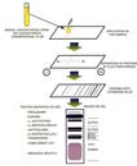
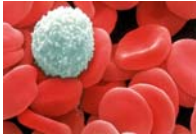


## Intro to Clinical Laboratory Medicine

Prof. Steven S. Sallterman, <http://sallterman.umn.edu/>



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## Topics

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

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## Specimen Collection

Obtaining a Specimen by Venipuncture



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## Specimen Processing...



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<https://youtu.be/k4W9DcgDeD8>

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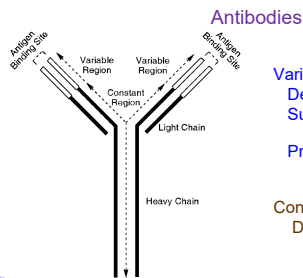
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## 1. Chemistries & Immunology



**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.

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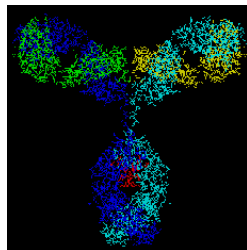
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## e.g. IgG Antibody...

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



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<http://www.umass.edu/microbio/rasmol/padlan.htm>

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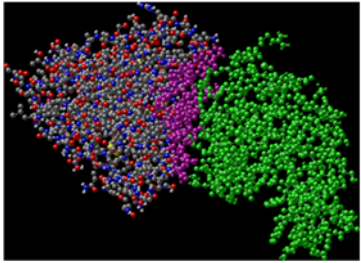
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## Antibody-Antigen Interaction...



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The Biology Project, University of Arizona

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## Antibodies at Work...



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[https://youtu.be/\\_N1xK48AqWQ](https://youtu.be/_N1xK48AqWQ)

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## ELISA



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- 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.

Barnett, C. ELISA Application. News Medical Life Sciences. 2020  
<https://www.news-medical.net/life-sciences/ELISA-Applications.aspx>

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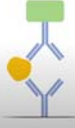
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## ELISA...

# ELISA



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<https://youtu.be/ERk0hwqyDw>

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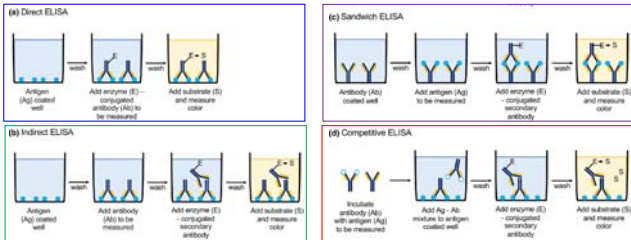
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## Methodologies...



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Karolina Bogaczewska, Review: immunoassays in DNA damage and instability detection in Cellular and Molecular Life Sciences CML 5 76(1) July 2019

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## 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.

Cinquanta L, Fontana DE, Bizzaro N. Chemiluminescent immunoassay technology: what does it change in autoantibody detection? *Auto Immun Highlights*. 2017;8(1):9-9.

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Chemiluminescence...



<https://youtu.be/SCK2XWSKEM>

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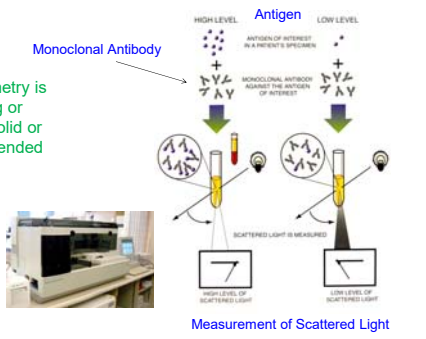
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Nephelometry

- 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.



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

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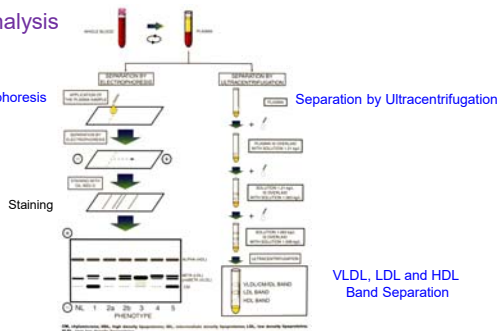
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Lipoprotein Analysis

Separation by Electrophoresis

Separation by Ultracentrifugation



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

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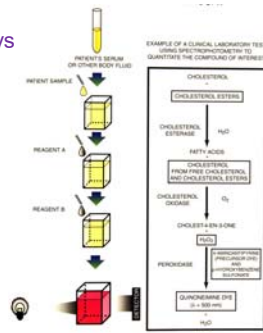
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## Spectrophotometric Assays



Laposata M. Laboratory medicine, Clinical pathology in the practice of medicine, ASCP Press, Chicago (2002).

