Techniques in Molecular Genetics

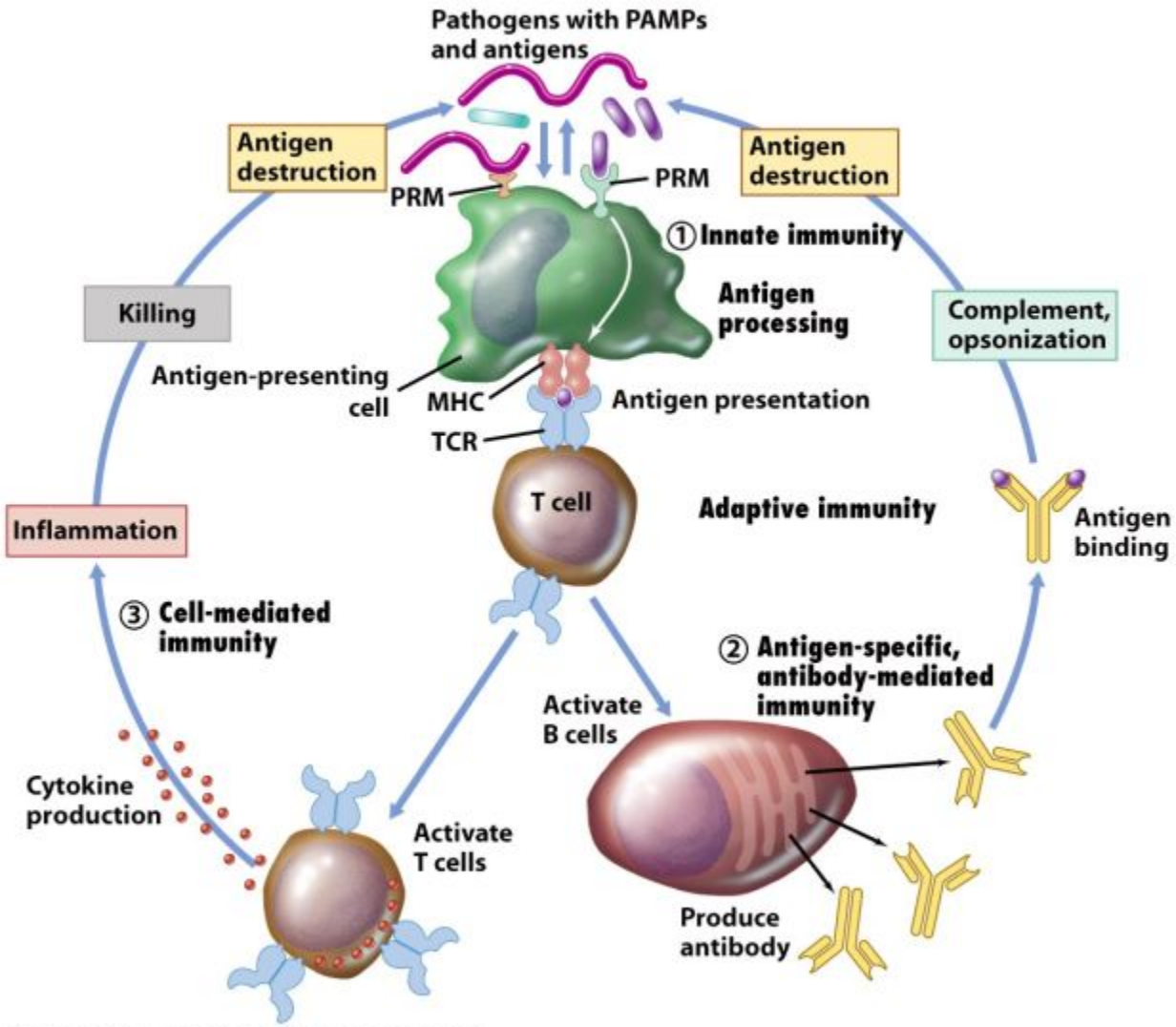
Immunological Methods And Western Analysis

