

# 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

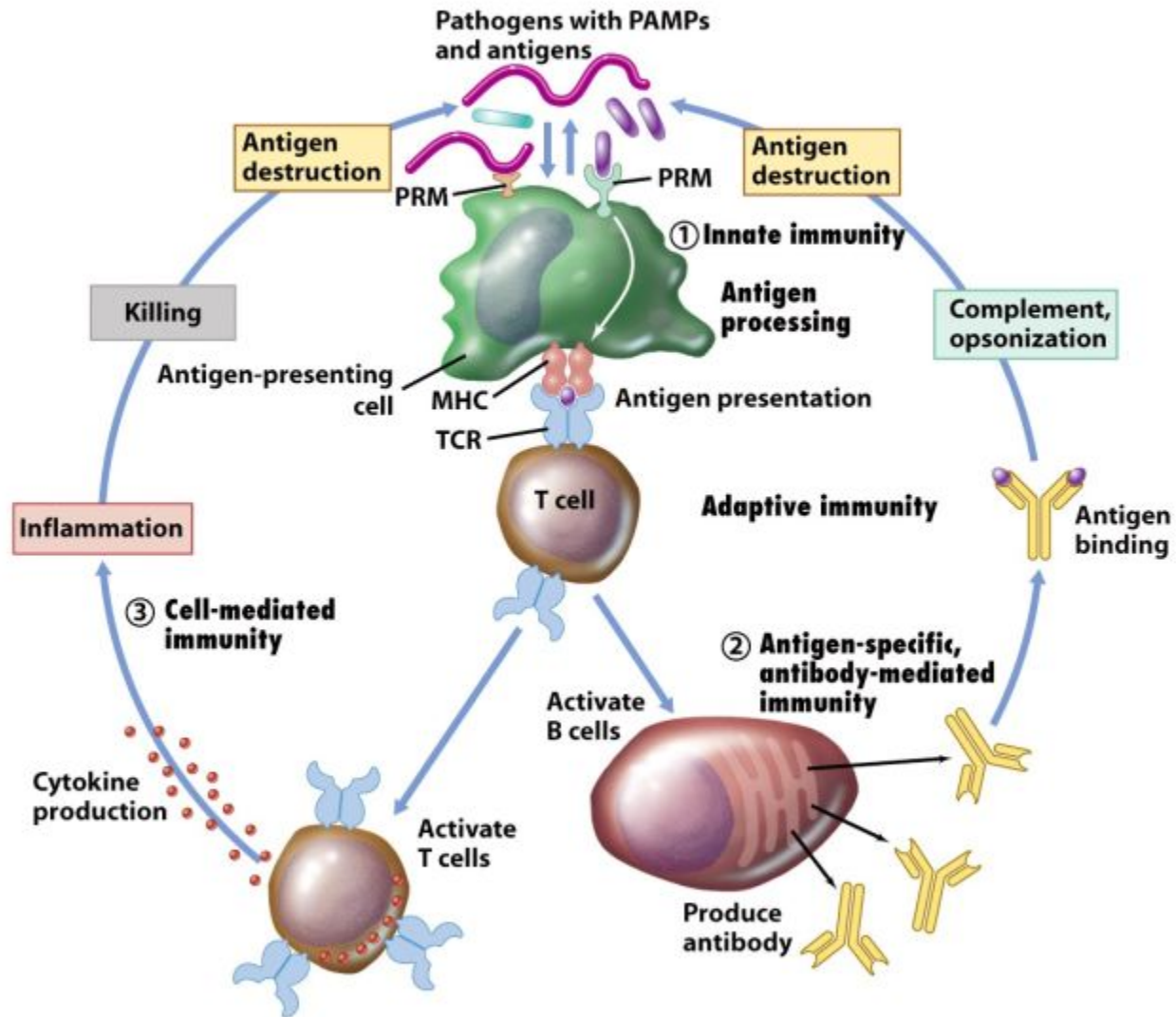


Figure 22-5 Brock Biology of Microorganisms 11/e  
© 2006 Pearson Prentice Hall, Inc.