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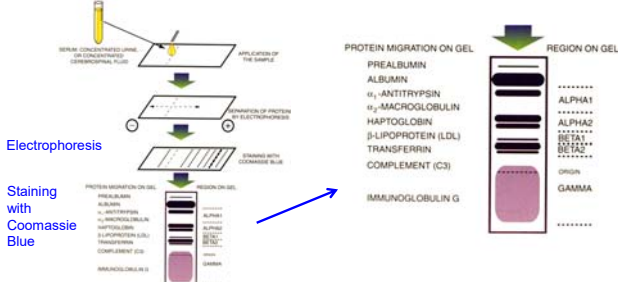
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## Protein Electrophoresis



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

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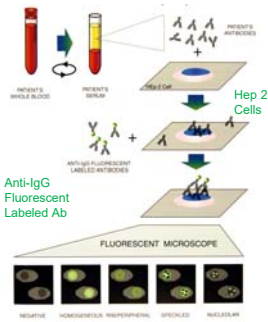
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## Antinuclear Antibodies (ANAs)

- 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.



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

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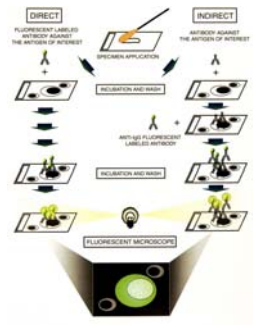
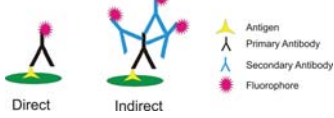
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### Direct & Indirect Immunofluorescence

- 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: Lapostolle M, Laboratory Medicine, Clinical Pathology in the Practice of Medicine, ASCP Press, Chicago (2002).

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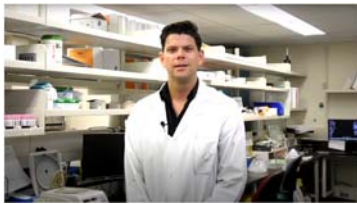
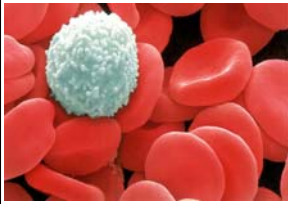
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## 2. Hematology



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msn. Encarta

<https://youtu.be/4DCCm5o92q8>

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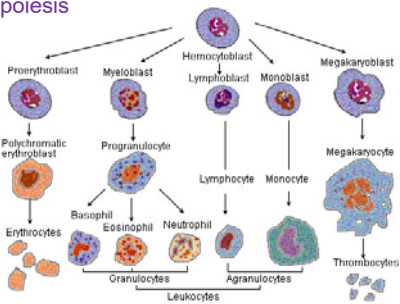
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### Hemopoiesis



training.seer.cancer.gov/

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### Complete Blood Count

**Coulter Counter Principle**

**Flow Cytometry**

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

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### Basic Flow Cytometry

(For white blood cell counts & differential.)

**Side Scatter – Cell Granularity**

**Forward Scatter - Cell Volume**

**Combined Presentation**

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

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### Flow Cytometry with Cell Markers

**Fluorescent Labeled Ab**

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

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

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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.

Sample preparation: Labeled sample, Sheath fluid, Hydro-foculation.

Laser beams, Photomultiplier, Optical bench (fluorescence settings), Mirrors, Side scatter, Forward scatter, Fluorescence.

Flow cytometry plots showing sequential gating: All Events (Lympho gate), Lympho T CD4+, Lympho T CD4+ CD8 APC-Cy5-A, Lympho T CD4+ CD8 APC-Cy5-A CD8 APC-Cy5-A.

A.-E. Deprince-Berger et al. New Tools in Cytometry, Morphologie, 100(331) 2016

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StarCellBio presents FLOW CYTOMETRY

<https://youtu.be/EQXPJ7eeesQ>

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### Peripheral Blood Smear

Application of Whole Blood

Blood is Smearred

Wright Stain

Pusher Slide makes Feather Edge

Microscope

WHOLE BLOOD

APPLICATION OF A DROP OF WHOLE BLOOD ONTO A SLIDE

THE BLOOD DROP IS SPREAD TO THE SLIDE USING A TECHNIQUE KNOWN AS "FEATHERING" TO OBTAIN A MONOLAYER OF CELLS

THE SLIDE IS STAINED WITH WRIGHT STAIN AND COVER SLEED

THICK PART OF THE SLIDE

FEATHER EDGE

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

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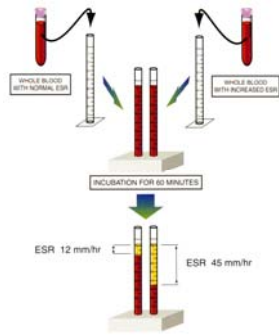
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### Sedimentation Rate or ESR

Comparison of high and low sedimentation rate.



