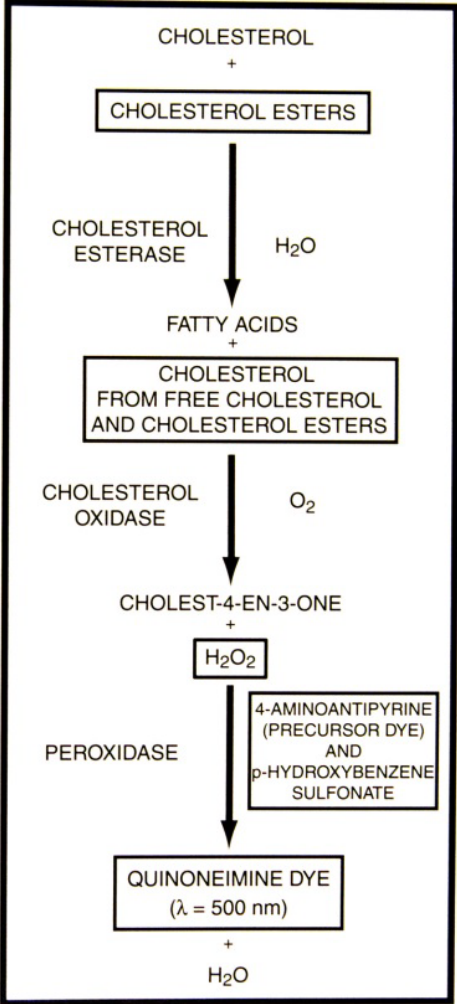
## VLDL, LDL and HDL Band Separation

CM, chylomicrons; HDL, high density lipoproteins; IDL, intermediate density lipoproteins; LDL, low density lipoproteins; VLDL, very low density lipoproteins.

# Spectrophotometric Assays

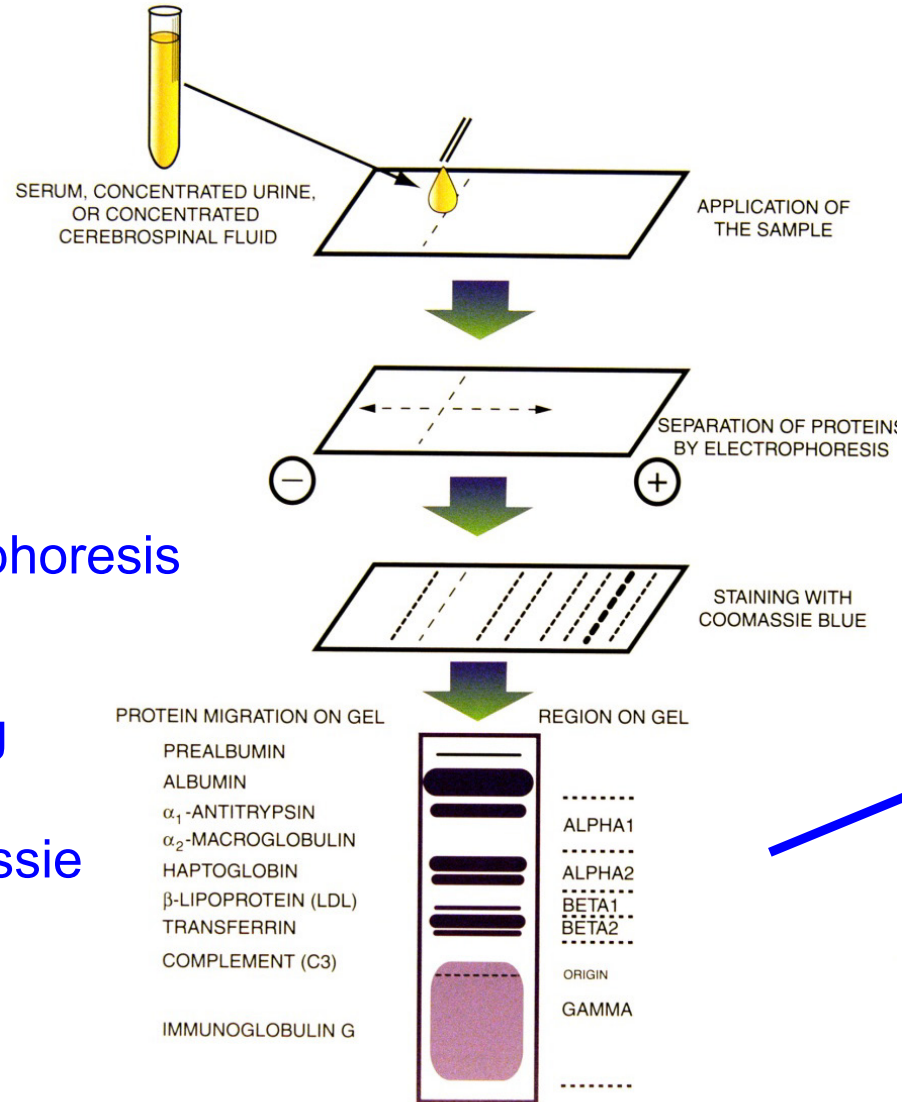


EXAMPLE OF A CLINICAL LABORATORY TEST USING SPECTROPHOTOMETRY TO QUANTITATE THE COMPOUND OF INTEREST



Laposata M, *Laboratory medicine, Clinical Pathology in the Practice of Medicine*, ASCP Press, Chicago (2002).

# Protein Electrophoresis



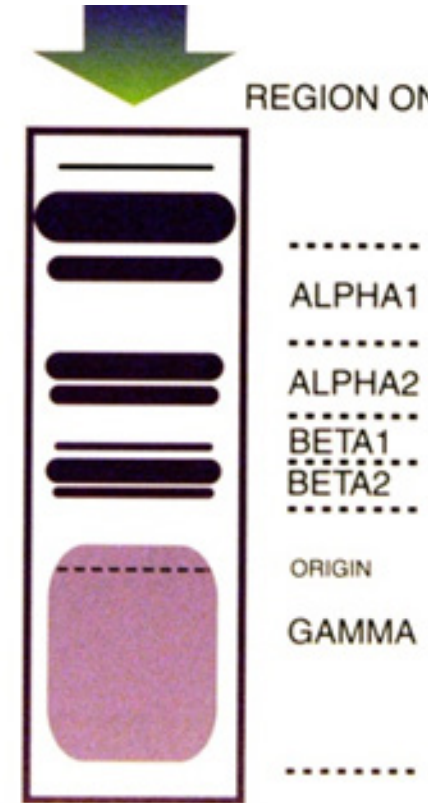
Electrophoresis

Staining with Coomassie Blue

PROTEIN MIGRATION ON GEL

PREALBUMIN  
ALBUMIN  
 $\alpha_1$ -ANTITRYPSIN  
 $\alpha_2$ -MACROGLOBULIN  
HAPTOGLOBIN  
 $\beta$ -LIPOPROTEIN (LDL)  
TRANSFERRIN  
COMPLEMENT (C3)  
IMMUNOGLOBULIN G