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

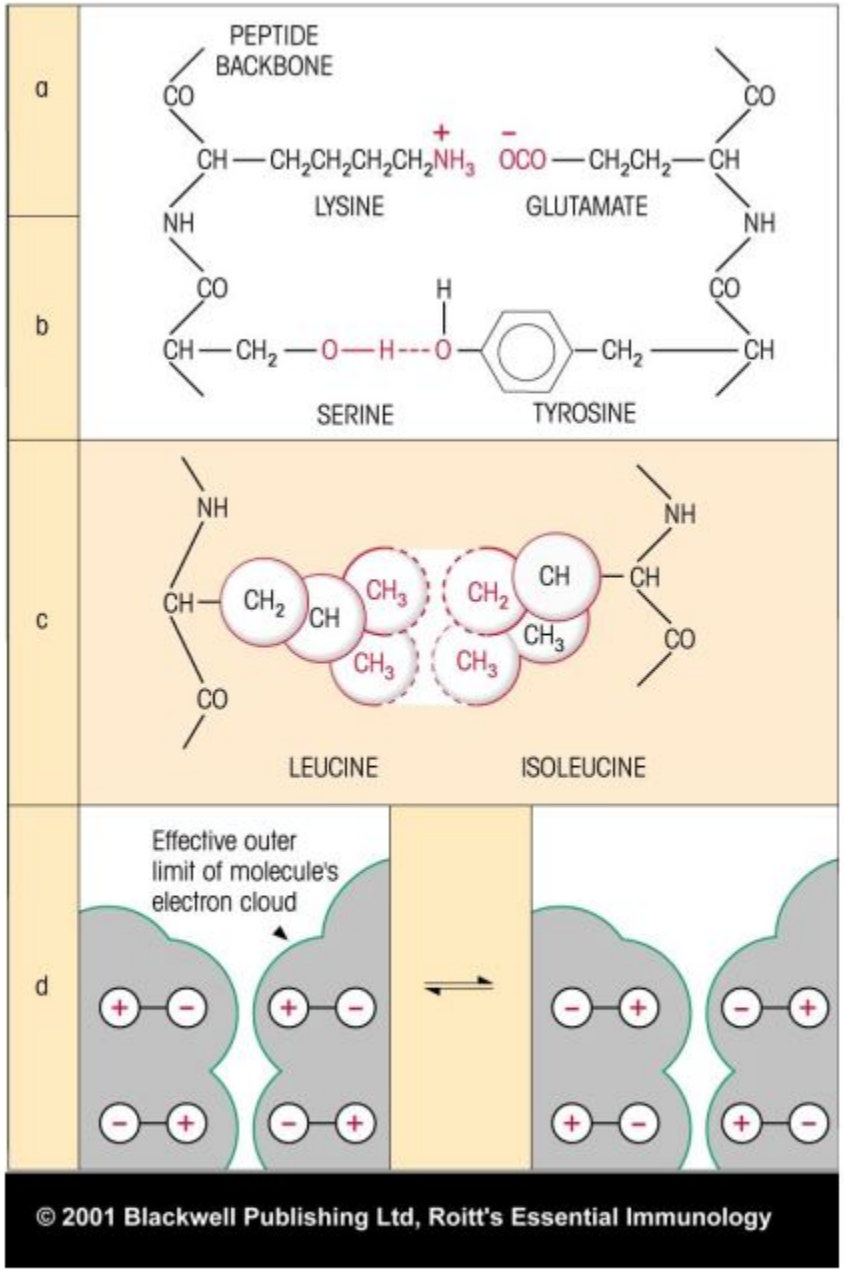
Cell-mediated immune response

→ Defence against intracellular pathogens (viruses and bacteria)

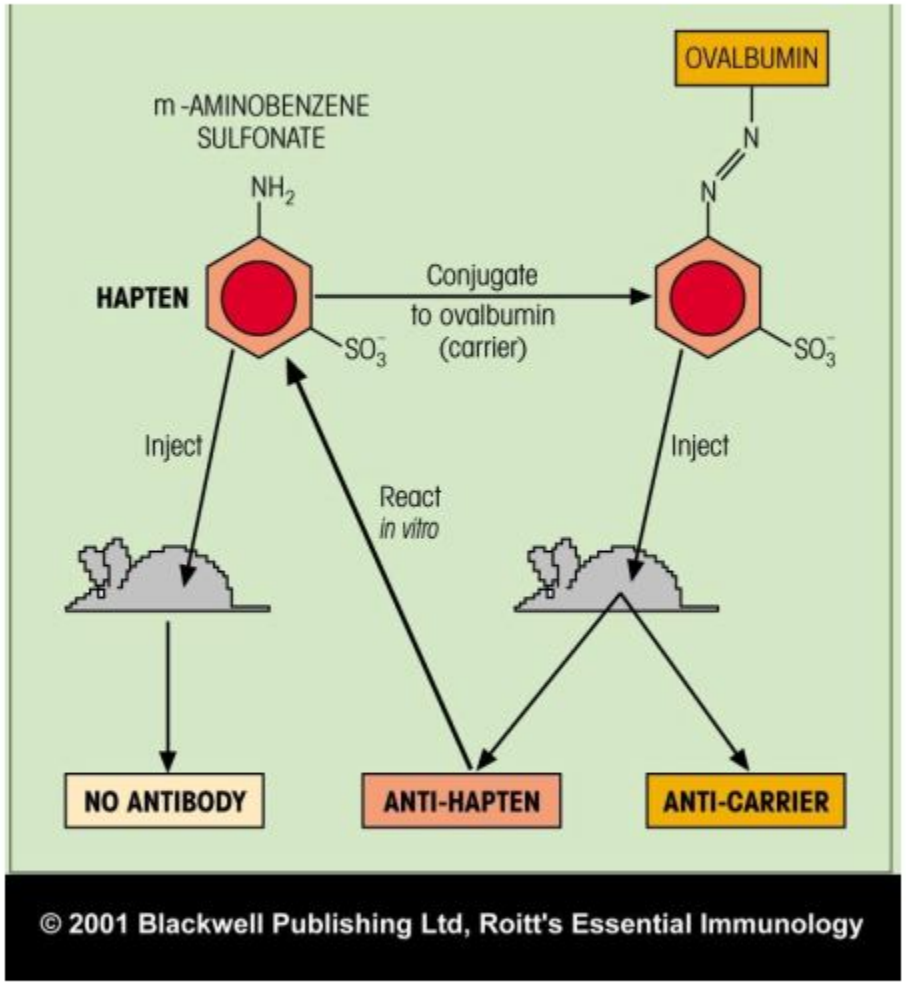
→ Effector T cell (CD4 helper and CD8 cytotoxic)

