Techniques in Molecular Genetics

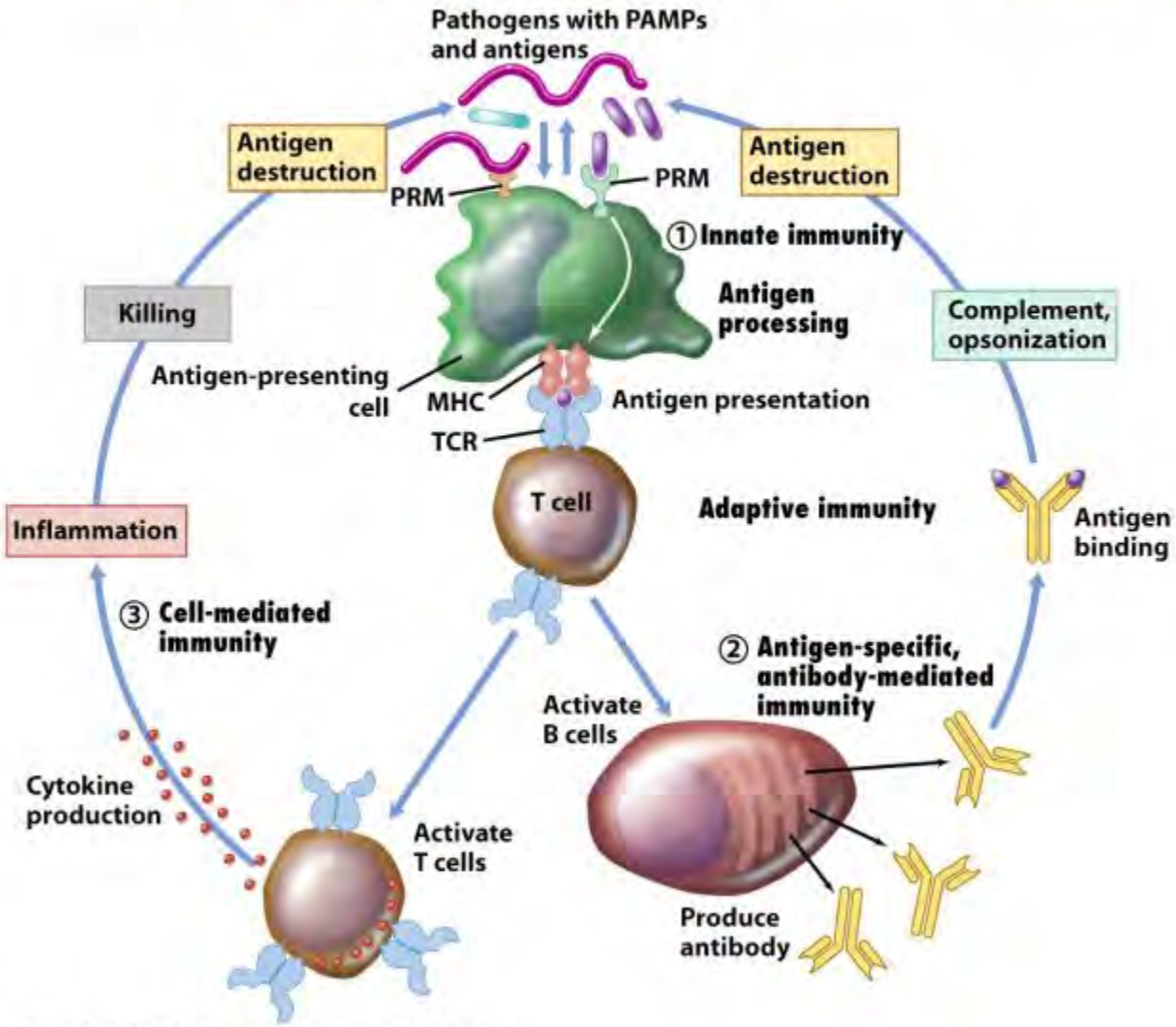
Immunological Methods And Western Analysis

H.E. Schellhorn

Day 4

- Immunology and Westerns
- PAGE
- Western

The Different Branches of the Immune Response



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Two Sides of the Adaptive Immune Response

Humoral immune response

→ Defence against extracellular pathogens

→ Soluble Effectors: Antibodies produced by B cells

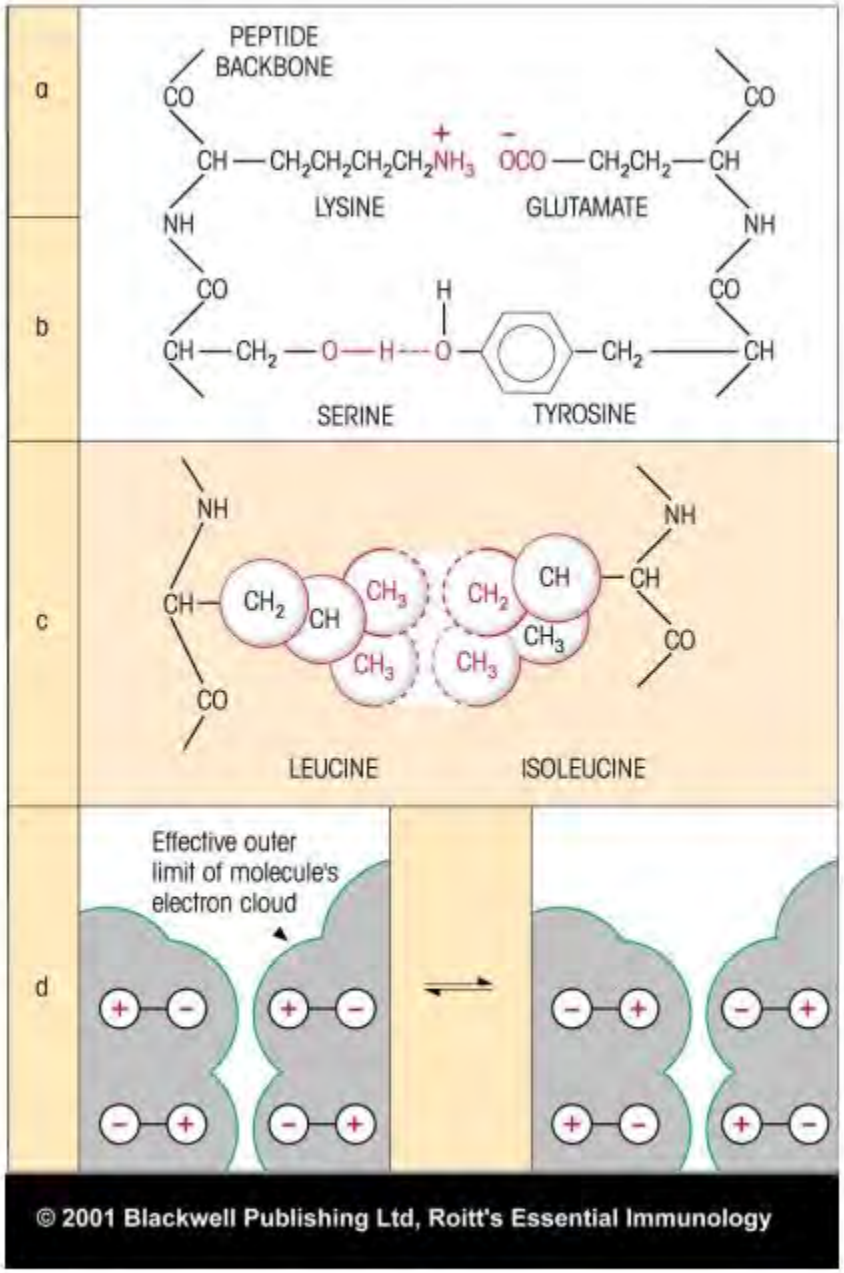
Cell-mediated immune response

→ Defence against intracellular pathogens (viruses and bacteria)