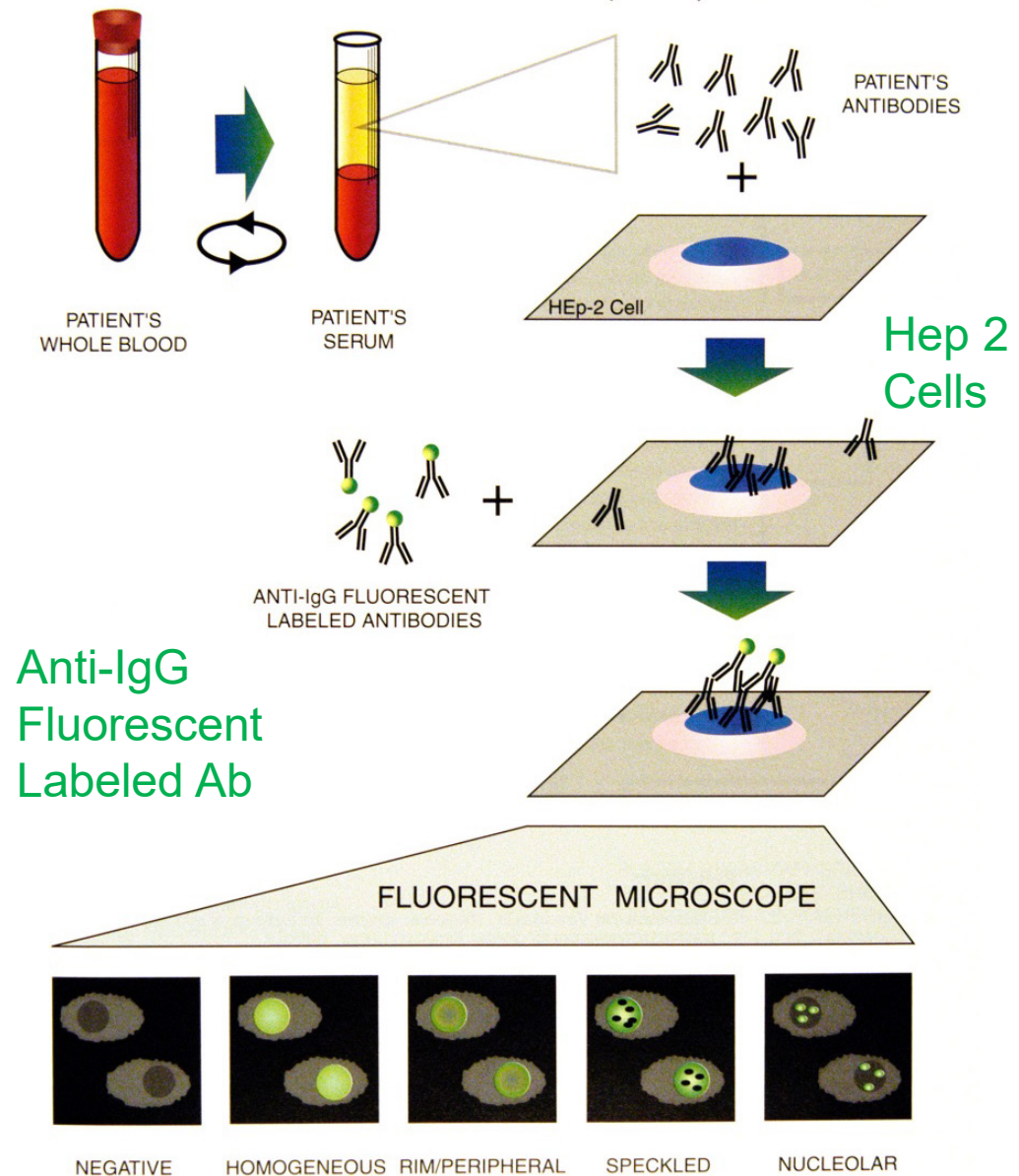
REGION ON GEL





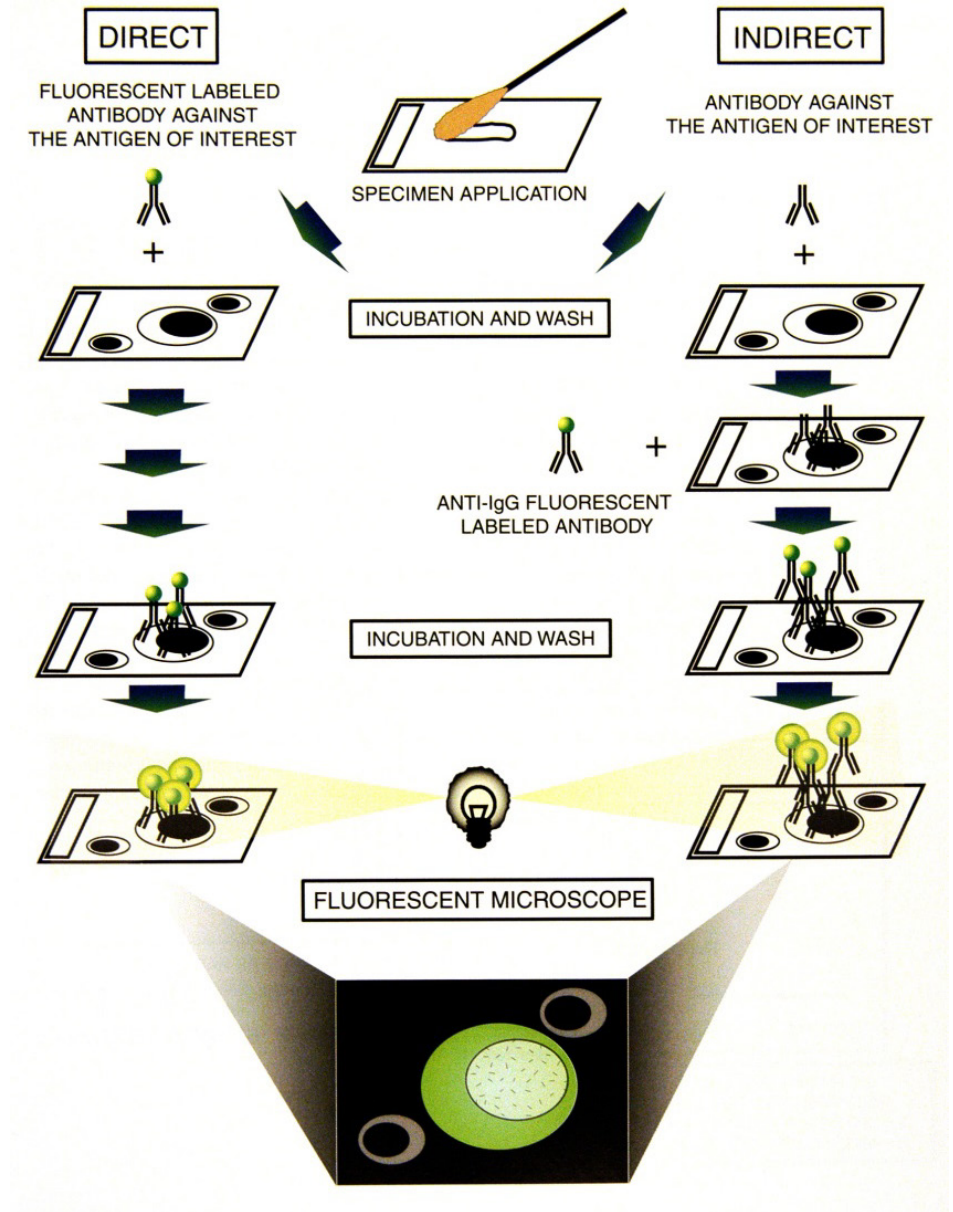
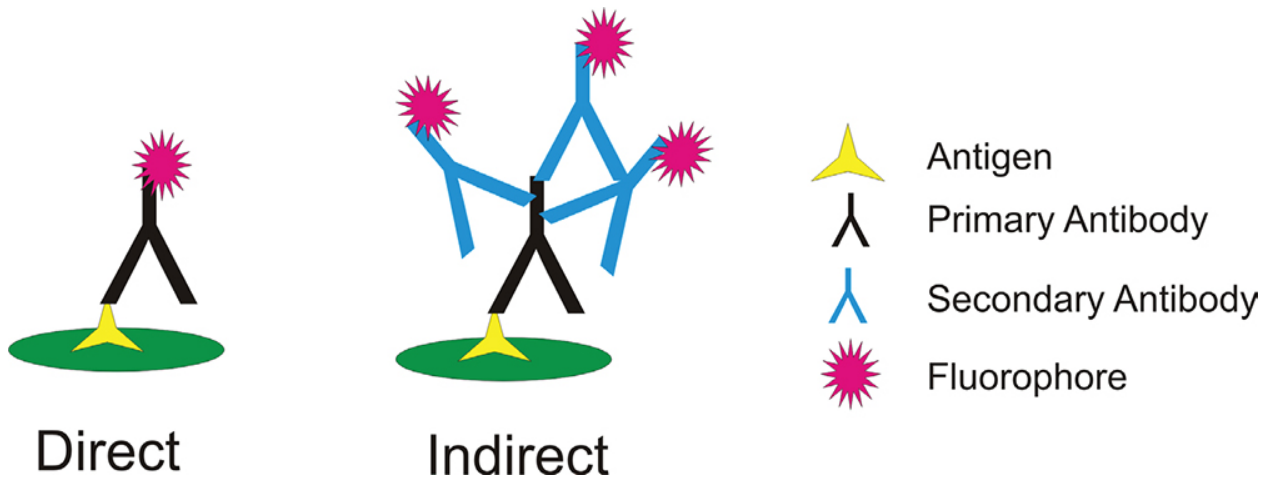
# Antinuclear Antibodies (ANAs)

- Normally antibodies, produced by white blood cells (B cells) recognize and combat infectious organisms in the body.
- ANAs are produced by a person's immune system, and mistakenly directed towards normal, naturally-occurring proteins in our bodies.
- By itself, a positive ANA test does not indicate the presence of an autoimmune disease or the need for therapy.
- Diseases include lupus, scleroderma, Sjögren's syndrome, polymyositis/dermatomyositis, mixed connective tissue disease, drug-induced lupus, autoimmune hepatitis, and in juvenile arthritis.



# Direct & Indirect Immunofluorescence

- Direct immunofluorescence uses a fluorophore-conjugated antibody to stain the target protein.
- Indirect immunofluorescence involves first binding the primary antibody to the target, then detecting the primary antibody using a conjugated secondary antibody.

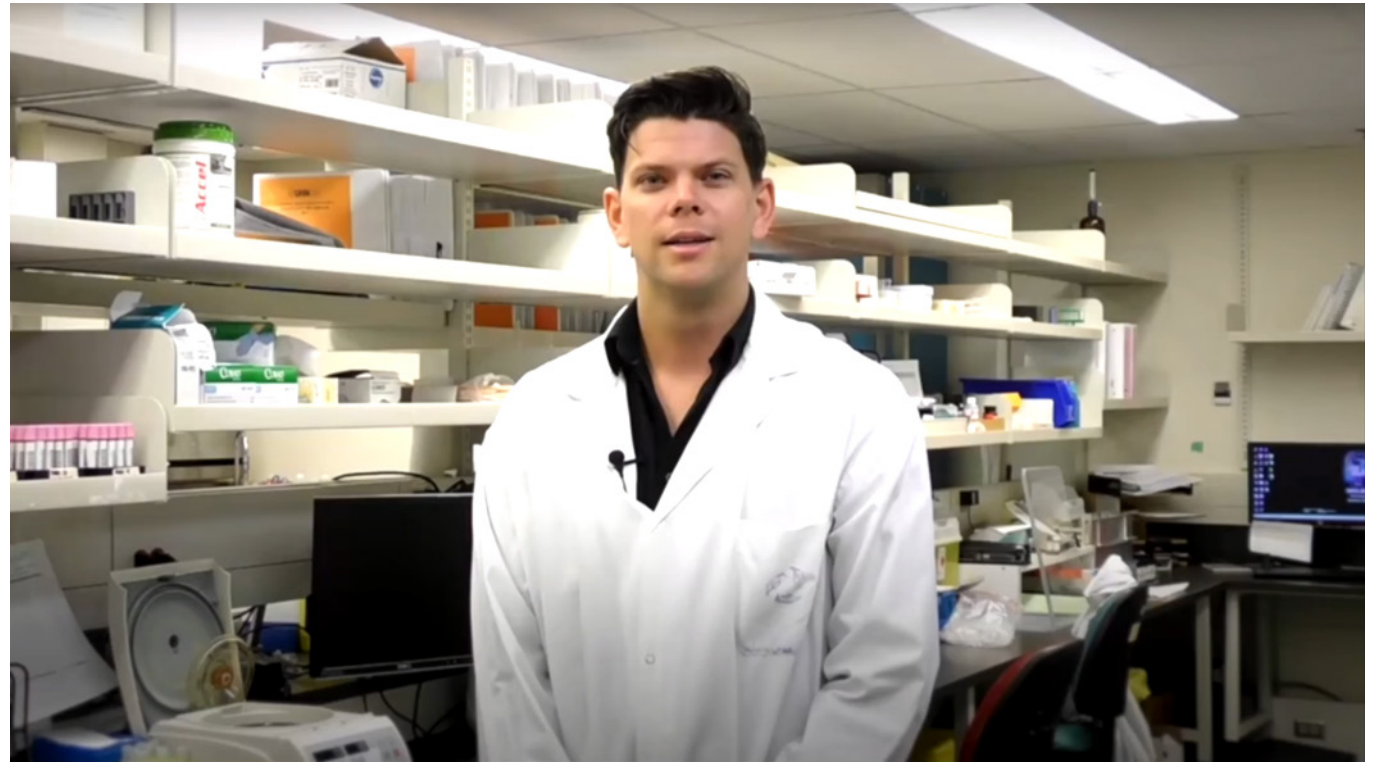
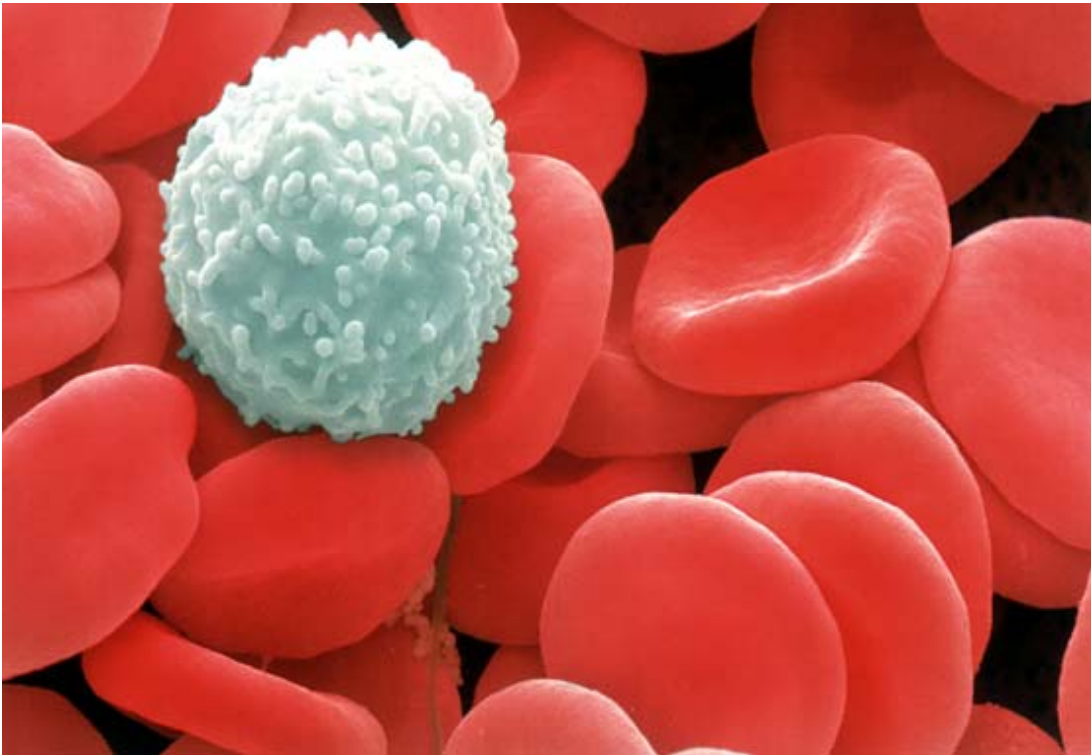