→ Dependence on antigen presentation by MHC I or II

Immunological Methods: Stabilization of Antigen-Antibody Interactions

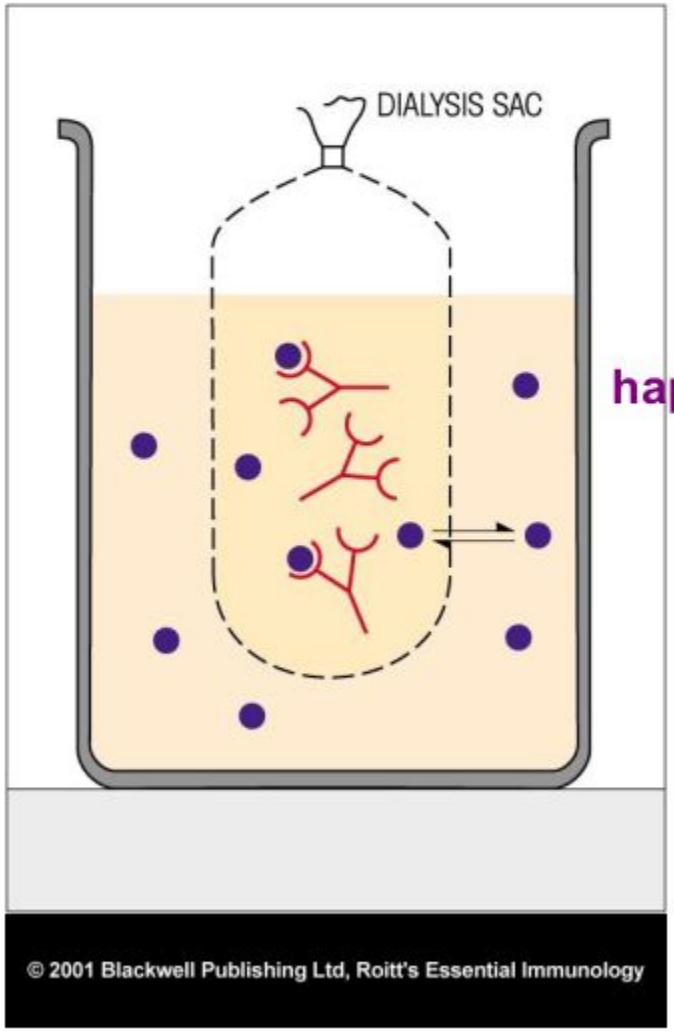


Immunological Methods: Immunization with Haptens

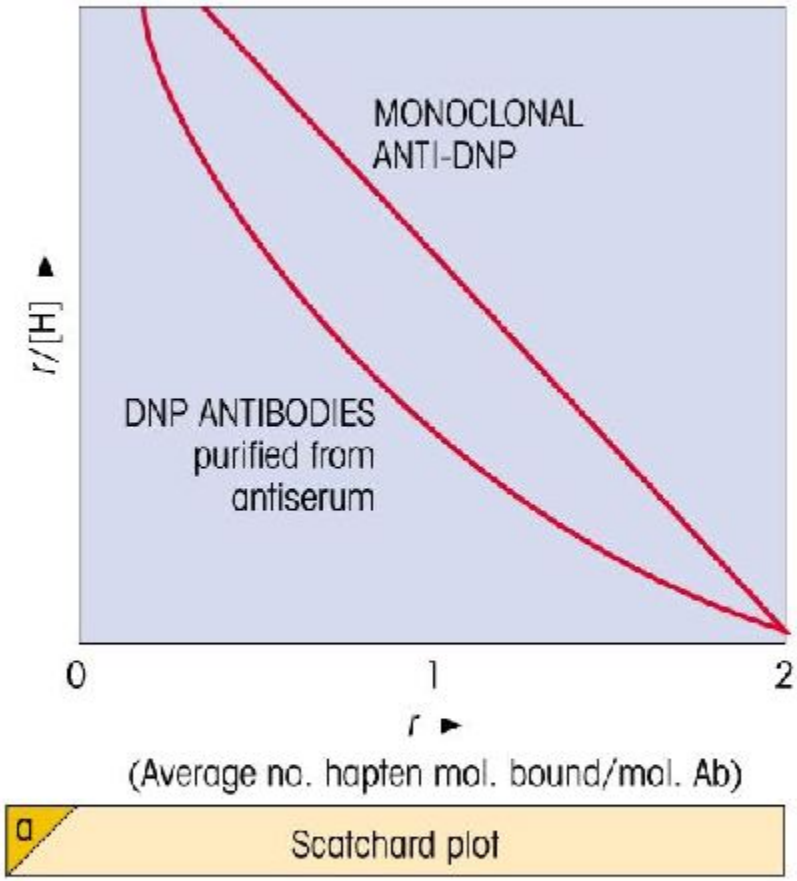


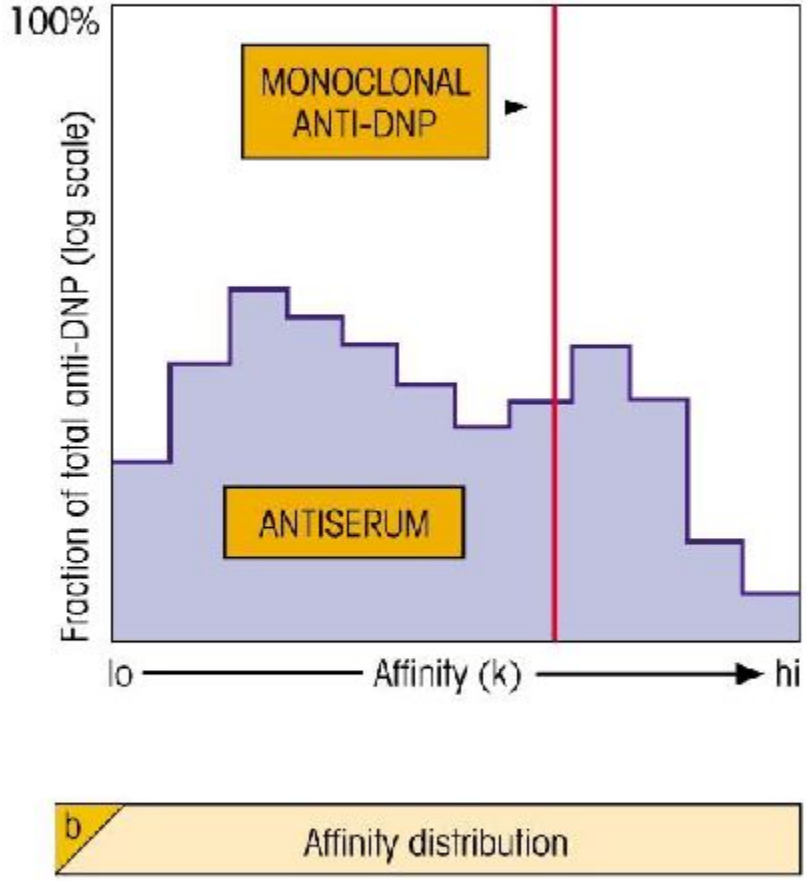
Application: Creation of peptide antisera