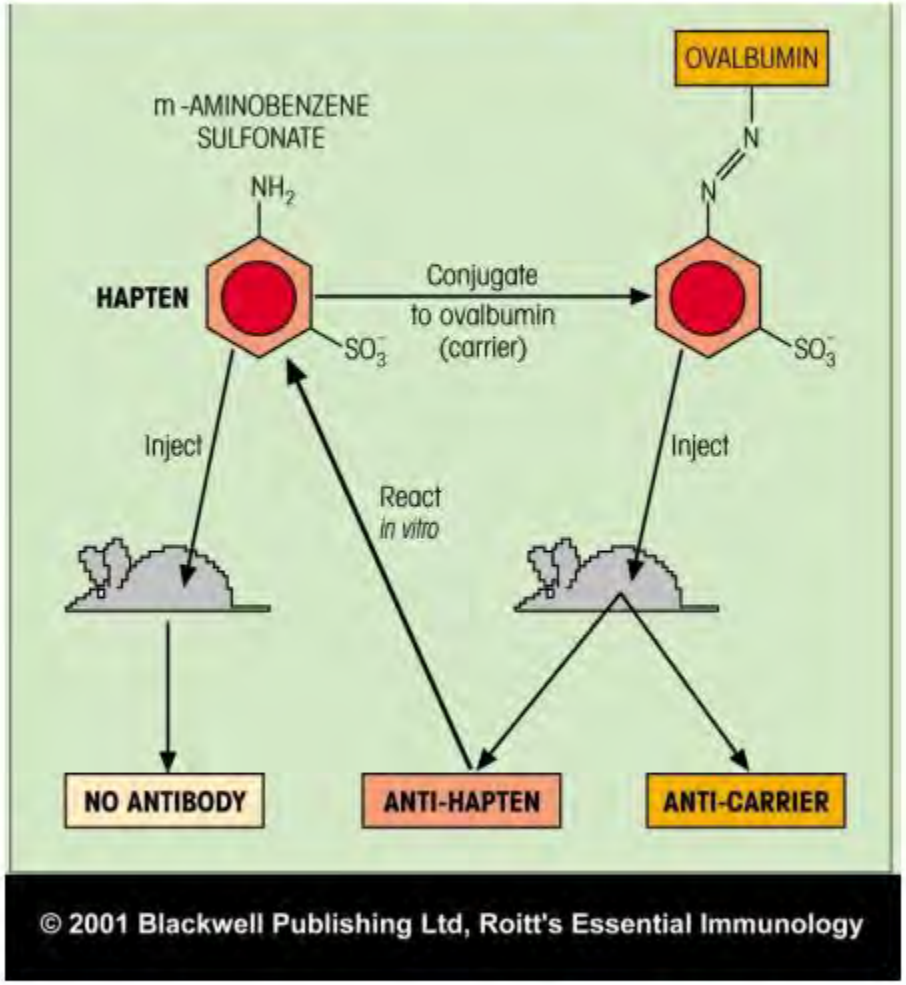
→ Effector T cell (CD4 helper and CD8 cytotoxic)