Left: Image courtesy of BioTEK

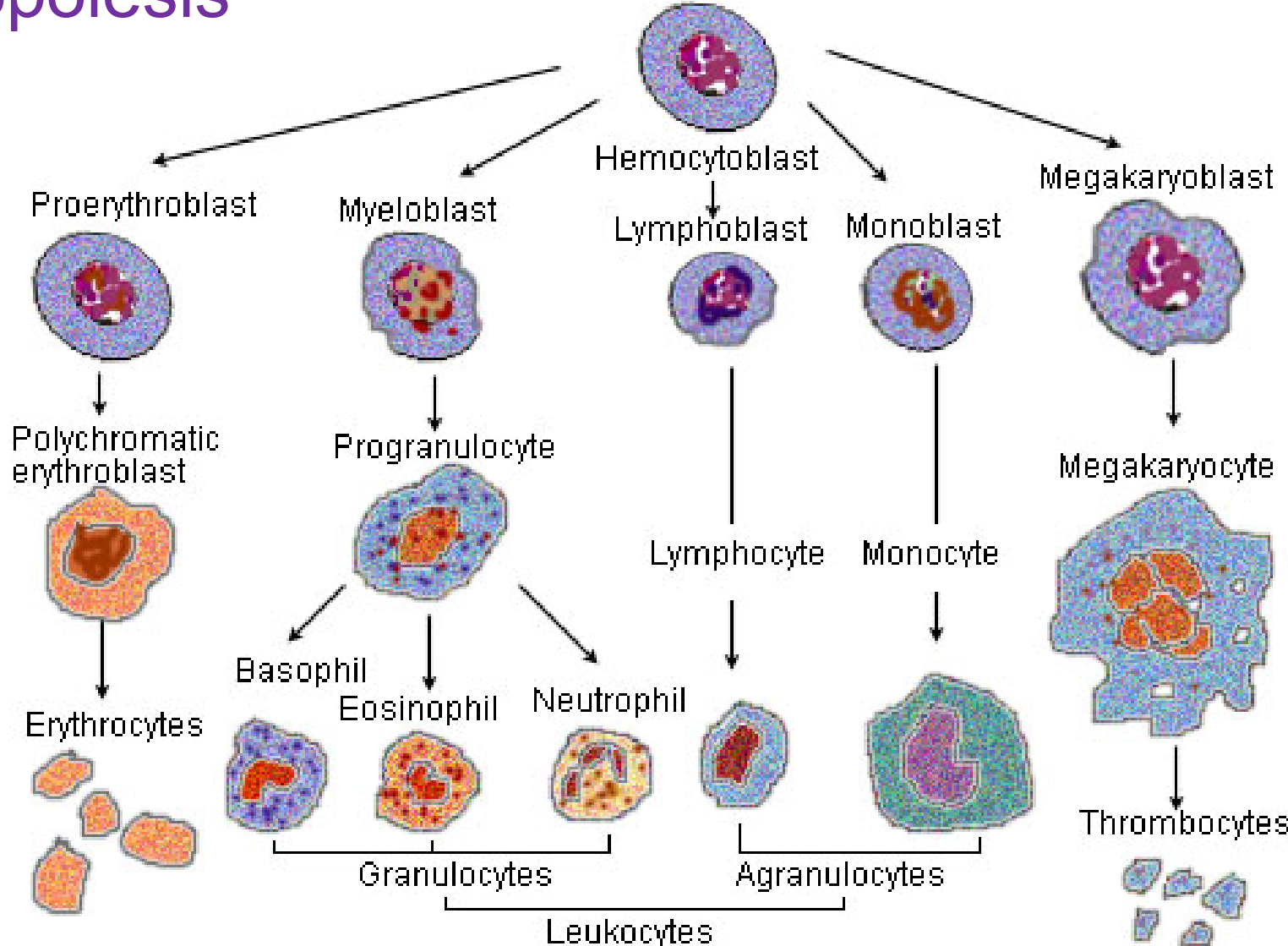
Right: Laposata M, *Laboratory Medicine, Clinical Pathology in the Practice of Medicine*, ASCP Press, Chicago (2002).



## 2. Hematology



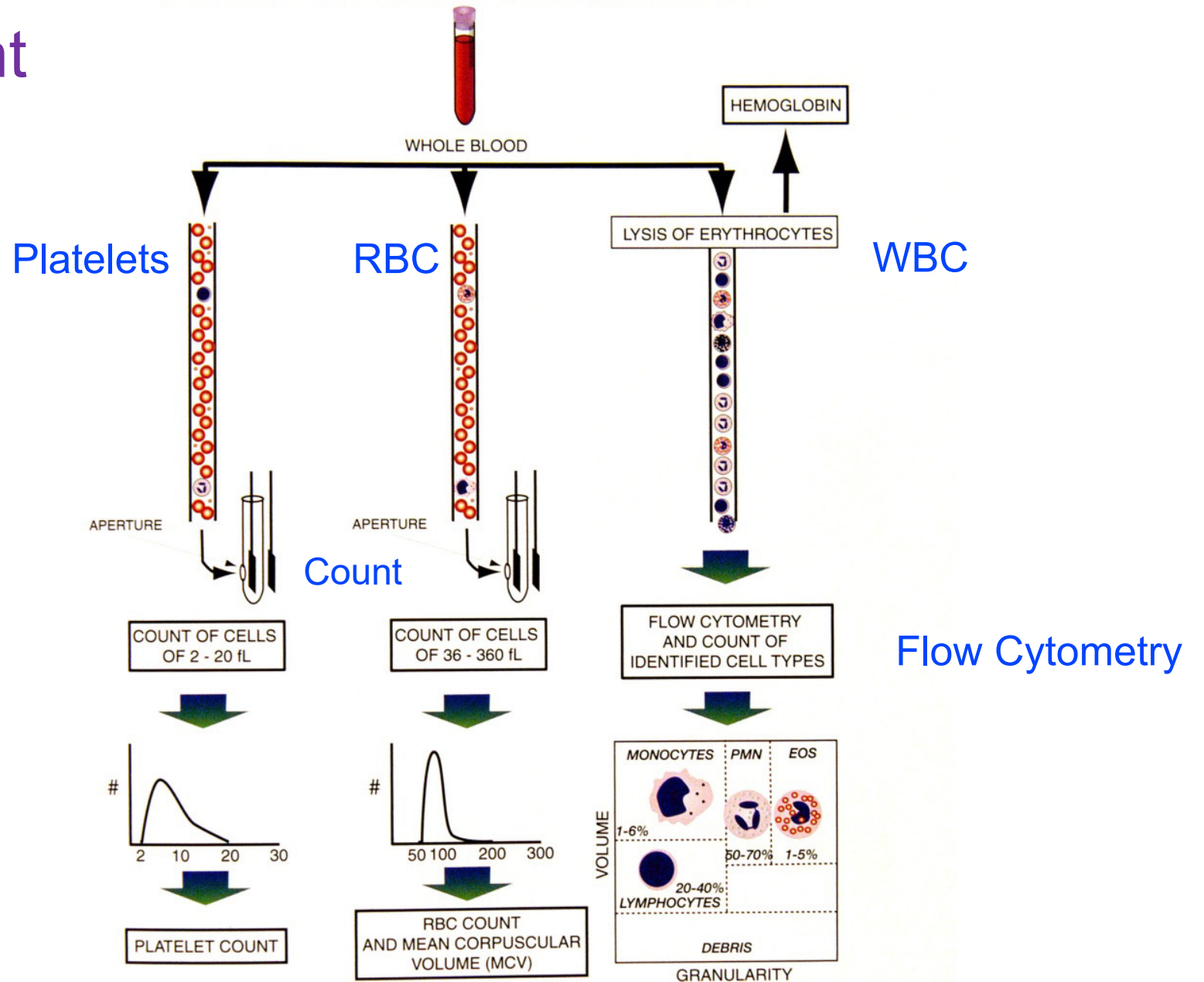
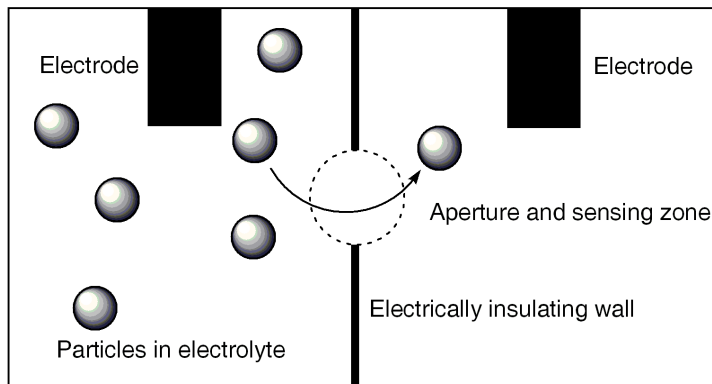
# Hemopoiesis