Immunological Methods:
Characterization of Antigen-Antibody Interactions



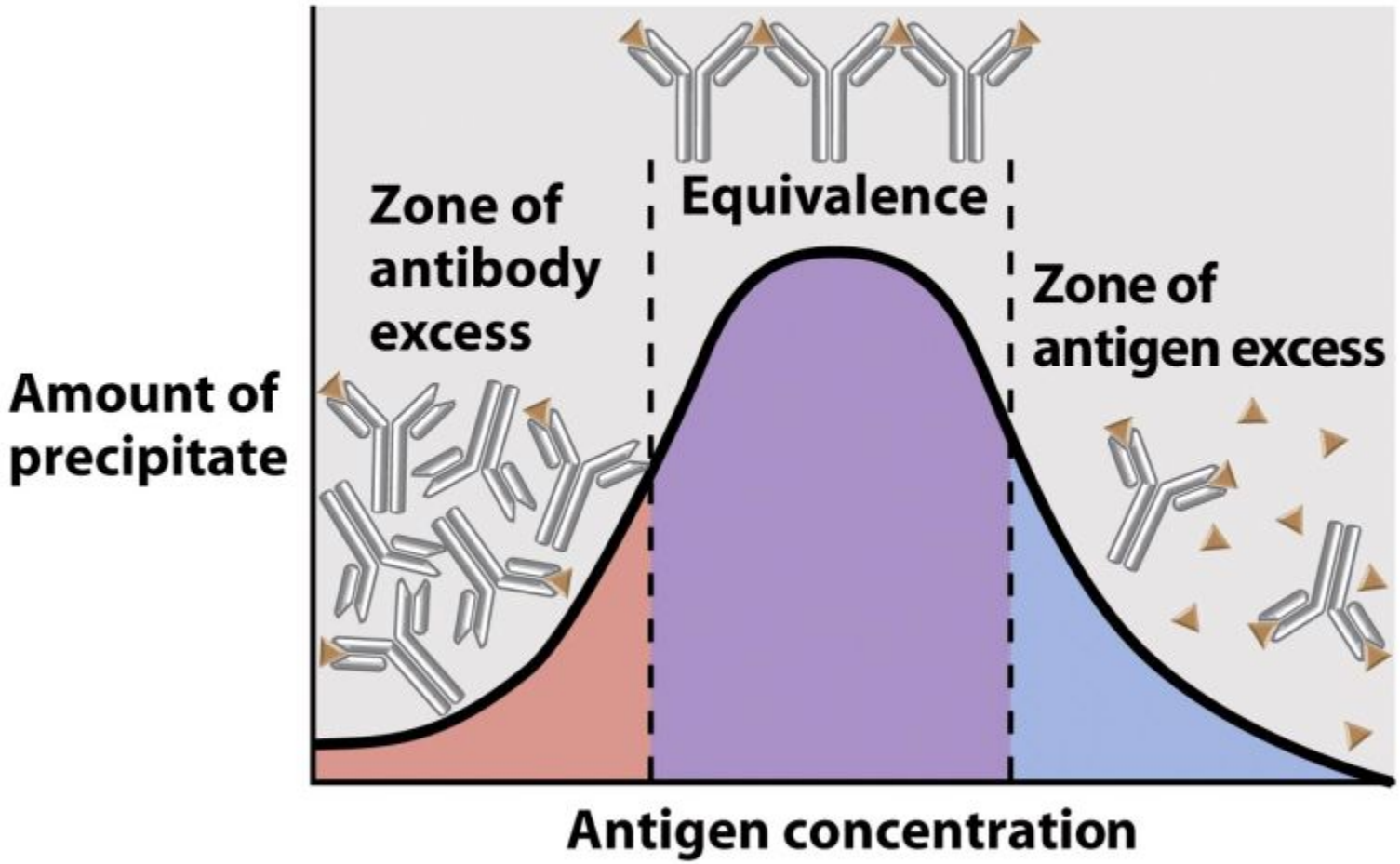
Immunological Methods:
Antisera Contain Antibodies with Different Affinities





Monoclonal antibodies vs polyclonal antiserum

Immunological Methods:
Principle of Immunoprecipitation - IP



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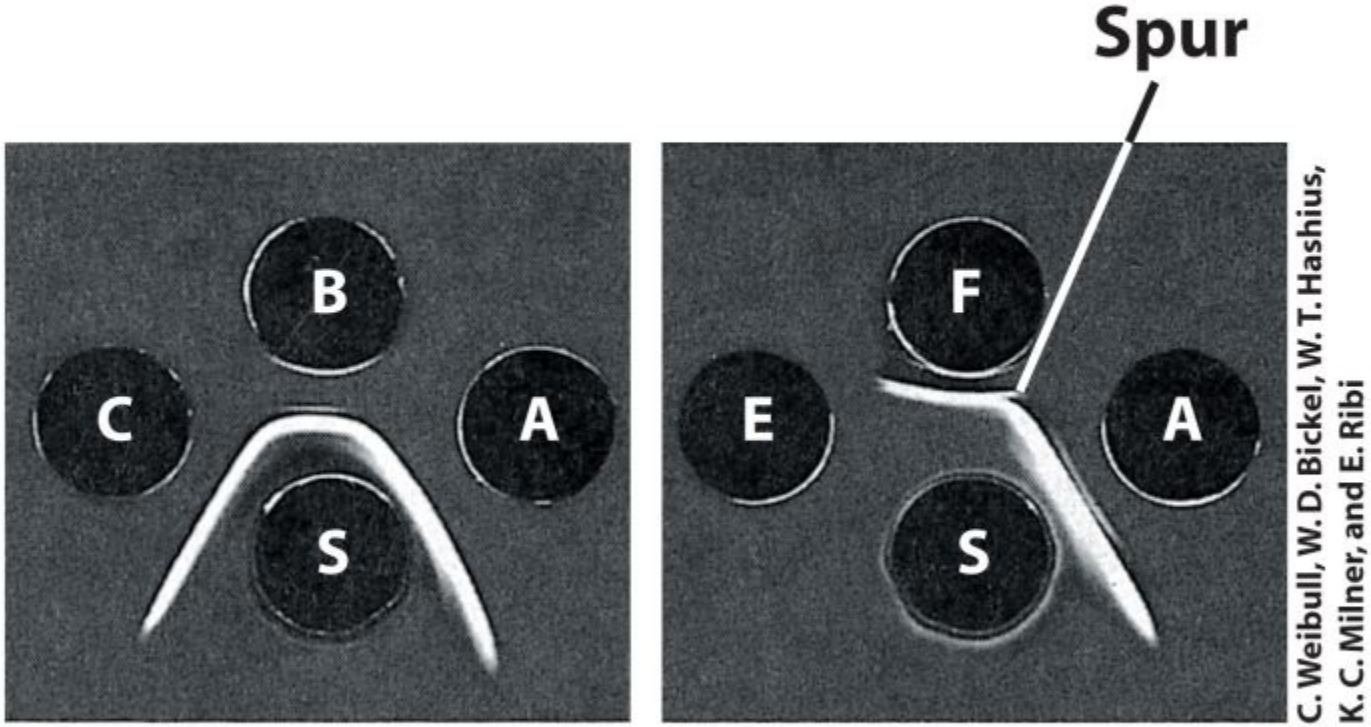
Immunological Methods:
IP Application - Immunodiffusion