→ Dependence on antigen presentation by MHC I or II

Immunological Methods: Stabilization of Antigen-Antibody Interactions

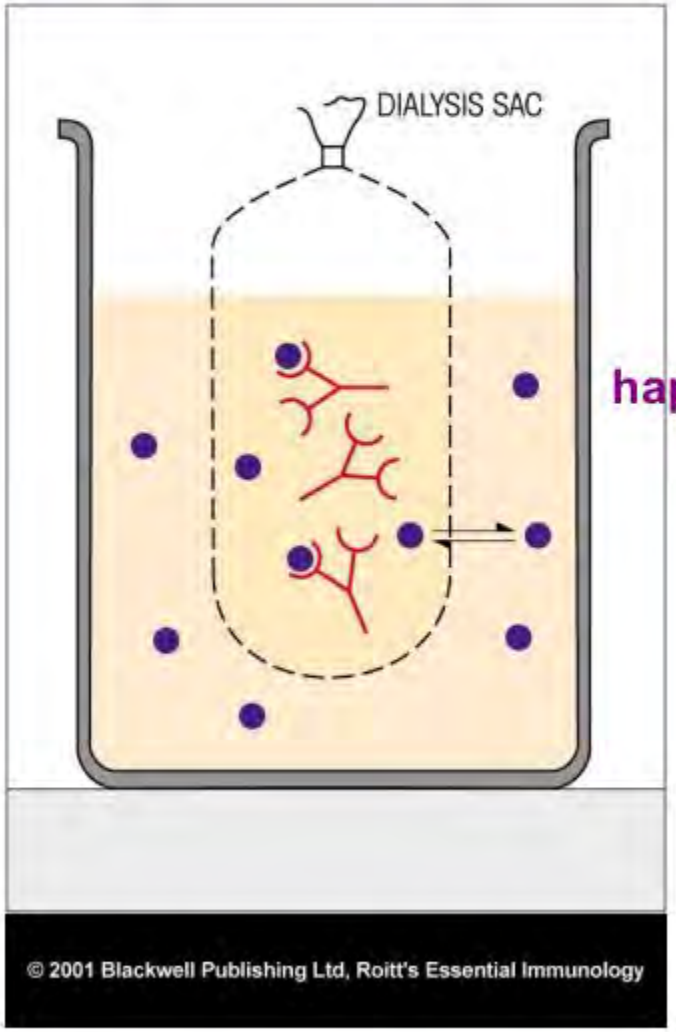


Immunological Methods: Immunization with Haptens

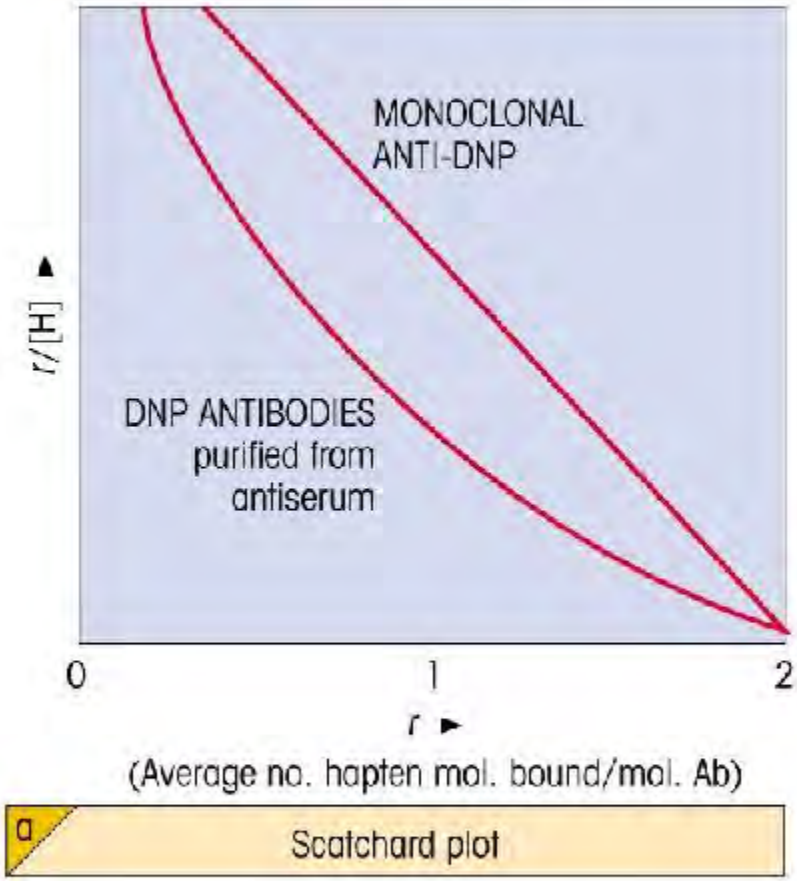


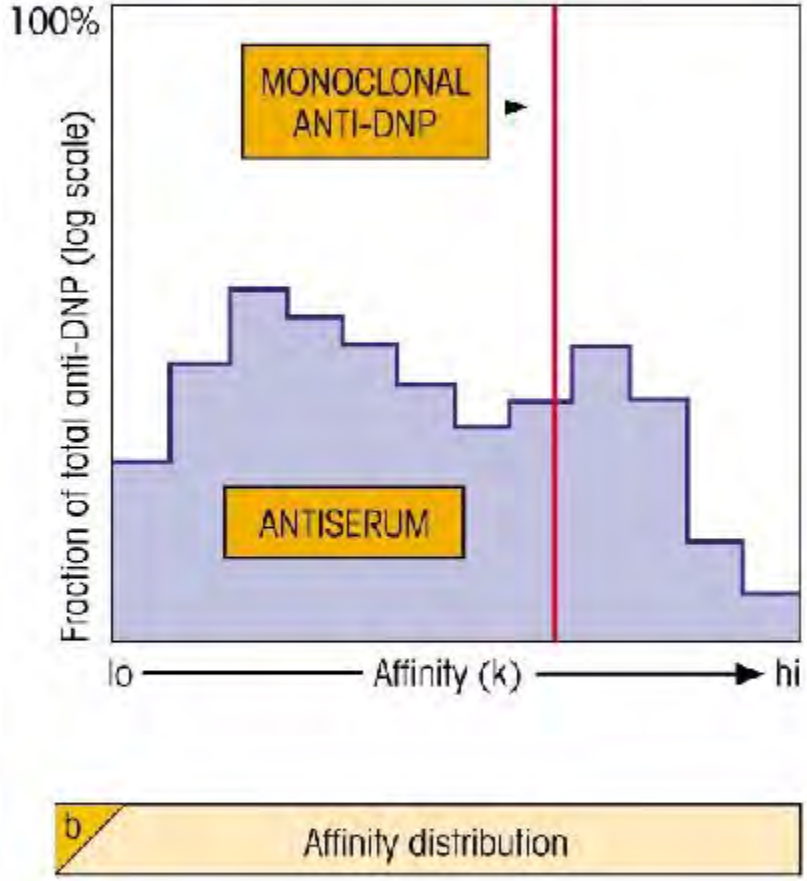
Application: Creation of peptide antisera

Immunological Methods:
Characterization of Antigen-Antibody Interactions



Immunological Methods:
Antisera Contain Antibodies with Different Affinities





Monoclonal antibodies vs polyclonal antiserum

Immunological Methods:
Principle of Immunoprecipitation - IP

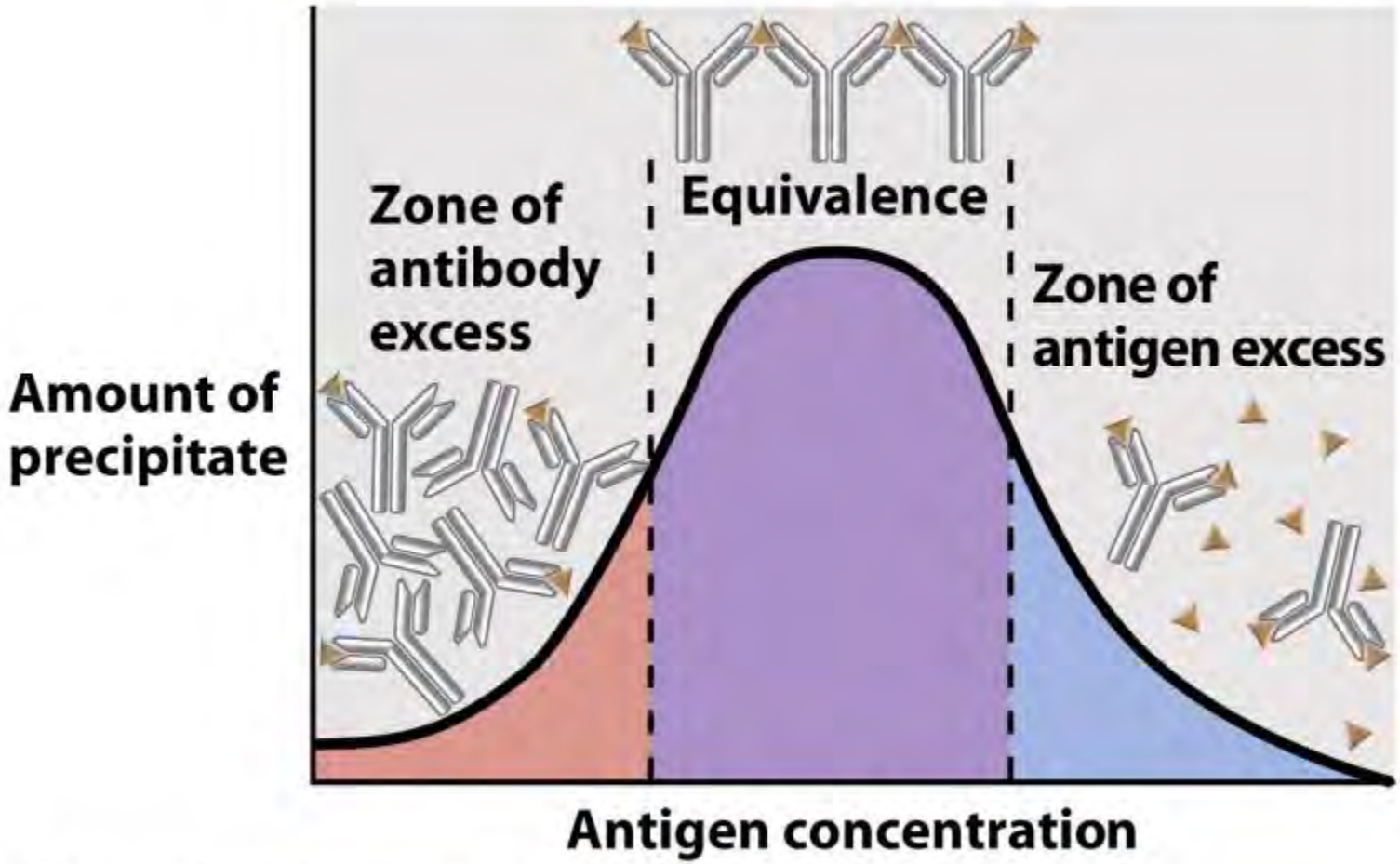
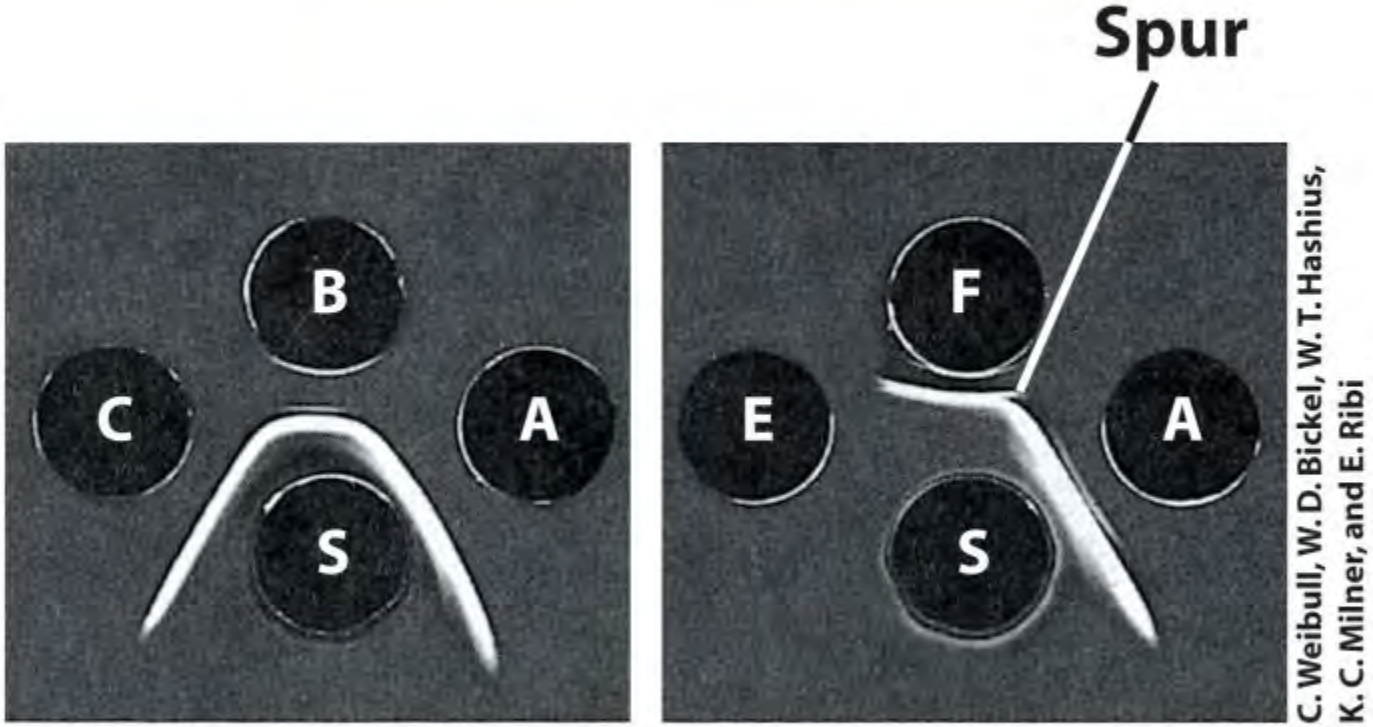