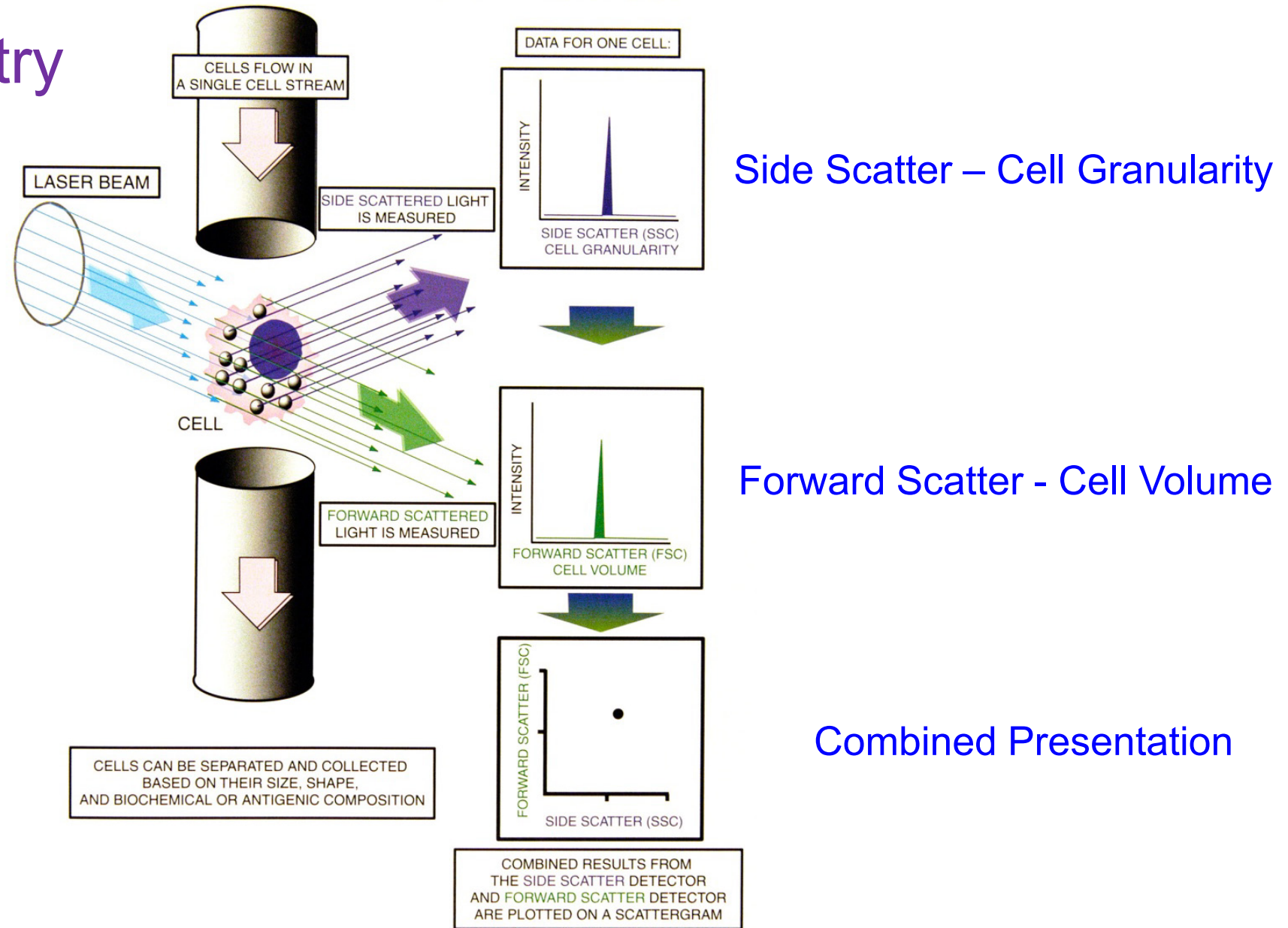
# Complete Blood Count



## Coulter Counter Principle



# Basic Flow Cytometry

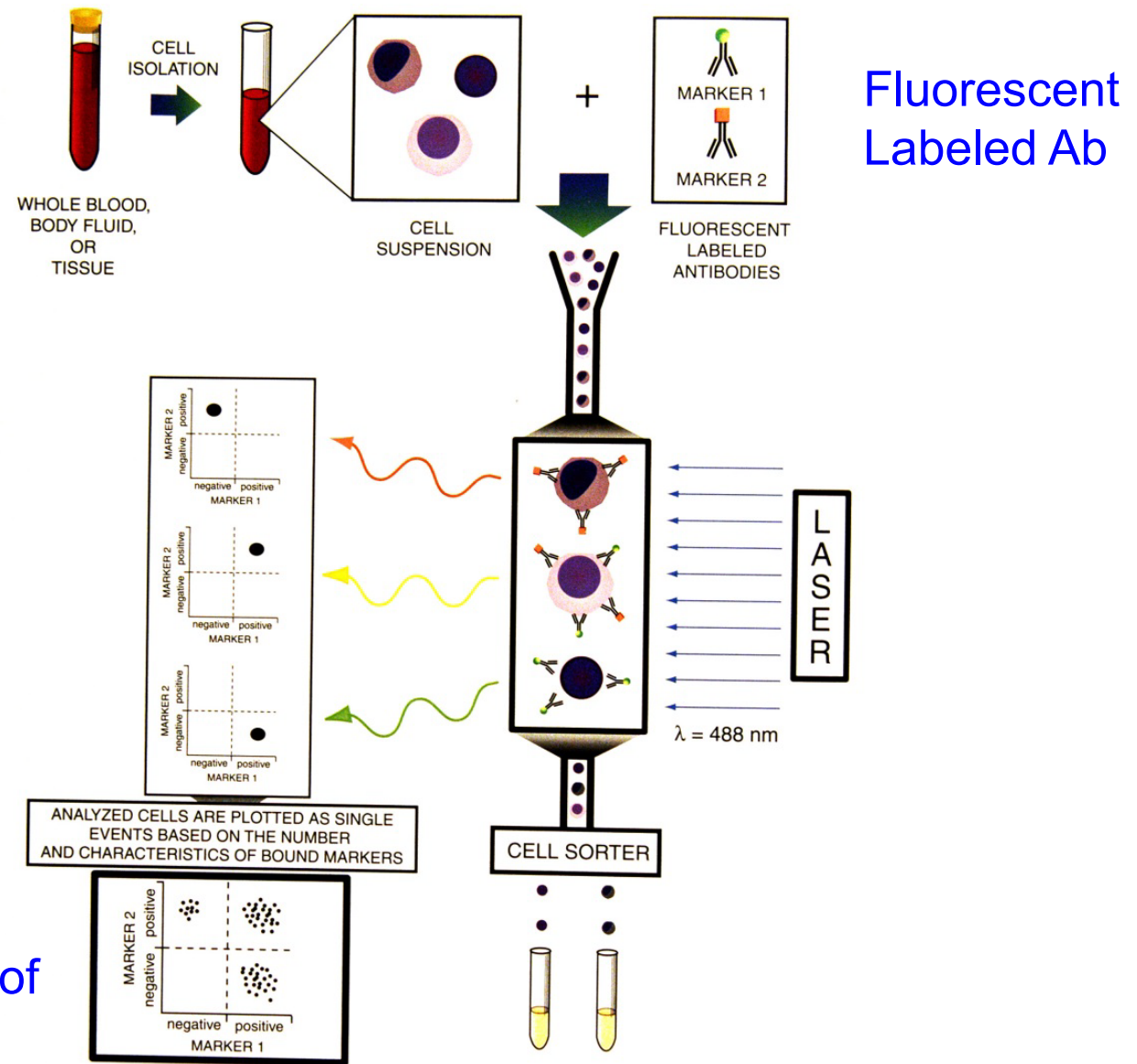




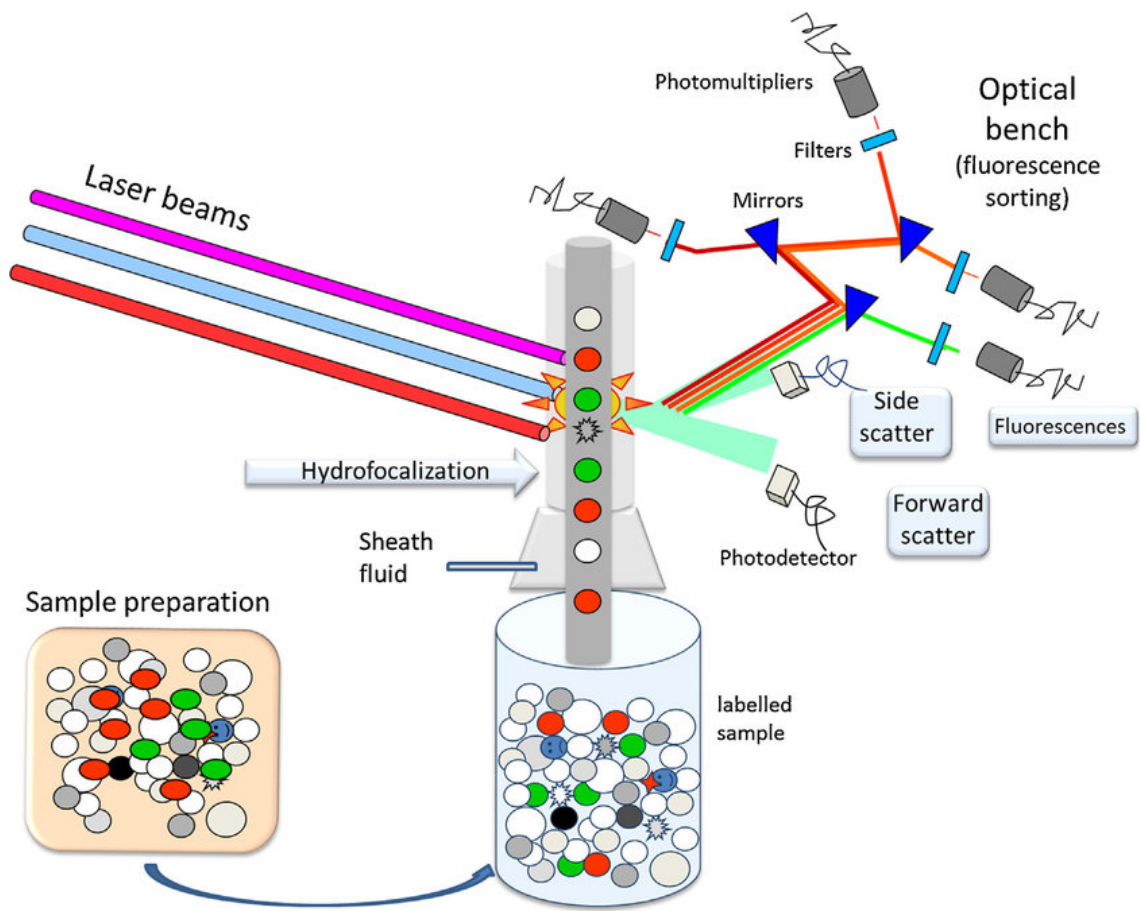
# Flow Cytometry with Cell Markers



Analyzed cells are plotted as single events based on the number and characterization of bound markers.