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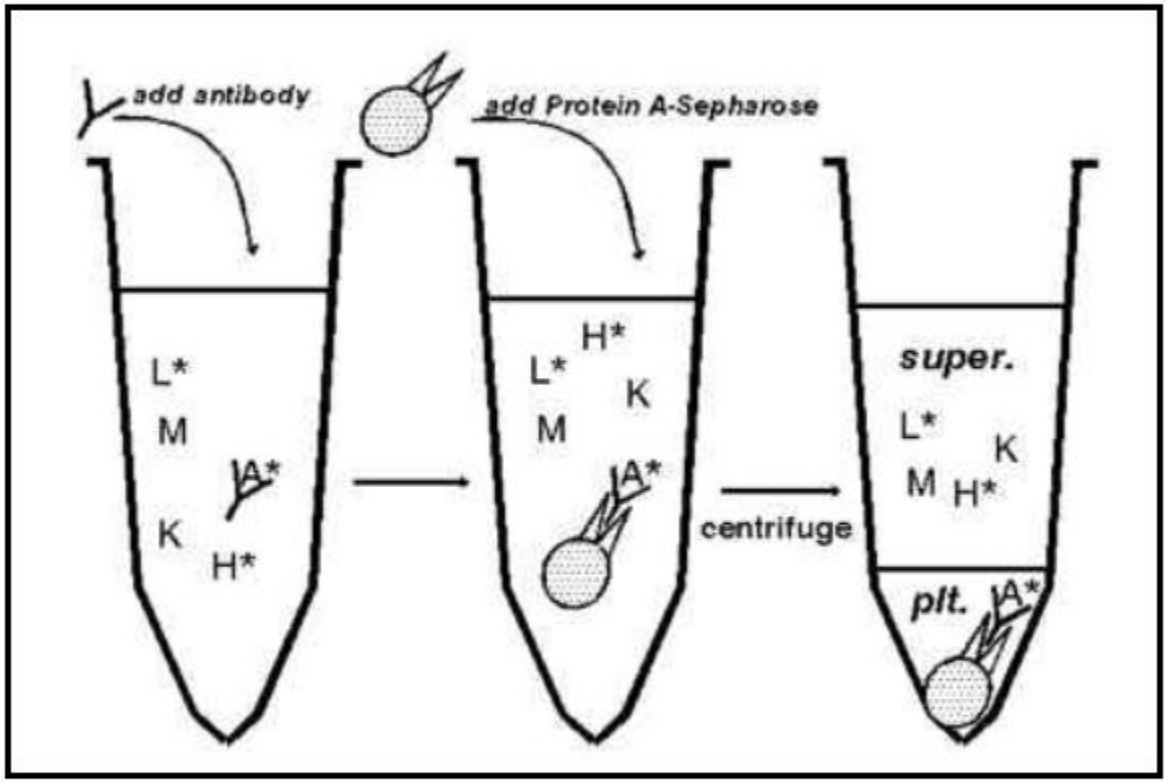
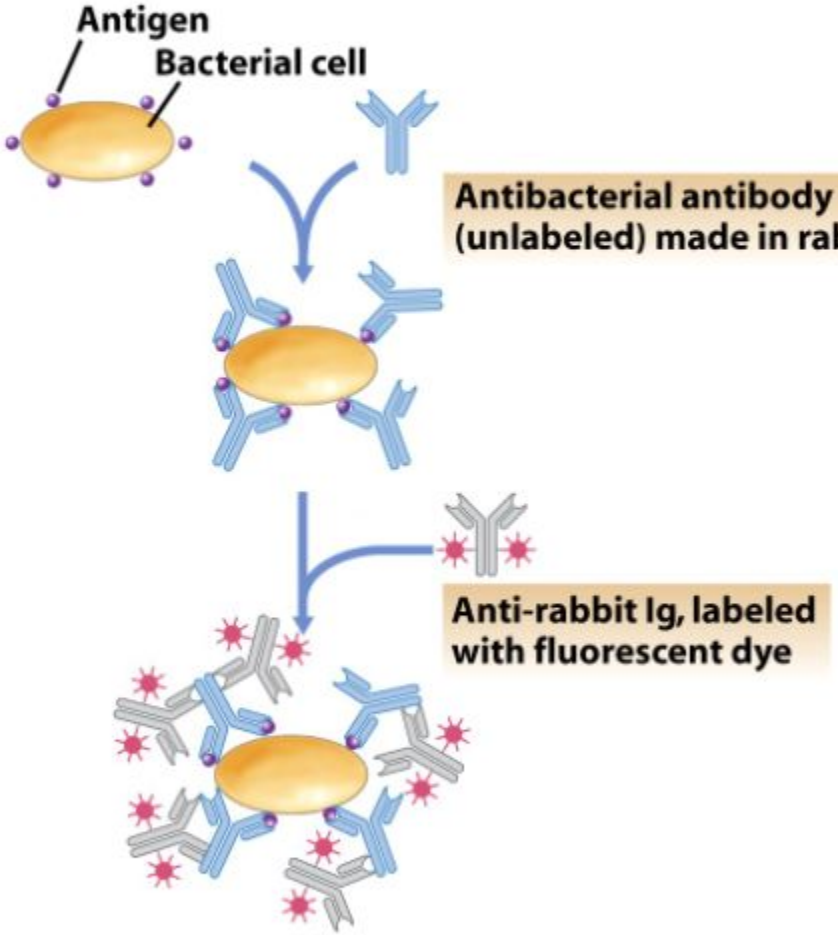
Immunological Methods:
IP Application - Immunoprecipitation



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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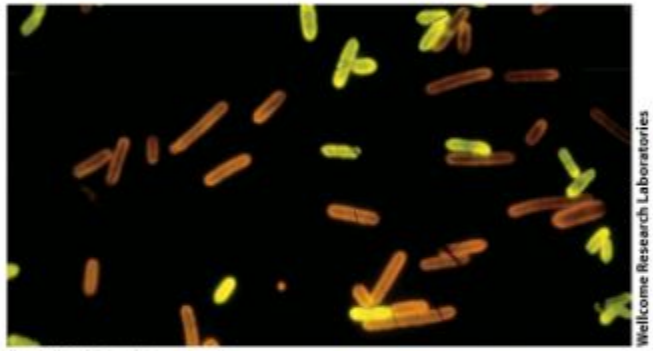