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

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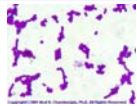
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### 3. Microbiology: Bacteria...



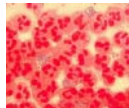
Staphylococcus aureus



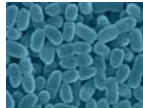
Bacillus anthracis



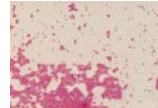
Clostridium difficile



Neisseria gonorrhoeae



Bordetella pertussis



Bacteroides fragilis

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Neil Chamberlain, Kirksville College of Osteopathic Medicine

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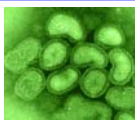
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### Viruses, Fungi and Parasites...



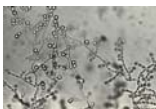
Influenza virus



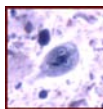
Herpes simplex



Aspergillus flavus



Candida albicans



Giardia lamblia



Malaria

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Viruses - Katholieke Universiteit Leuven; Fungi - RC-PPMT, Chiba University, Japan; Parasites - The Ohio State University

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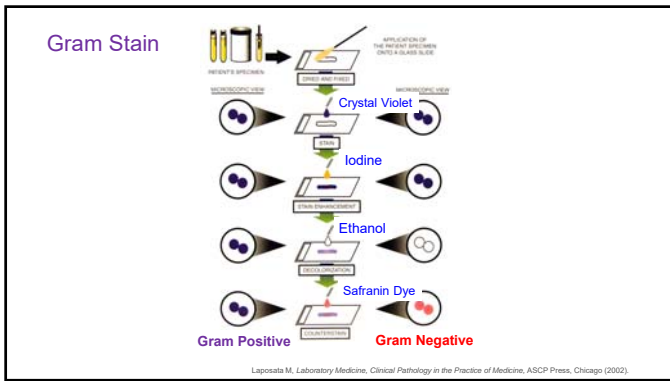
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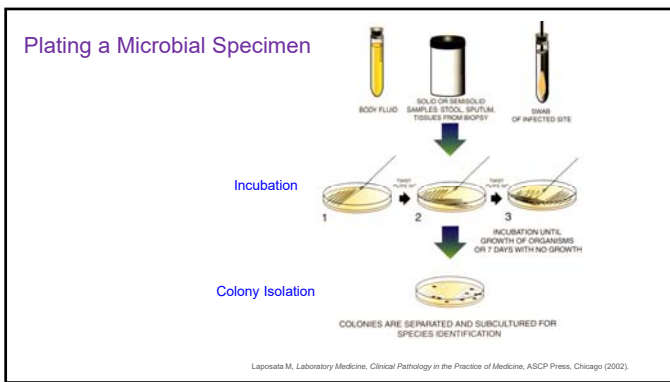
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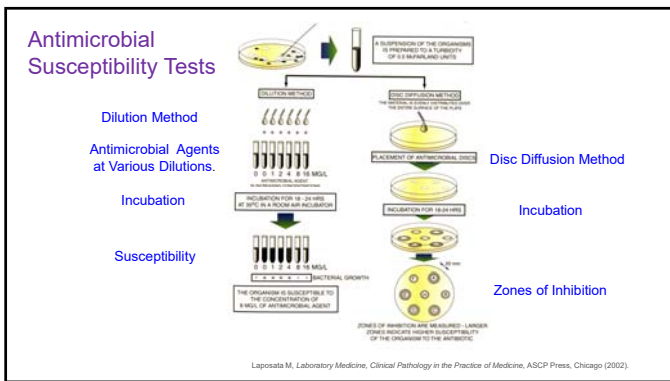
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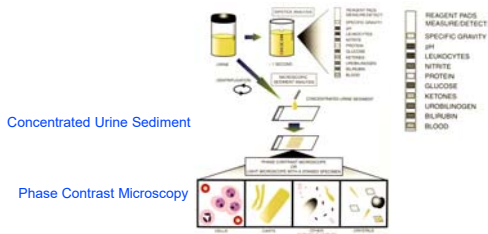
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## 4. Urinalysis



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

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## 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.

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## 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.

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## PT & INR Measurement...



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Image courtesy of HemoSense Inc

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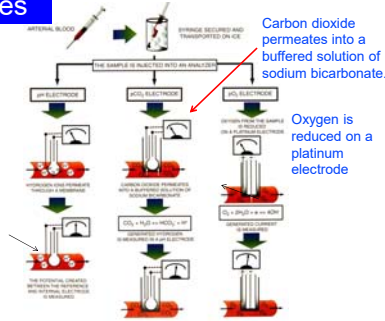
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## 6. Arterial Blood Gases

Start with an Arterial Sample on Ice

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

Hydrogen ions permeate through a membrane.



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

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## Summary

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

Steven S. Sallterman

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