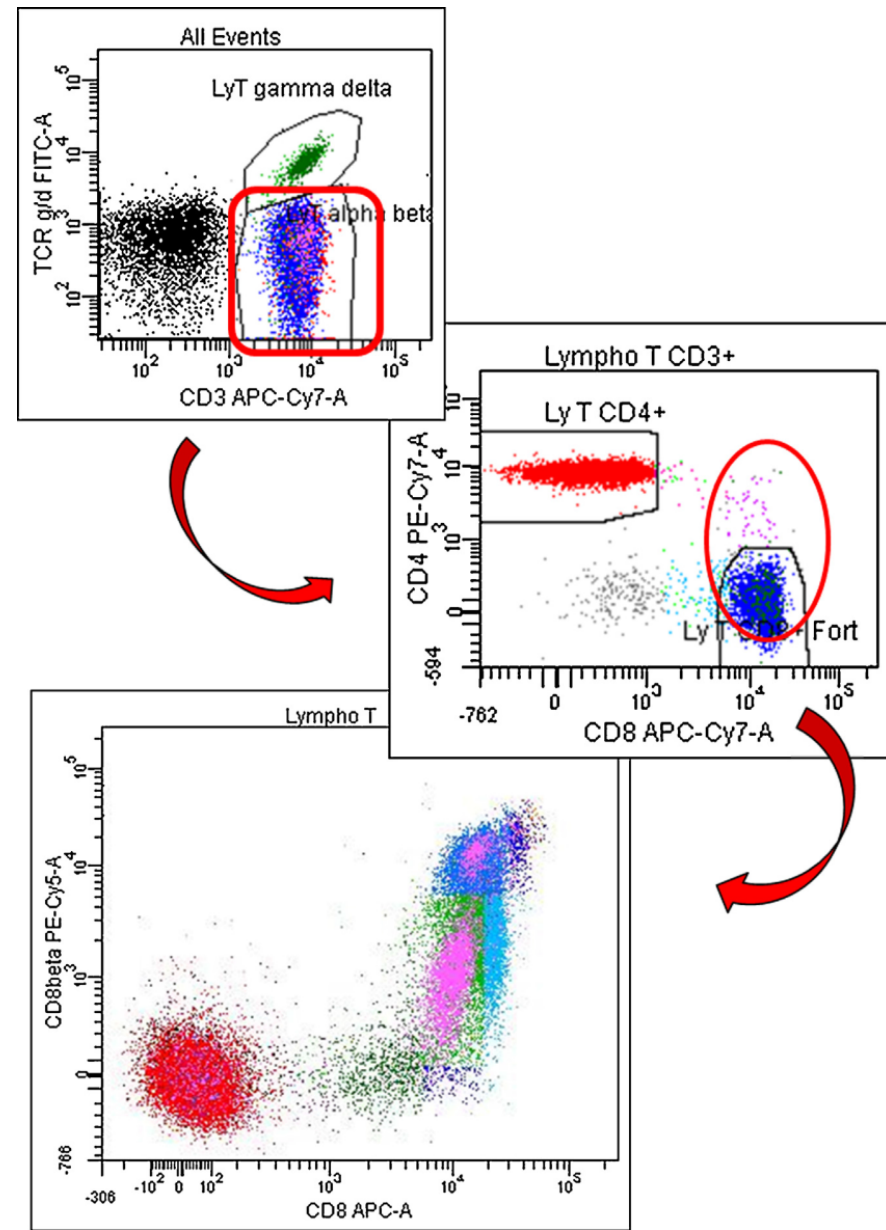




Fluorescence is with dichroic mirrors and filters and detected, amplified by photomultipliers.

Sequential determination of numerous T cell subtypes in one analysis. Each dot represents one event.

Cells are identified by direct immunofluorescence.