Wellcome Research Laboratories

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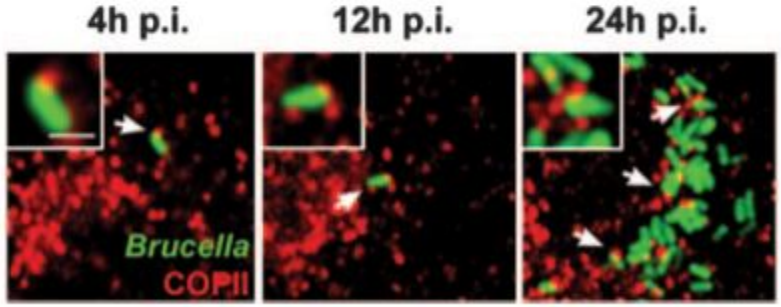
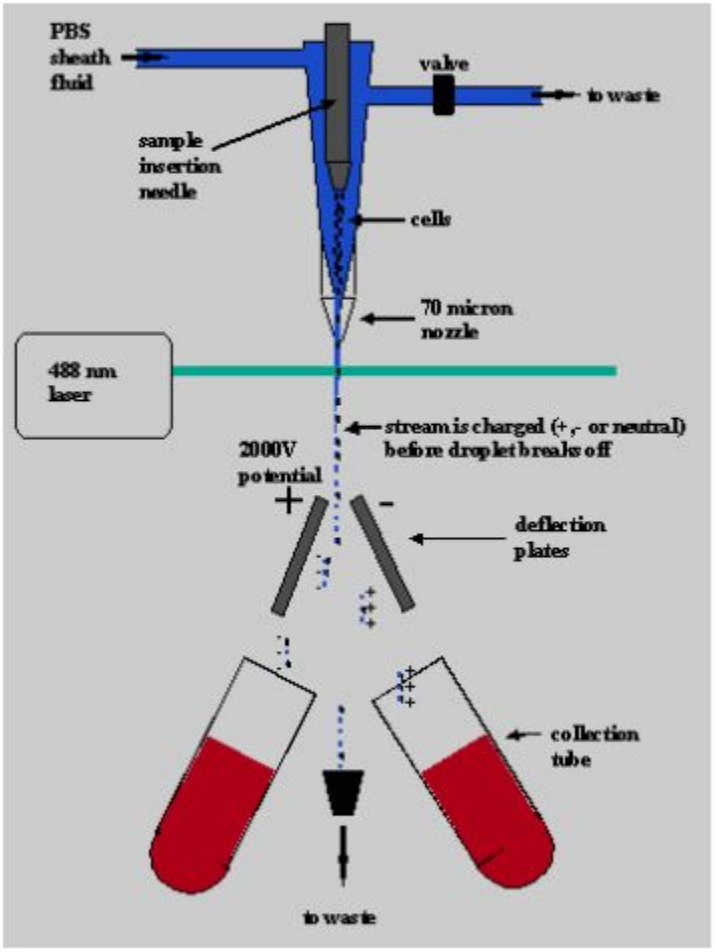
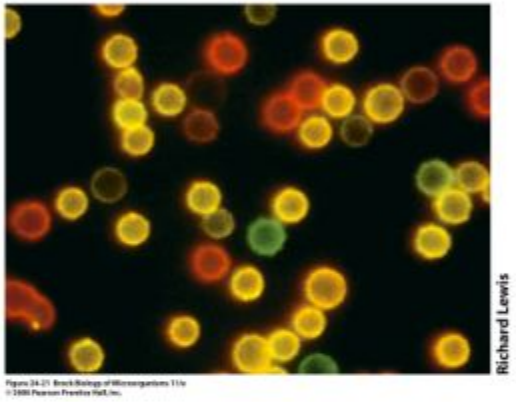


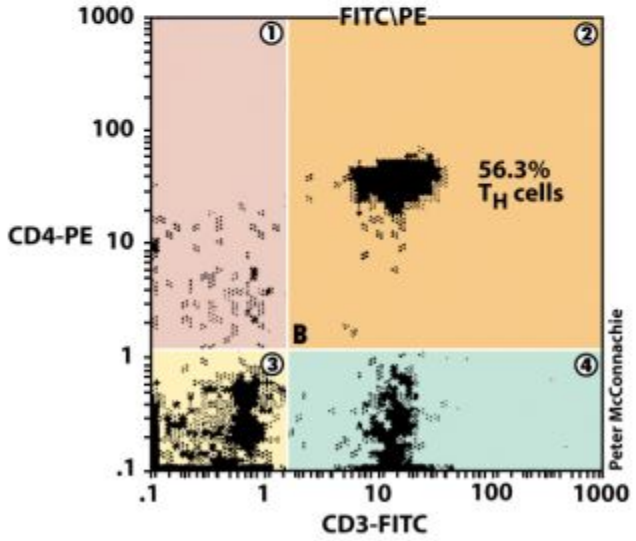
Immunological Methods:
Fluorescence-Activated Cell Sorter - FACS



Courtesy Vanderbilt Medical Center

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





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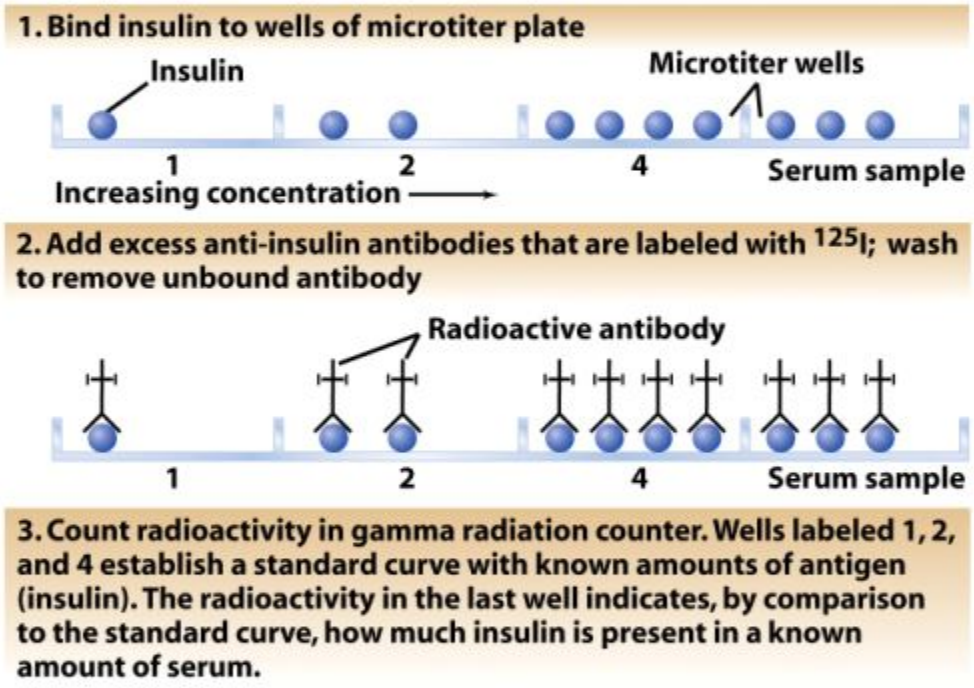
Immunological Methods: Radioimmunoassay - RIA