Figure 24-14a Brock Biology of Microorganisms 11/e

Immunological Methods:
IP Application - Immunodiffusion



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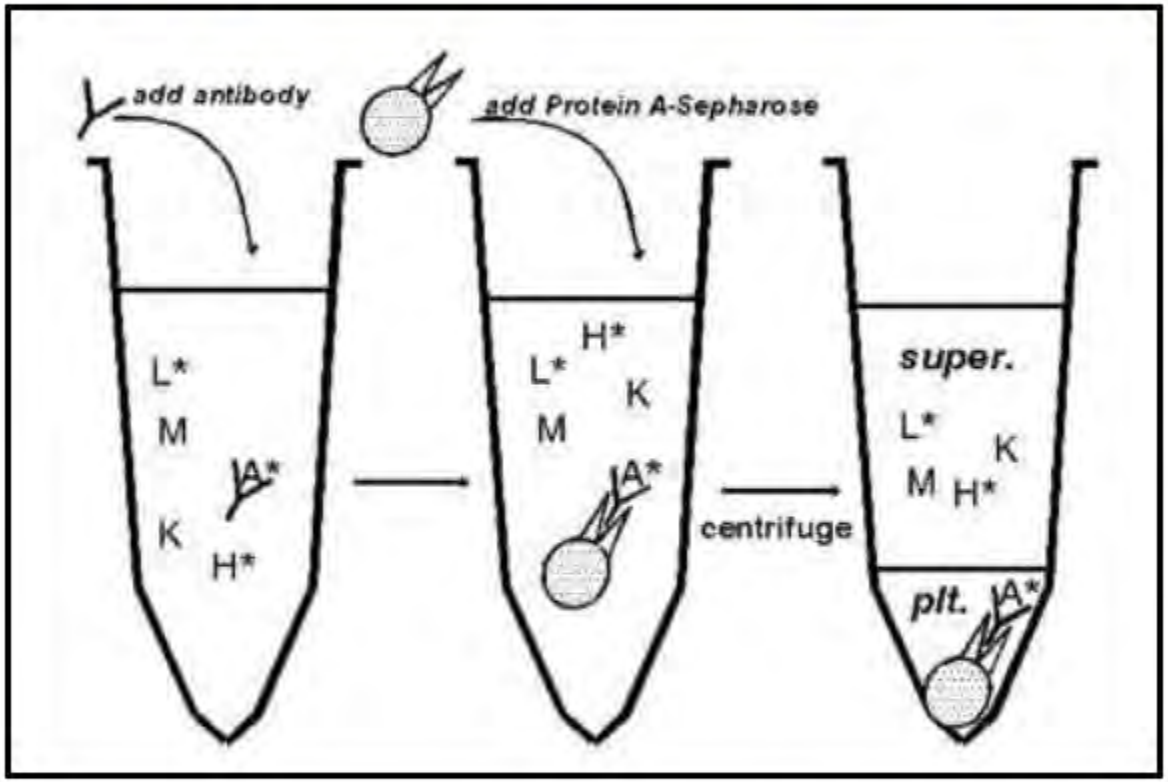
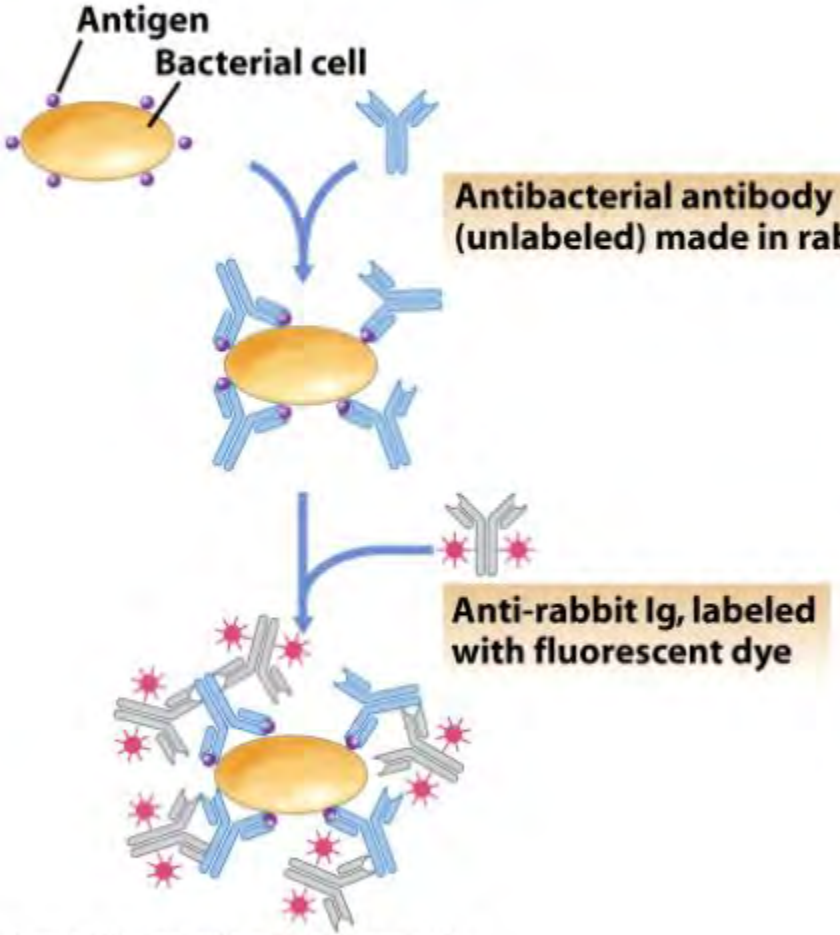
Immunological Methods:
IP Application - Immunoprecipitation