Star CellBio

presents

FLOW CYTOMETRY

# Peripheral Blood Smear

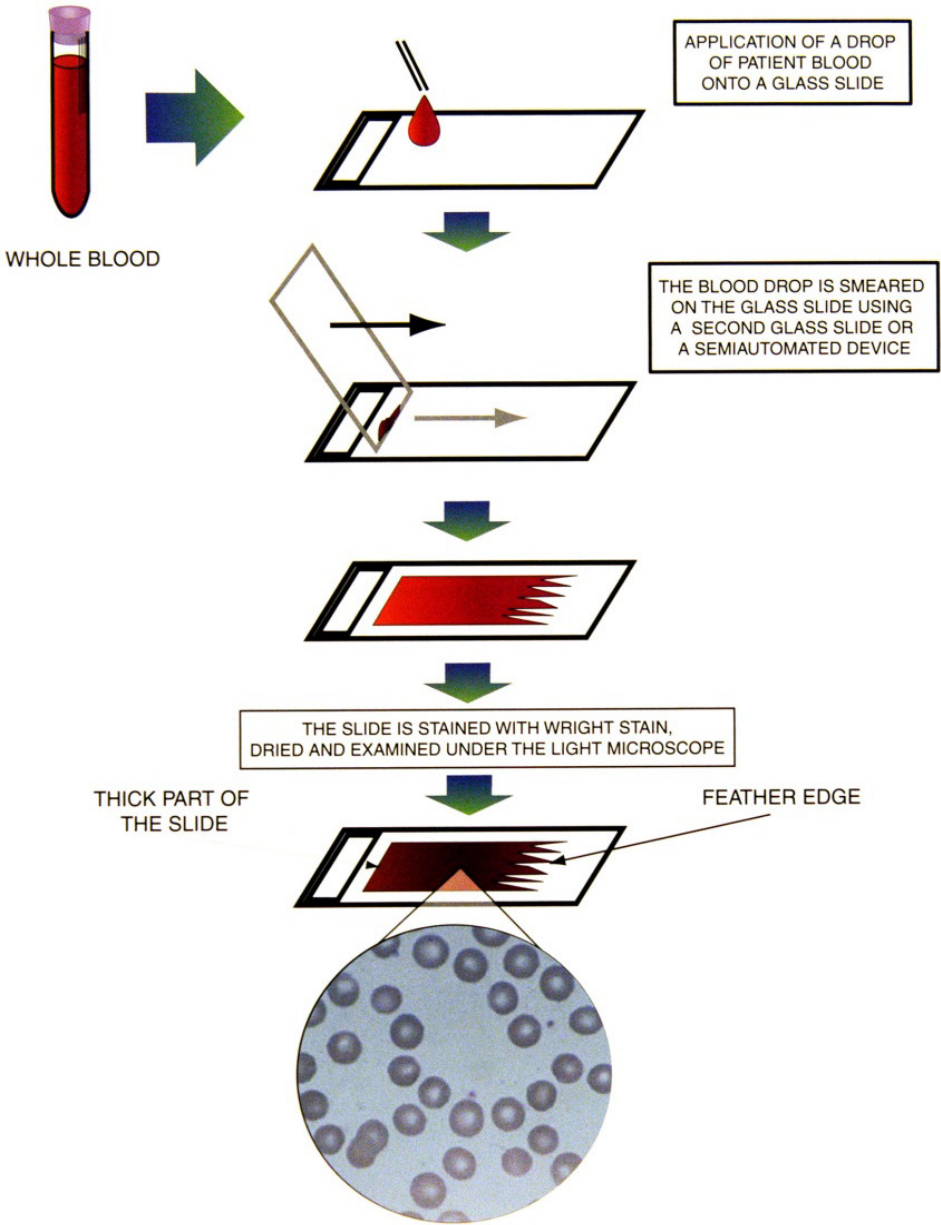
Application of Whole Blood

Blood is Smear

Wright Stain

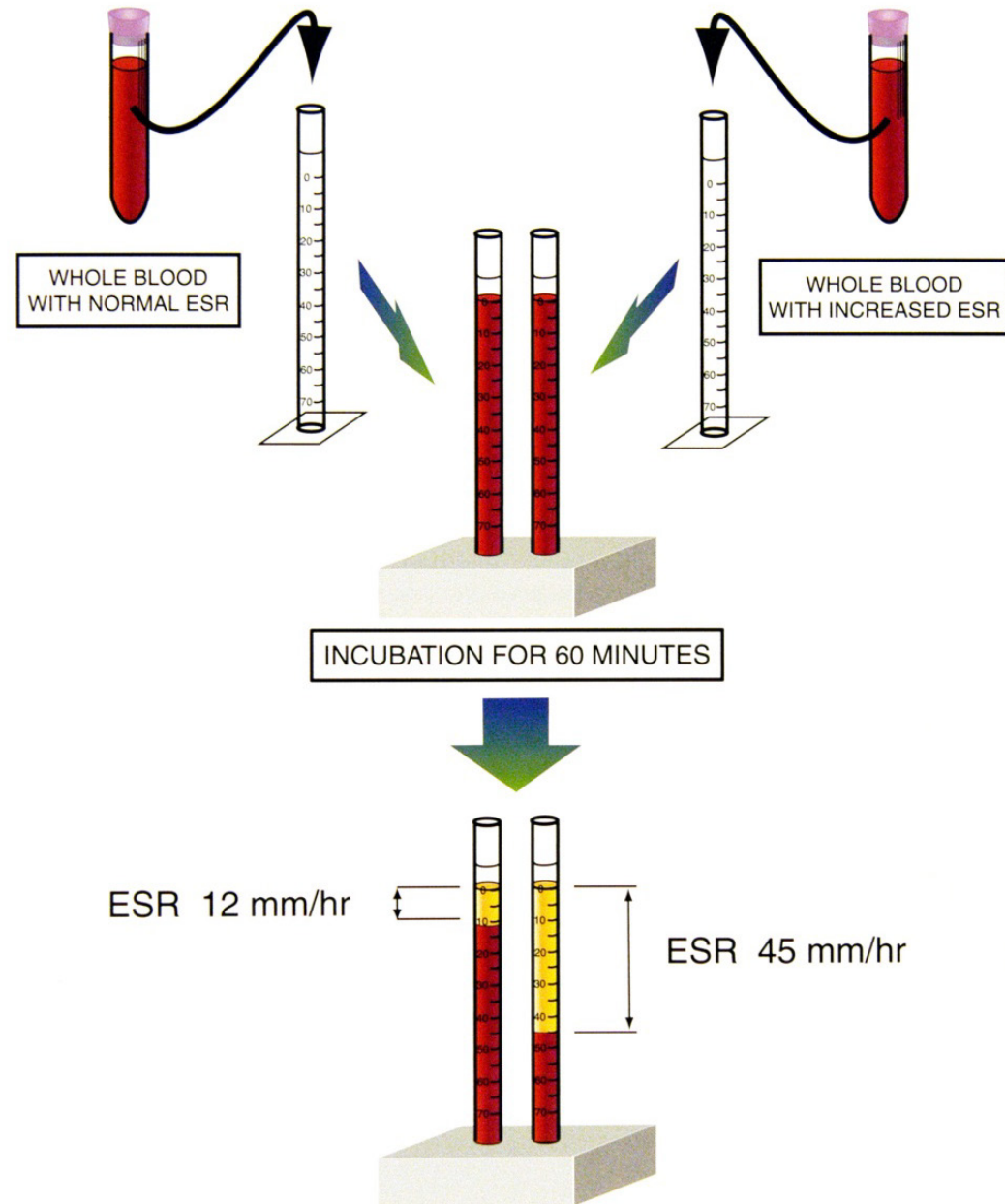
Pusher Slide makes Feather Edge

Microscope



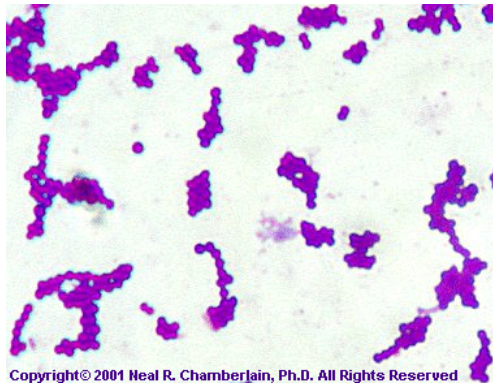
# Sedimentation Rate or ESR

Comparison of high and  
low sedimentation rate.





