Introduction to BioMEMS & Medical Microdevices

Intro to Clinical Laboratory Medicine

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Laboratory Studies by Category

- Chemistries & Immunology
- Hematology
- Microbiology
- Urinalysis
- Anticoagulation
- Blood gas
- Cytology
- Pathology
Specimen Collection

- Obtaining a specimen by venipuncture:
Tracking and Handling…

- The robotic LAB InterLink® automated specimen processor control station:
Recall 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 region:
- 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.

http://www.umass.edu/microbio/rasmol/padlan.htm
Antibody-Antigen Interaction...
A fluorophore is a fluorescent chemical compound that may re-emit light upon light excitation.

Fluorophores typically contain several combined aromatic groups, or plane or cyclic molecules with several π bonds.

Generally covalently bonded to a macromolecule, serving as a marker (or dye, or tag, or reporter) for affine or bioactive reagents (antibodies, peptides nucleic acids).

Fluorophores are notably used to stain tissues, cells, or materials in a variety of analytical methods.
Clinical Techniques:
- ELISA
- Western Blot
- Immunofluorescence
- Immunohistochemistry
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.
Methodologies...

(a) Direct ELISA

Antigen (Ag) coated well
Add enzyme (E) - conjugated antibody (Ab) to be measured
Add substrate (S) and measure color

(b) Indirect ELISA

Antigen (Ag) coated well
Add antibody (Ab) to be measured
Add enzyme (E) - conjugated secondary antibody
Add substrate (S) and measure color

(c) Sandwich ELISA

Antibody (Ab) coated well
Add antigen (Ag) to be measured
Add enzyme (E) - conjugated secondary antibody
Add substrate (S) and measure color

(d) Competitive ELISA

Incubate antibody (Ab) with antigen (Ag) to be measured
Add Ag - Ab mixture to antigen coated well
Add enzyme (E) - conjugated secondary antibody
Add substrate (S) and measure color

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Karolina Boguszewska, Review: immunoassays in DNA damage and instability detection in Cellular and Molecular Life Sciences CMLS 76(1) - July 2019
Chemiluminescence Immunoassays (CLIA)

- 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 (alka-line 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.
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.

Lipoprotein Analysis

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

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

1. Patient's Serum or Other Body Fluid
2. Patient Sample
3. Reagent A
4. Reagent B
5. Cholesterol Oxidase
6. Peroxidase
7. Quinoneimine Dye

Cholesterol
- Cholesterol Esters
- Fatty Acids
- Cholesterol from Free Cholesterol and Cholesterol Esters
- Cholesterol-4-en-3-one
- L-AMINOPANTYRINE (PRECURSOR DYE) AND p-HYDROXYBENZENEME (ILUMINATE)

O₂, H₂O₂, H₂O
Protein Electrophoresis

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

• Normally antibodies, produced by white blood cells (9B 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.
- Used for identifying immunoglobulins in the blood.
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).
Hemopoiesis

Stem Cell

- Proerythroblast
- Polychromatic erythroblast
- Erythrocytes

- Myeloblast

- Hemocytoblast

- Progranulocyte

- Basophil
- Eosinophil
- Neutrophil

- Granulocytes
- Agranulocytes

- Leukocytes

- Lymphoblast

- Lymphocyte
- Monocyte

- Monoblast

- Megakaryoblast

- Megakaryocyte

- Thrombocytes
Complete Blood Count

Laposata M, Laboratory Medicine, Clinical Pathology in the Practice of Medicine, ASCP Press, Chicago (2002).
Flow Cytometry with Cell Markers

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

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Sedimentation Rate

Microbiology: Bacteria

Staphylococcus aureus  Bacillus anthracis  Clostridium difficile

Neisseria gonorrhoeae  Bordetella pertussis  Bacteroides fragilis
Viruses, Fungi and Parasites

Influenza virus
Herpes simplex
Aspergillus flavus
Candida albicans
Giardia lamblia
Malaria

Viruses – Katholieke Universiteit Leuven; Fungi – RC-PFMT, Chiba University, Japan; Parasites – The Ohio State University
Gram Stain

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

1. BODY FLUID
2. SOLID OR SEMISOLID SAMPLES: STOOL, SPUTUM, TISSUES FROM BIOPSY
3. SWAB OF INFECTED SITE

TWIST PLATE 90°

INCUBATION UNTIL GROWTH OF ORGANISMS OR 7 DAYS WITH NO GROWTH

COLONIES ARE SEPARATED AND SUBCULTURED FOR SPECIES IDENTIFICATION
Antimicrobial Susceptibility Tests

Dilution Method

Disc Diffusion Method

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Urinalysis

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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.
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

Image courtesy of HemoSense Inc
Arterial Blood Gases

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

- Antibodies & Fluorophores
- Studies by categories include:
  - Chemistries & Immunology
  - Hematology
  - Microbiology
  - Urinalysis
  - Anticoagulation
  - Blood gases