A* - antigen
L*, H*, M, K - other proteins

Source: http://www.animal.ufl.edu/hansen/protocols/imp98.prt.htm

Immunological Methods: Immunofluorescence Analysis - IF



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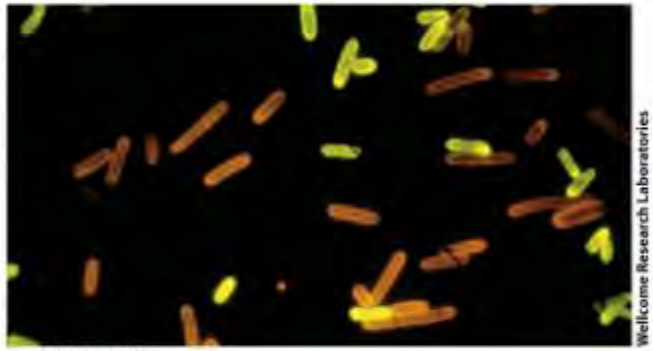
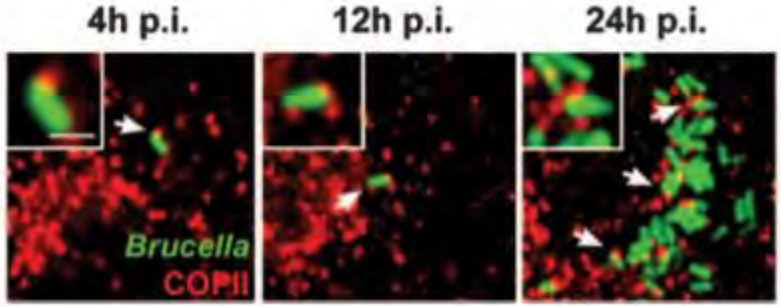
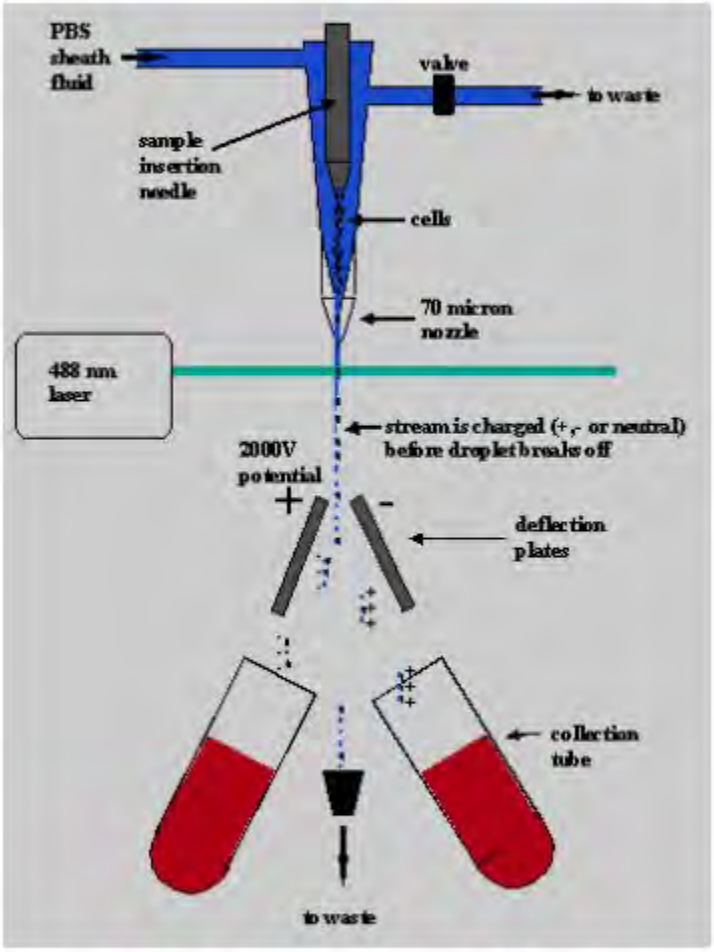


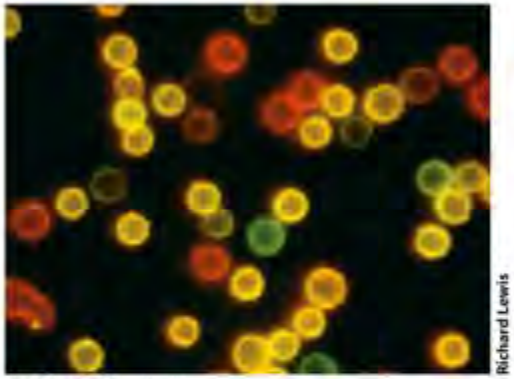


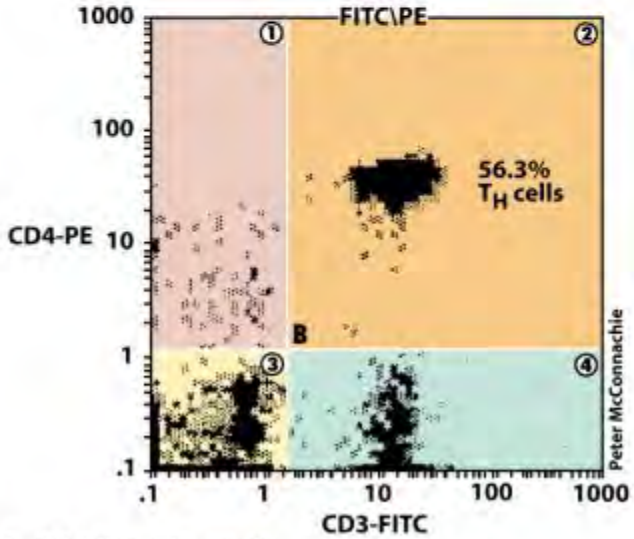
Immunological Methods:
Fluorescence-Activated Cell Sorter - FACS



Courtesy Vanderbilt Medical Center

www.mae.wmich.edu/faculty/liou/wp_MEmicro04p2_ManikK.ppt





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Immunological Methods: Radioimmunoassay - RIA