# 3. Microbiology: *Bacteria...*

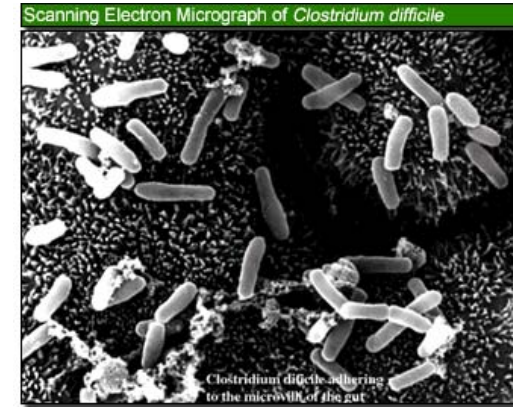


Copyright © 2001 Neal R. Chamberlain, Ph.D. All Rights Reserved

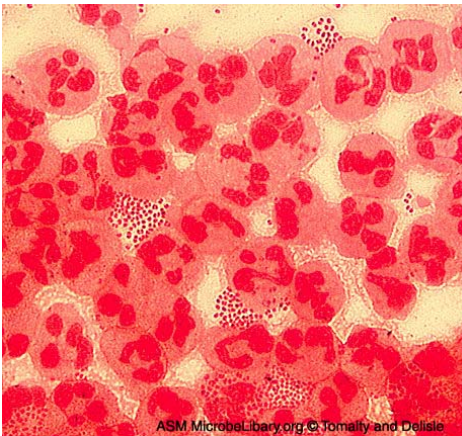
**Staphylococcus aureus**



**Bacillus anthracis**

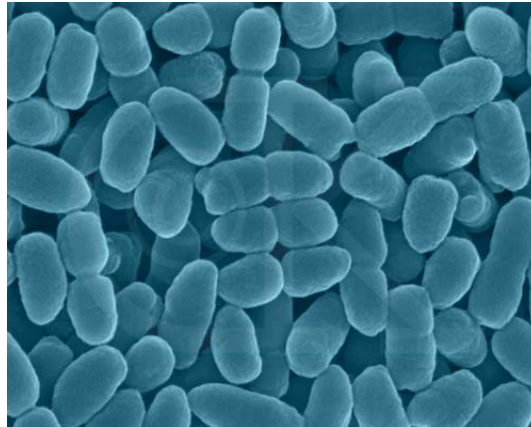


**Clostridium difficile**

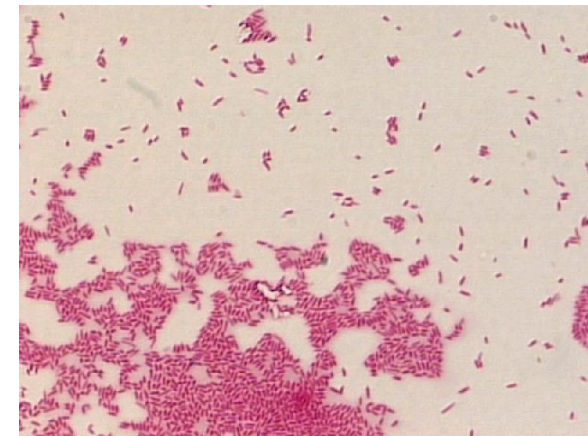


ASM MicrobeLibrary.org © Tomally and Delisle

**Neisseria gonorrhoeae**

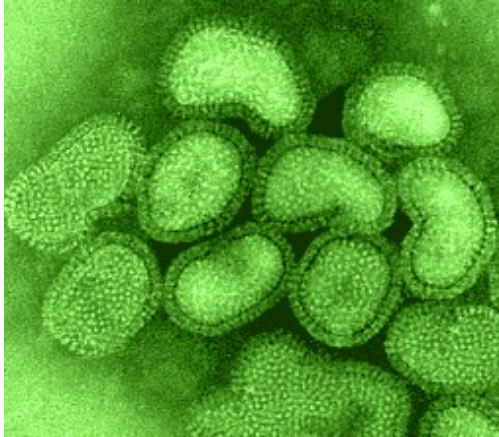


**Bordetella pertussis**

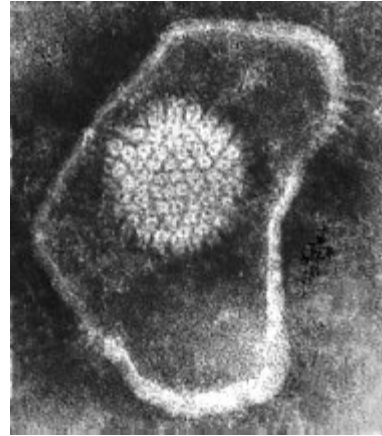


**Bacteroides fragilis**

# Viruses, Fungi and Parasites...



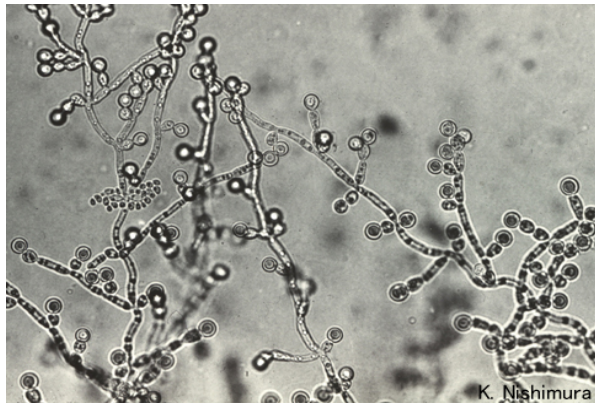
Influenza virus



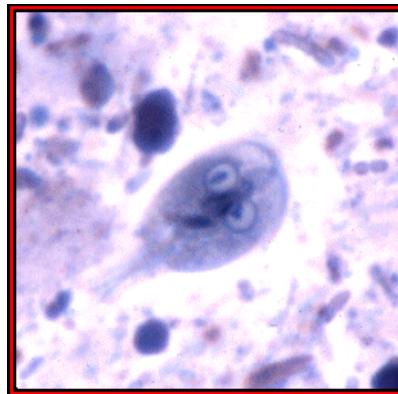
Herpes simplex



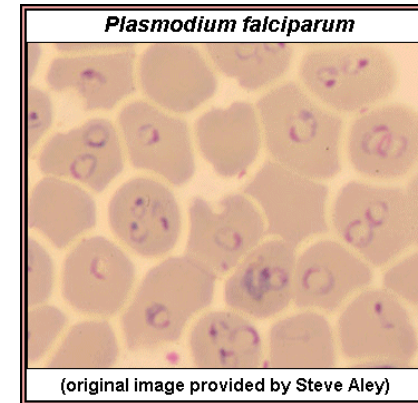
Aspergillus flavus



Candida albicans



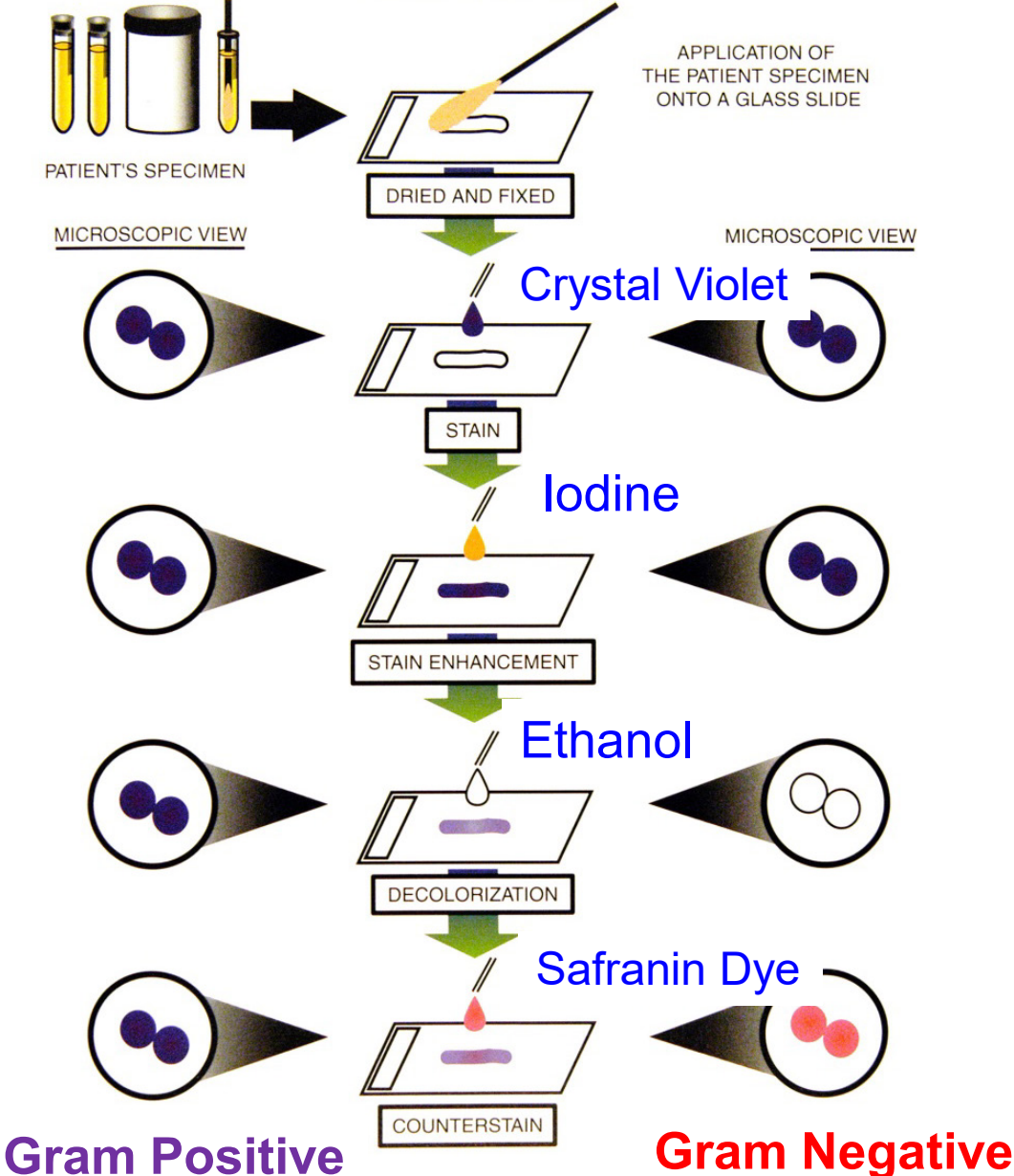
Giardia lamblia



Malaria

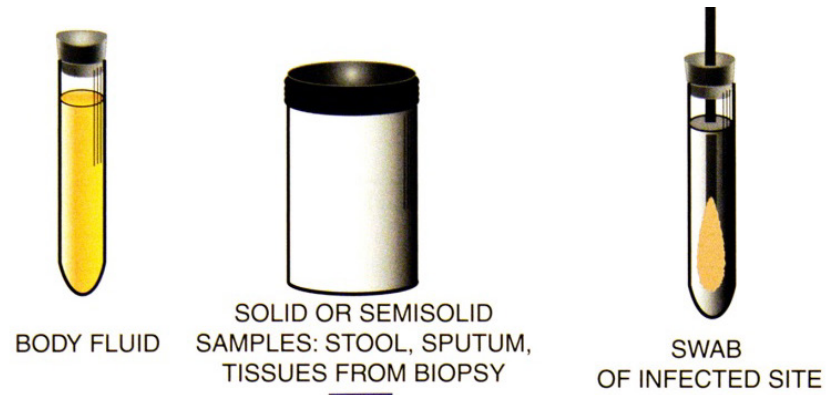


# Gram Stain

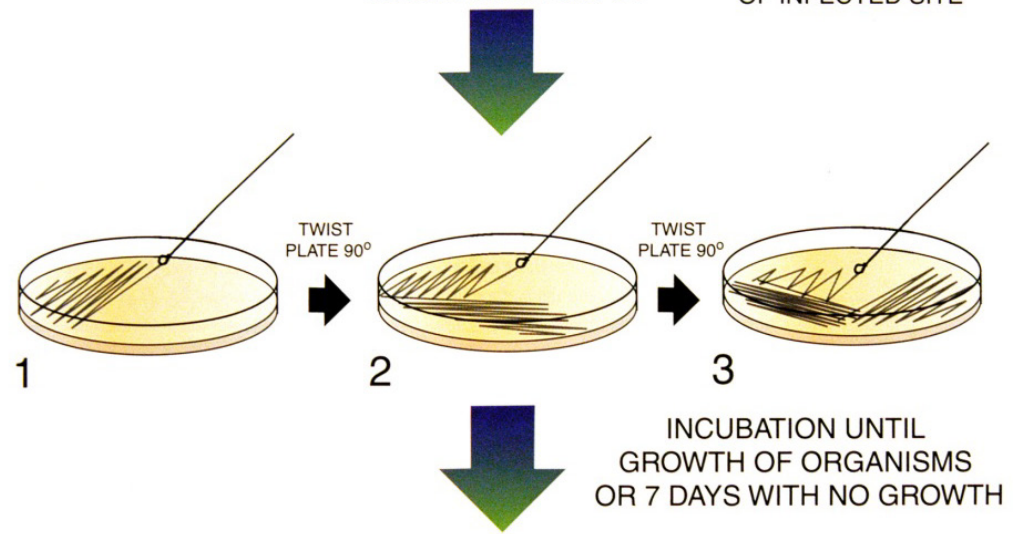




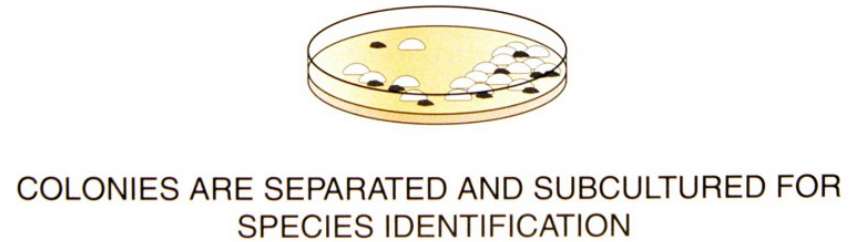
# Plating a Microbial Specimen



Incubation



Colony Isolation



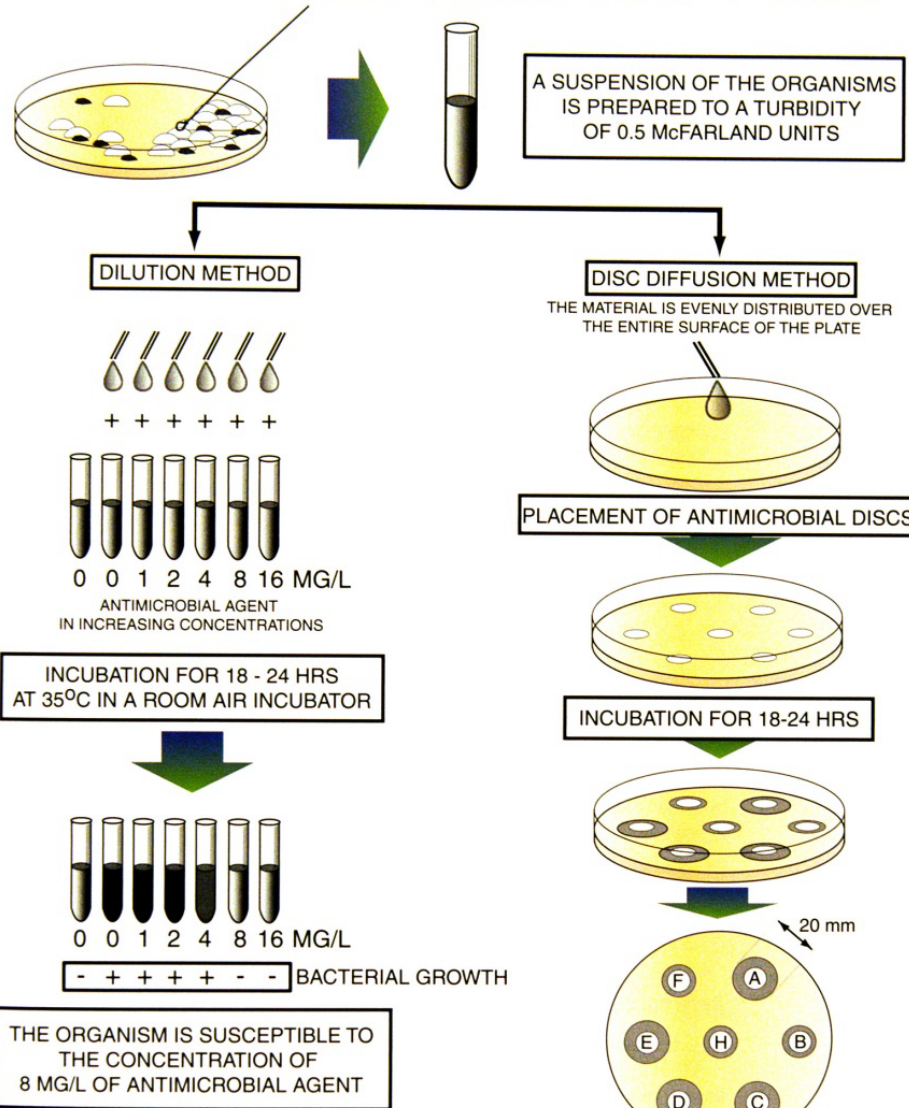
# Antimicrobial Susceptibility Tests

## Dilution Method

Antimicrobial Agents at Various Dilutions.