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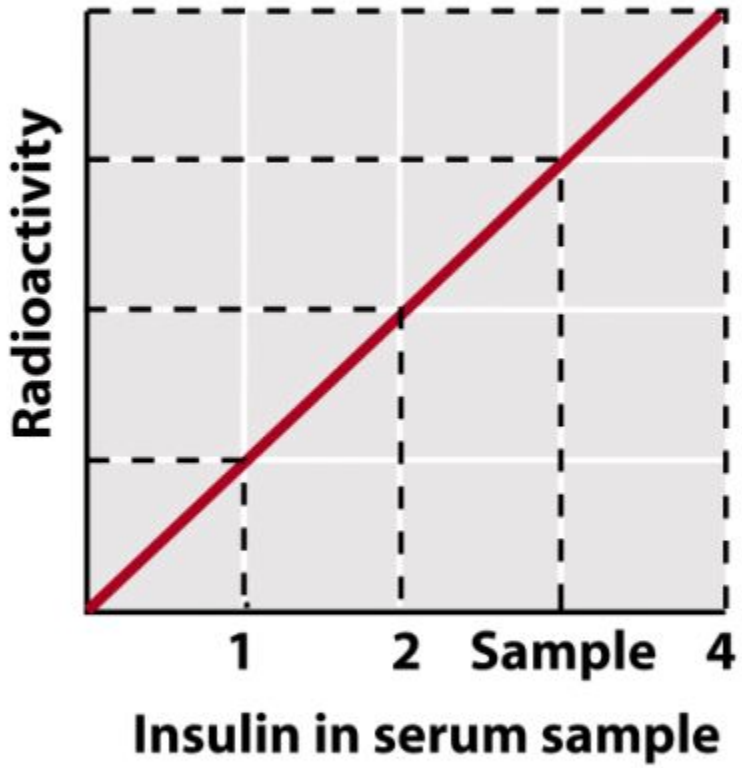

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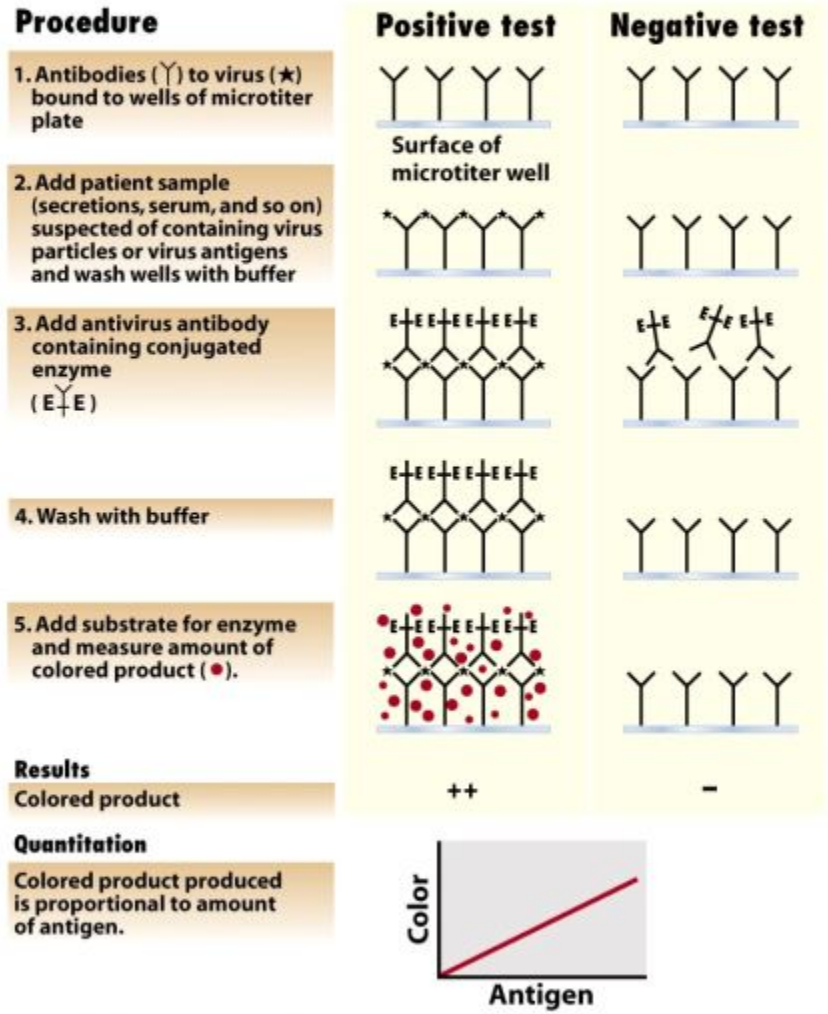
Immunological Methods:
Enzyme-Linked Immunosorbant Assay ELISA