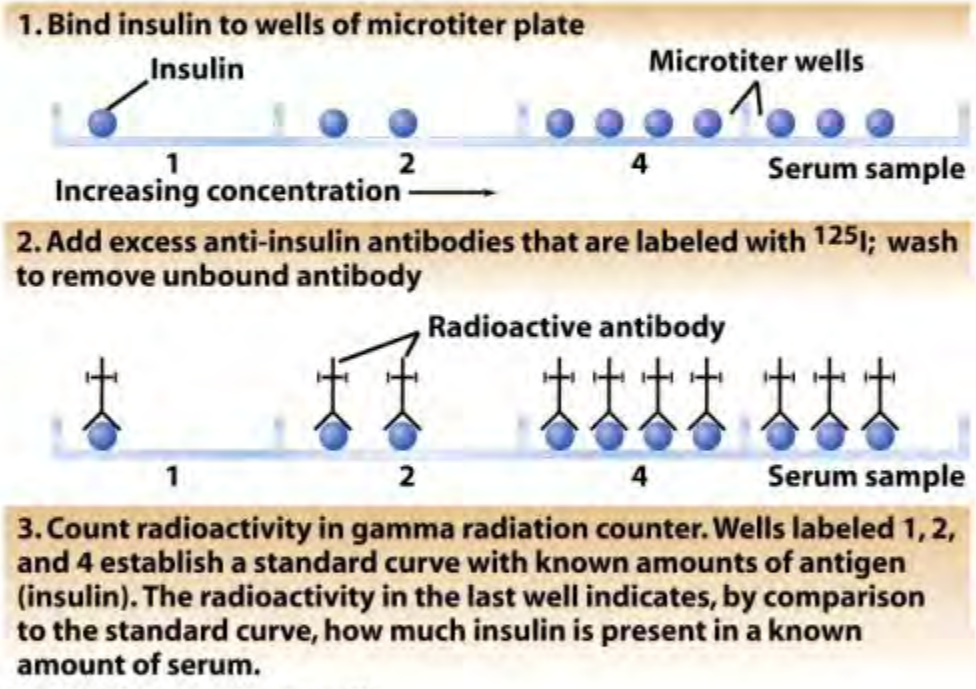


Figure 24-26 part 1 Brock Biology of Microorganisms 11/e

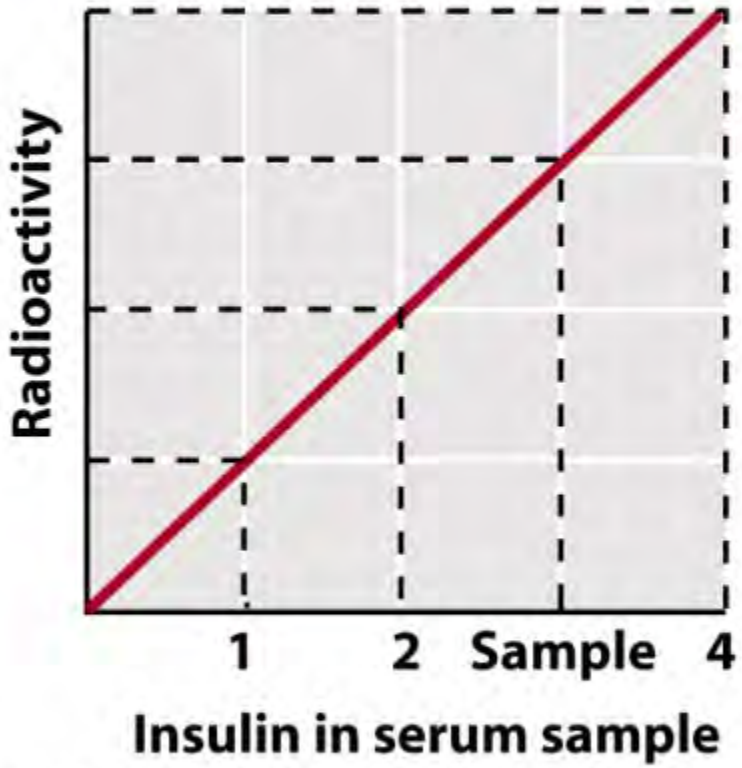
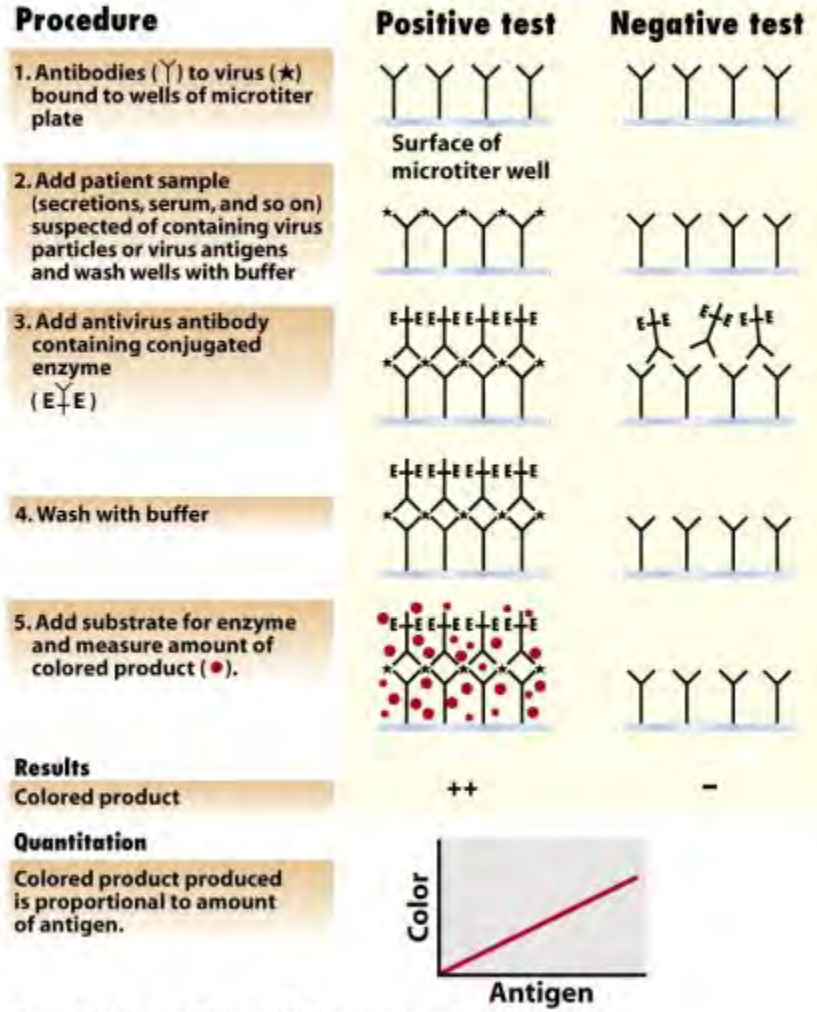