## Incubation

## Susceptibility



## Disc Diffusion Method

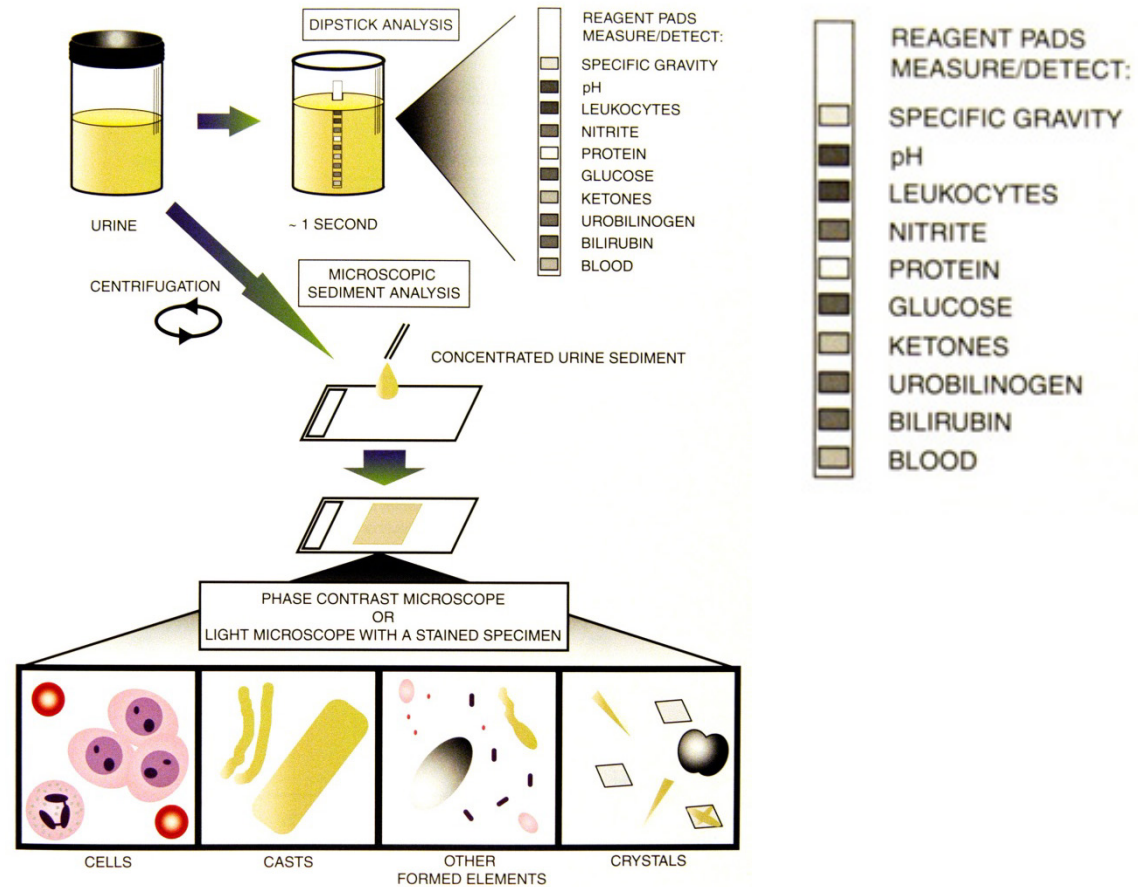
## Incubation

## Zones of Inhibition

# 4. Urinalysis

Concentrated Urine Sediment

Phase Contrast Microscopy



# 5. Anticoagulation

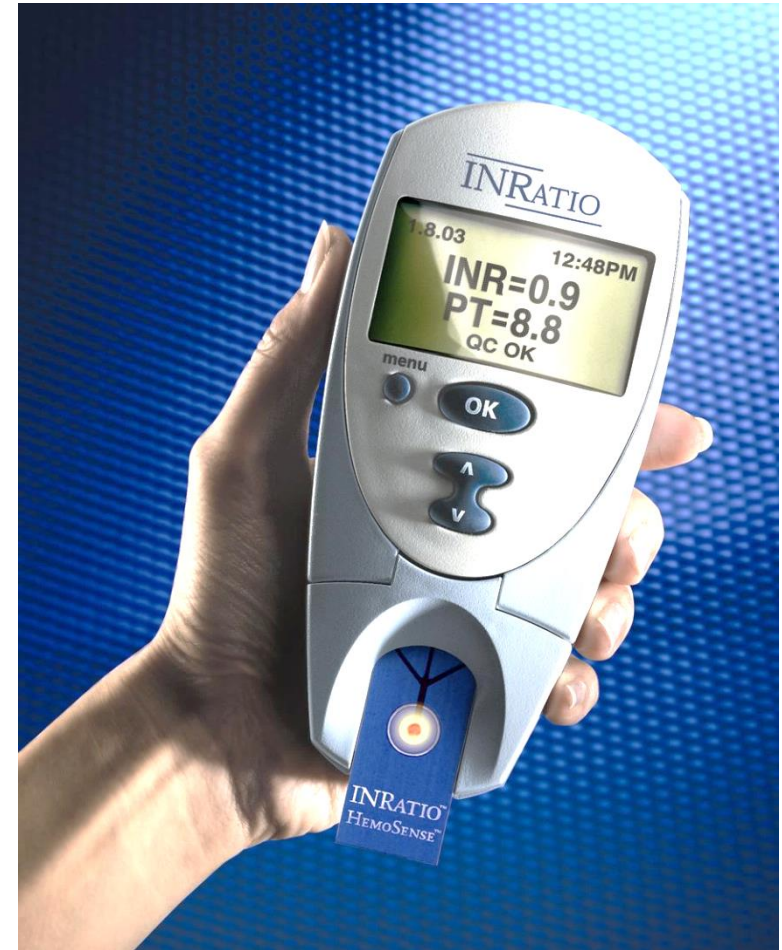
- Anticoagulant drugs are used in patients with:
  - Heart disease (including coronary artery disease and mural thrombi),
  - Atrial fibrillation (an arrhythmia),
  - Pulmonary embolism (clot in the lung vasculature),
  - Deep venous thrombophlebitis (DVT),
  - Artificial heart valves and other prosthetic cardiovascular devices, and
  - Other disorders.
- Coagulopathies include genetic and acquired deficiencies in coagulation factors, abnormal synthesis performance of the liver in hepatic (liver) diseases.

# INR...

- The International Normalized Ratio (INR)
  - Created by the World Health Organization (WHO) because PT results can vary depending on the thromboplastin reagent used.
  - The INR is a conversion unit that takes into account the different sensitivities of thromboplastins.
  - The INR is widely accepted as the standard unit for reporting PT results.



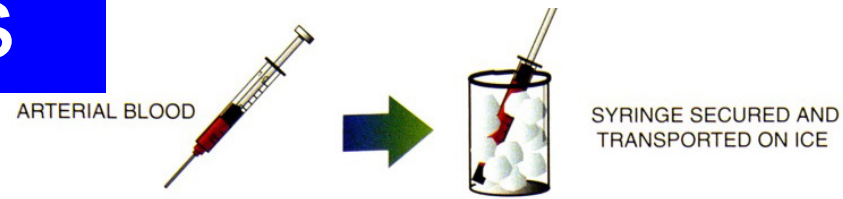
# PT & INR Measurement...





# 6. Arterial Blood Gases

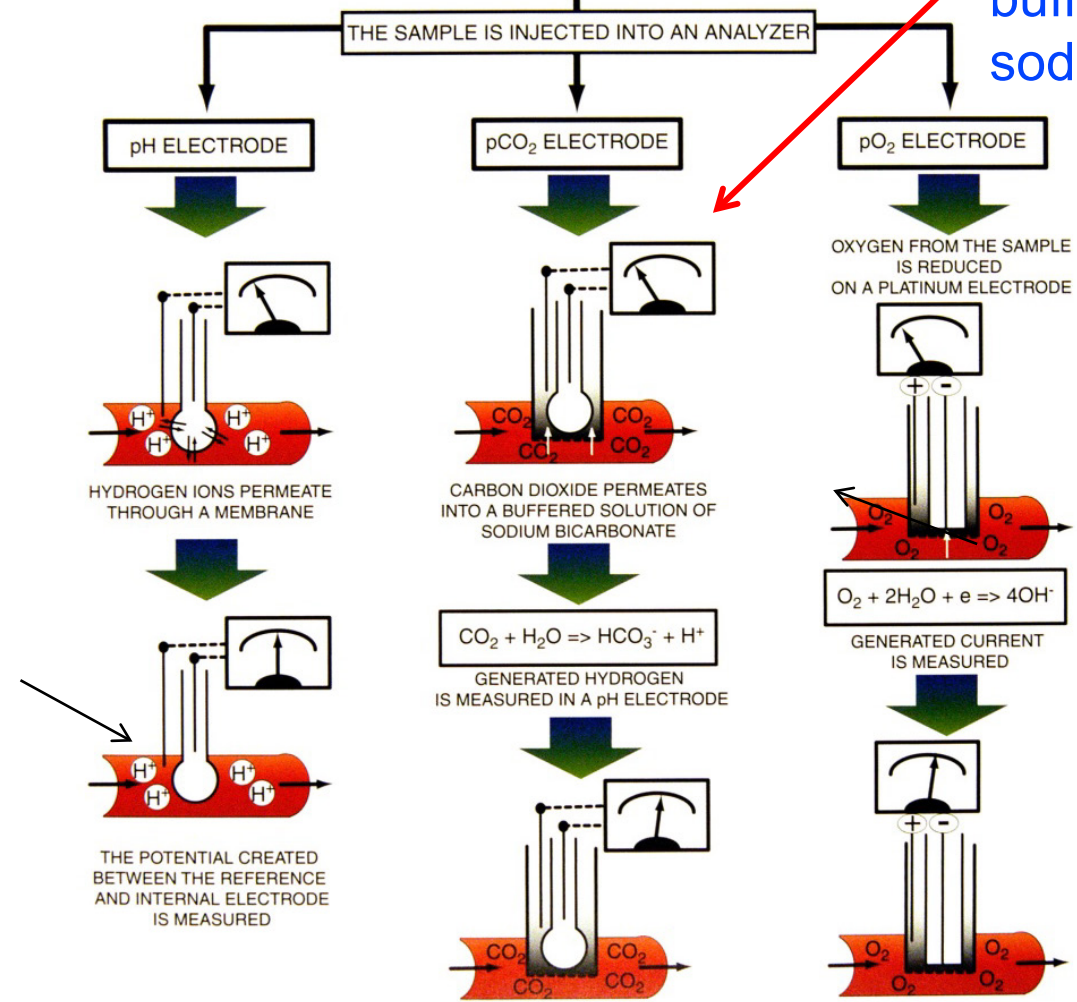
Start with an Arterial Sample on Ice



Carbon dioxide permeates into a buffered solution of sodium bicarbonate.

- pH Electrode
- pCO<sub>2</sub> Electrode
- pO<sub>2</sub> Electrode

Hydrogen ions permeate through a membrane.



Oxygen is reduced on a platinum electrode

# Summary

- Specimen collection.
- Studies by categories include:
  - Chemistries & Immunology.
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    - ELISA & other techniques.
  - Hematology
    - Hemopoiesis
  - Microbiology
  - Urinalysis
  - Anticoagulation
  - Arterial Blood Gases