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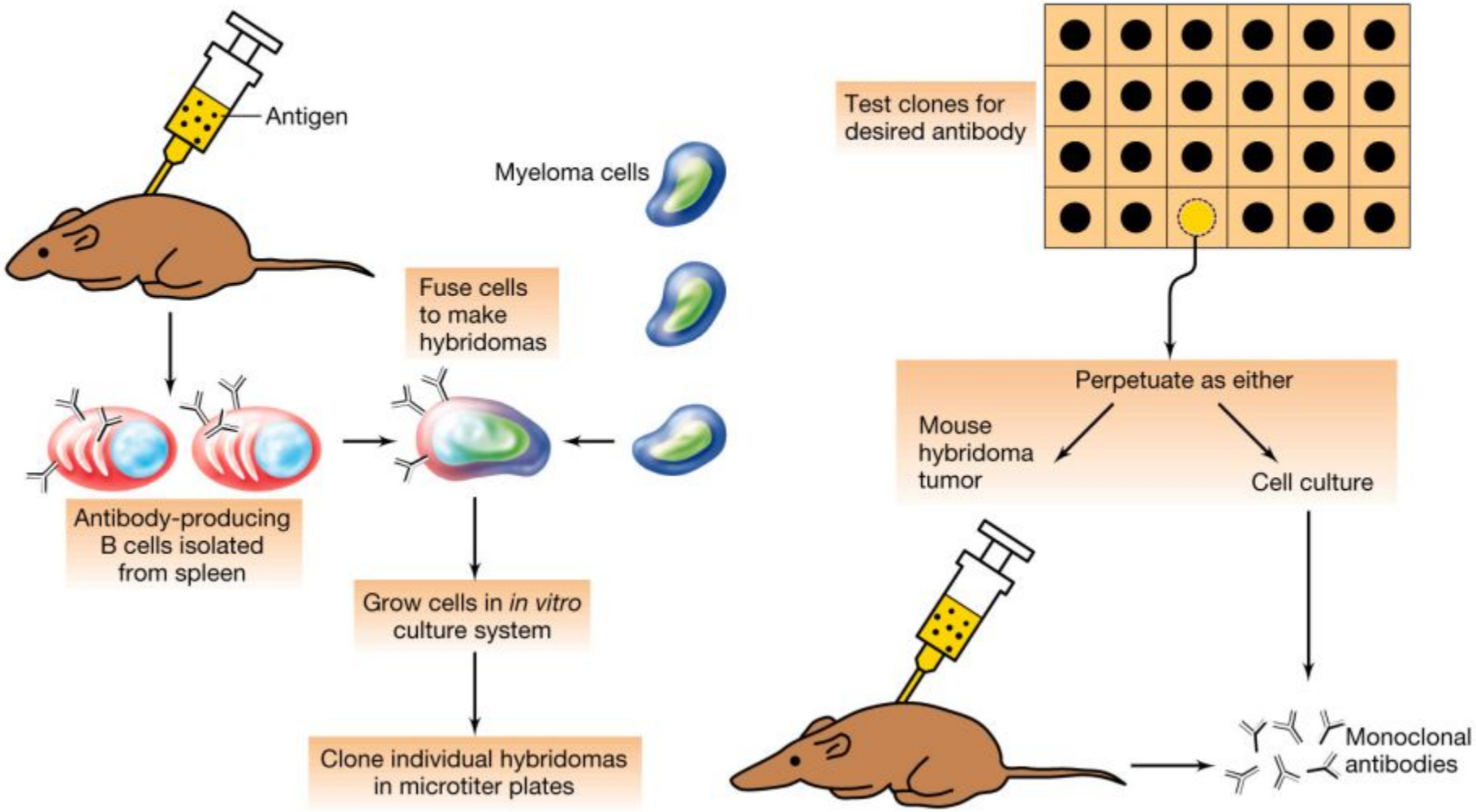
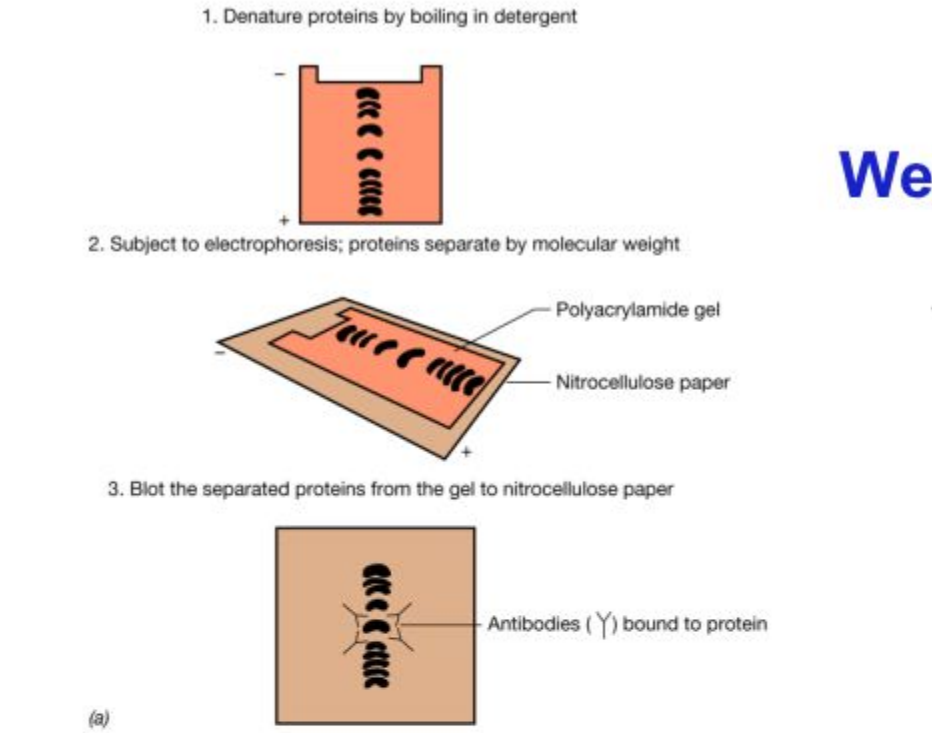
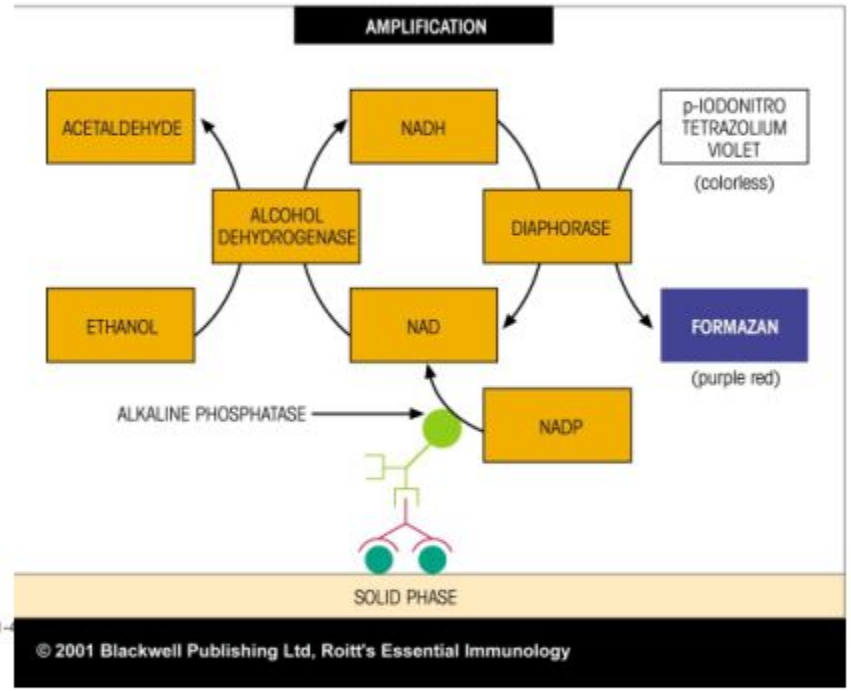
Immunological Methods: Generation of Monoclonal Antibodies



Immunological Methods: Western Blot as Diagnostic Tool





Alternative used in the course: Chemoluminescent reaction