Figure 24-26 part 2 Brock Biology of Microorganisms 11/e

Immunological Methods:
Enzyme-Linked Immunosorbant Assay ELISA



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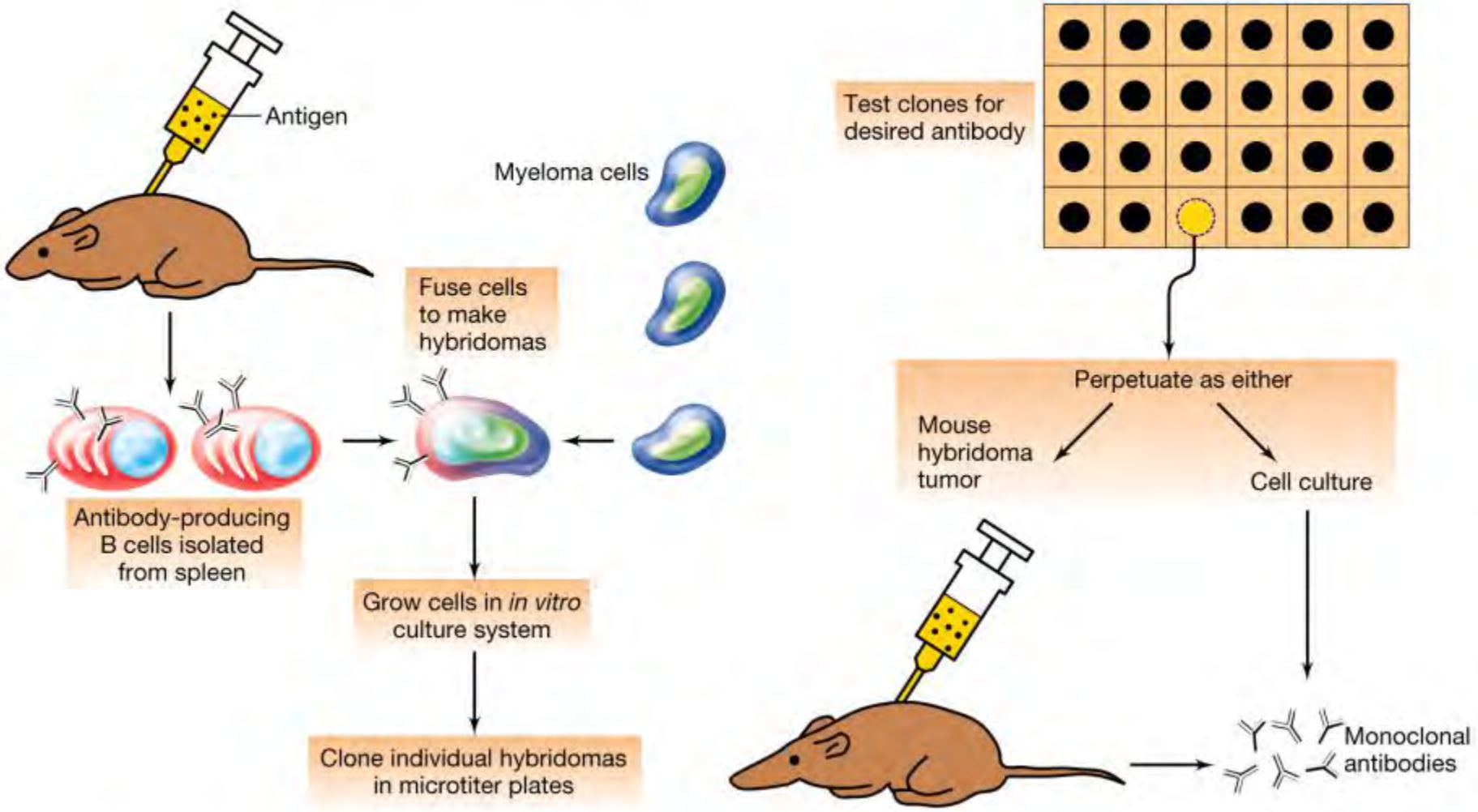
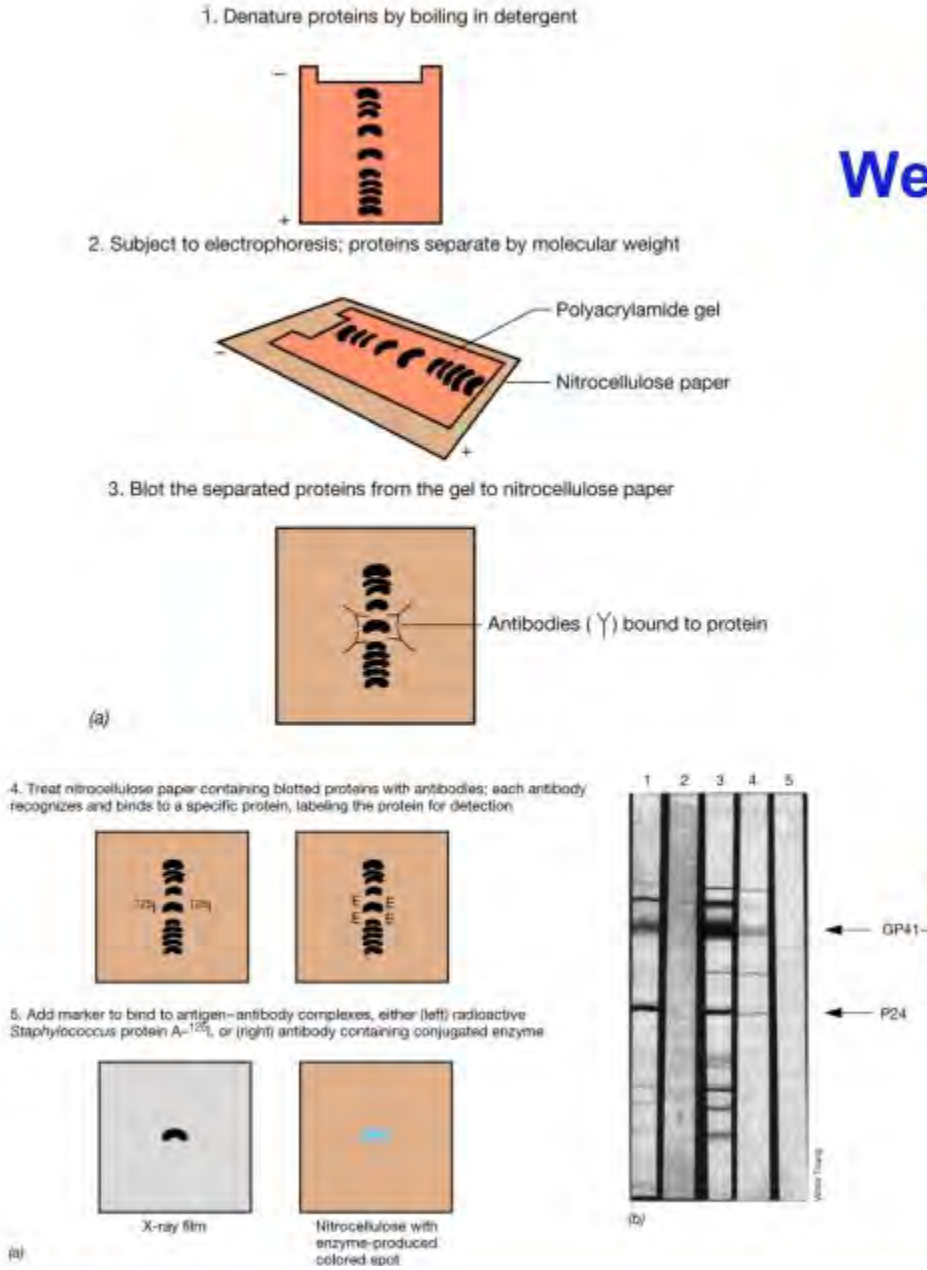
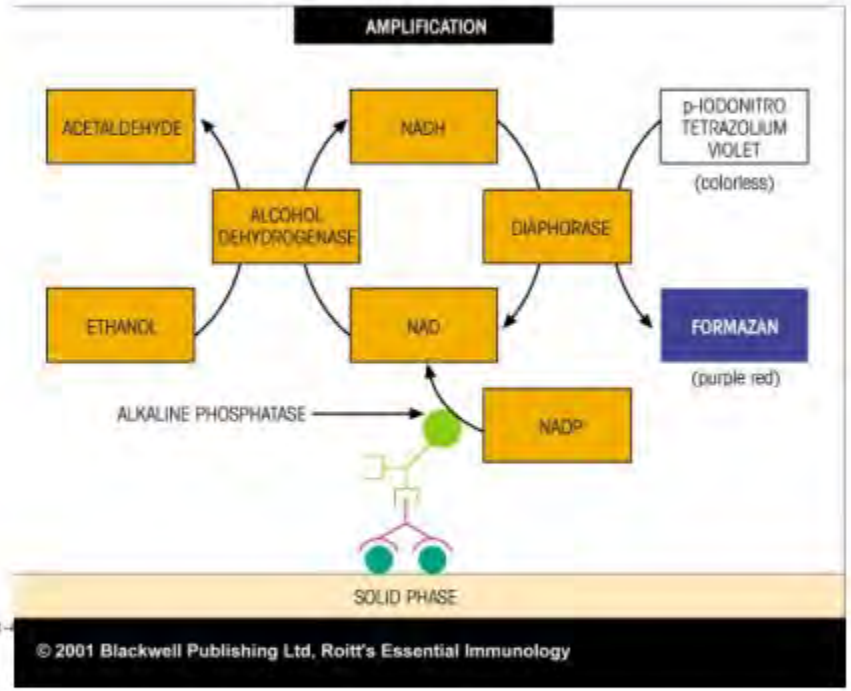
Immunological Methods: Generation of Monoclonal Antibodies



Immunological Methods: Western Blot as Diagnostic Tool





Alternative used in the course: Chemoluminescent reaction

Lecture Topic: Western Blot Analysis

Date: Thursday May 9th 2013

Introduction

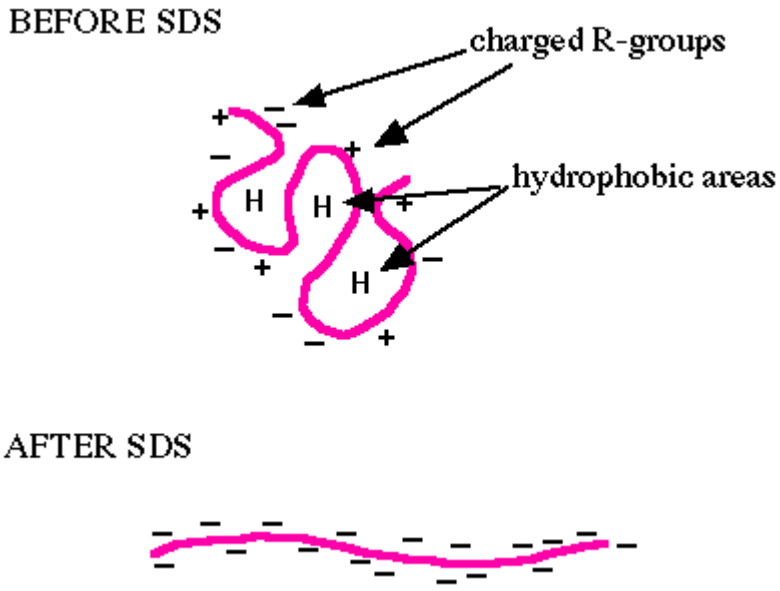
- Immuno (Western) Blotting is a commonly used technique to detect specific proteins from a complex mixture.
- It provides information on:
  - Protein expression (relative to a control sample)
  - Protein size (based on a marker protein run along with your sample)

Steps in Western Blotting

1. Sample Preparation
2. Polyacrylamide Gel Electrophoresis (PAGE)
3. Transfer from gel to membrane
4. Incubation with antibody
5. Detection

Sample Preparation

- Cells are grown to desired density (OD)
- Samples are centrifuged to collect cells and separate media (discard supernatant)
- Wash samples in buffer to remove salts
- Coat samples in SDS-loading buffer
- Boil samples for 5 minutes to denature proteins



SDS dissociates hydrophobic areas and renders proteins highly electronegative so that their migration through the gel is independent of their isoelectric point.

SDS-PAGE

- Discontinuous Gel
  - Top: Stacking Gel
  - Bottom: Resolving Gel
- Proteins run from negative (anode) end to positive (cathode) end
- The percentage of gel used determines the pore size, the larger the percentage the more cross linking and the smaller the pore size

Western Transfer

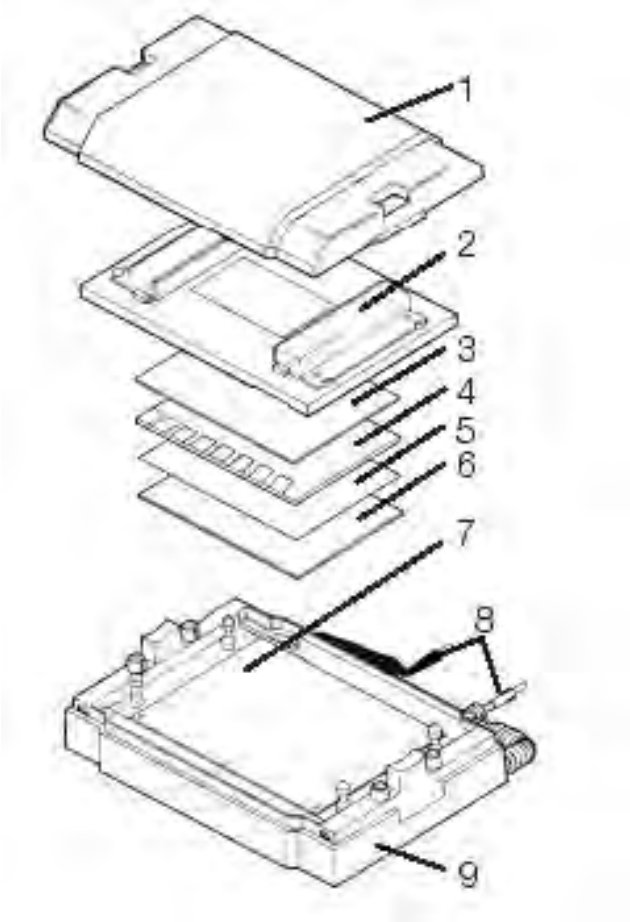
- Transfer from gel to PVDF (polyvinylidene fluoride membrane)
  - PVDF has good protein binding capacity (170-200ug/cm2), physical strength and enhanced binding in the presence of SDS
- Two Types of Transfer Units:

  1. Semi-dry Unit
  2. Mini Trans-Blot

Semi-Dry Electrophoretic Transfer Cell (BioRad)

- How to Set Up Transfer:

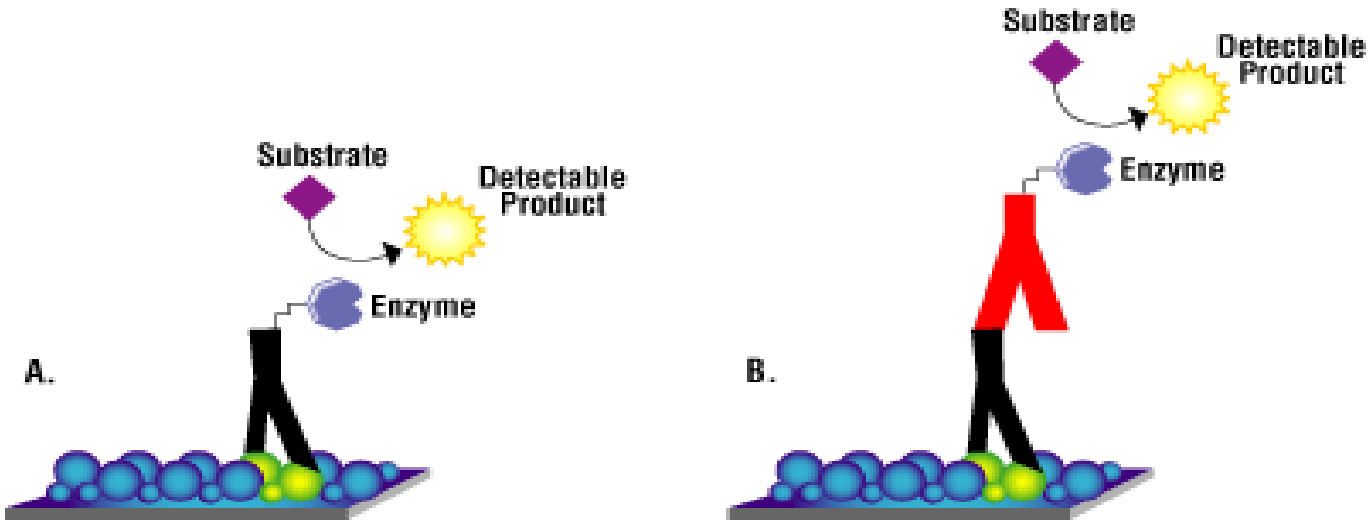
1. Safety Cover
2. Steel Cathode Assembly
3. Thick Blot Paper
4. Gel
5. Membrane (PVDF)
6. Thick Blot Paper
7. Platinum Anode
8. Power Cables
9. Base



Antibody Incubation

- After proteins are transferred from gel to membrane, the membrane is blocked using 5% milk.
- Blocking prevents non-specific interactions
- After blocking, the membrane is incubated in primary antibody

Direct and Indirect Detection



Draw this outshamr

Detection

- The secondary antibody is attached to HRP (horse radish peroxidase) enzyme
- HRP catalyzes the oxidation of luminol (substrate)
- Oxidation of luminol will put it in an excited state followed by decay to ground state accompanied by the emission of LIGHT
- The light is captured on a special film
- The intensity of the light is correlated with the abundance of protein present
- Enhanced Chemiluminescence occurs in the presence of chemical enhancers such as phenol.
- Signal is increased by 1000 fold