## Two Sides of the Adaptive Immune Response

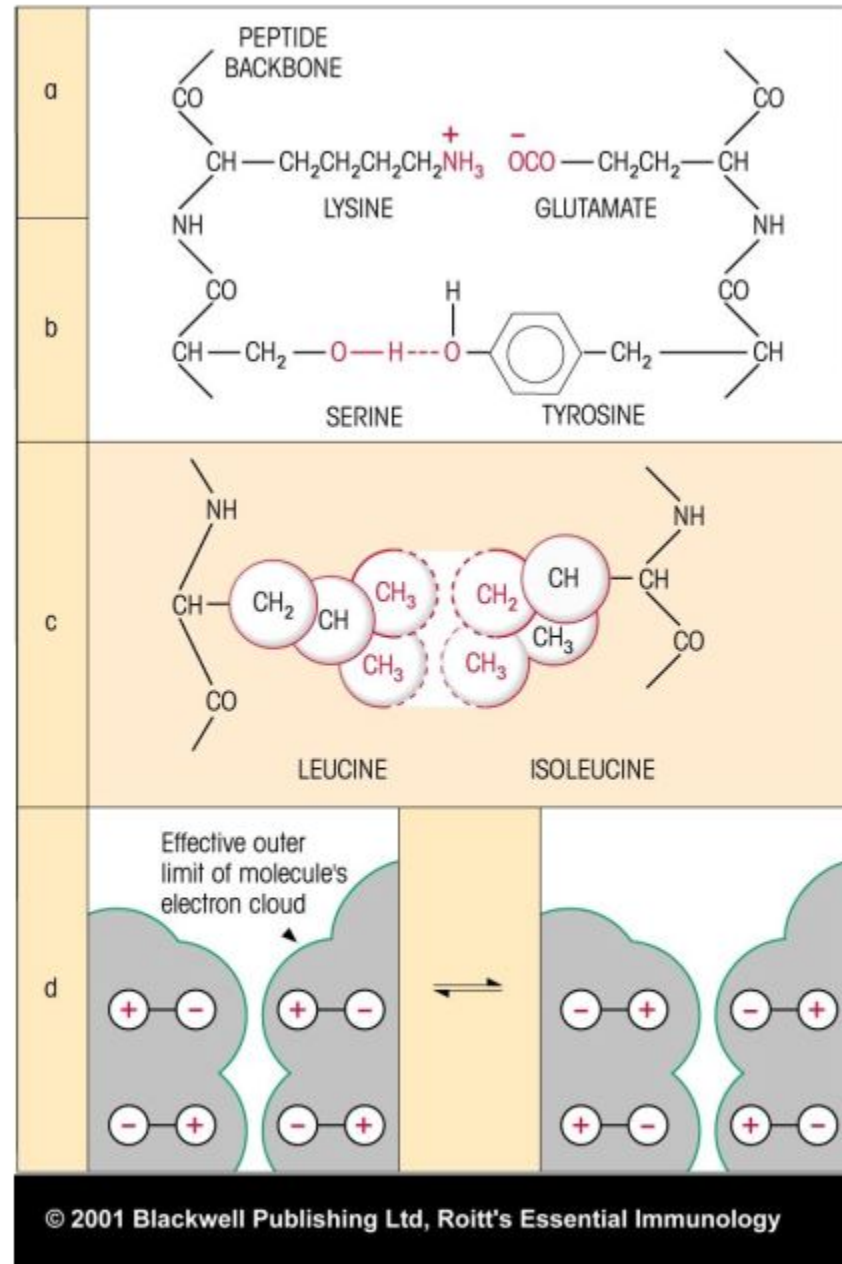
### *Humoral immune response*

- Defence against extracellular pathogens
- Soluble Effectors: Antibodies produced by B cells

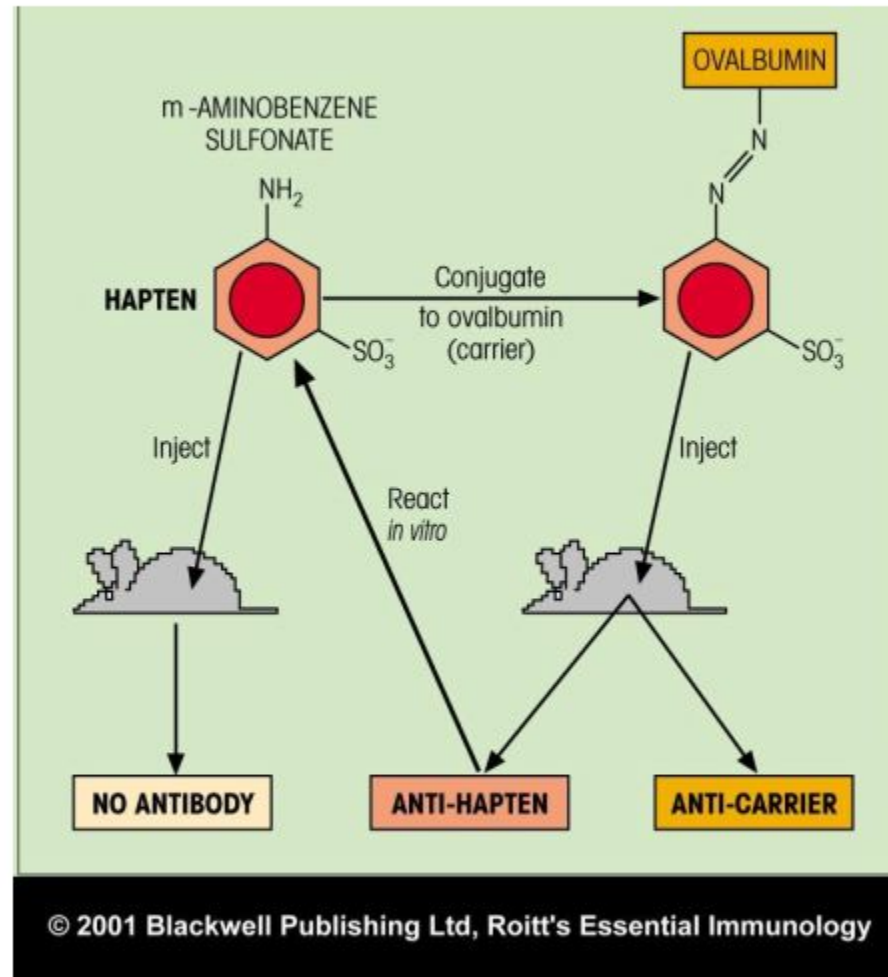
### *Cell-mediated immune response*

- 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

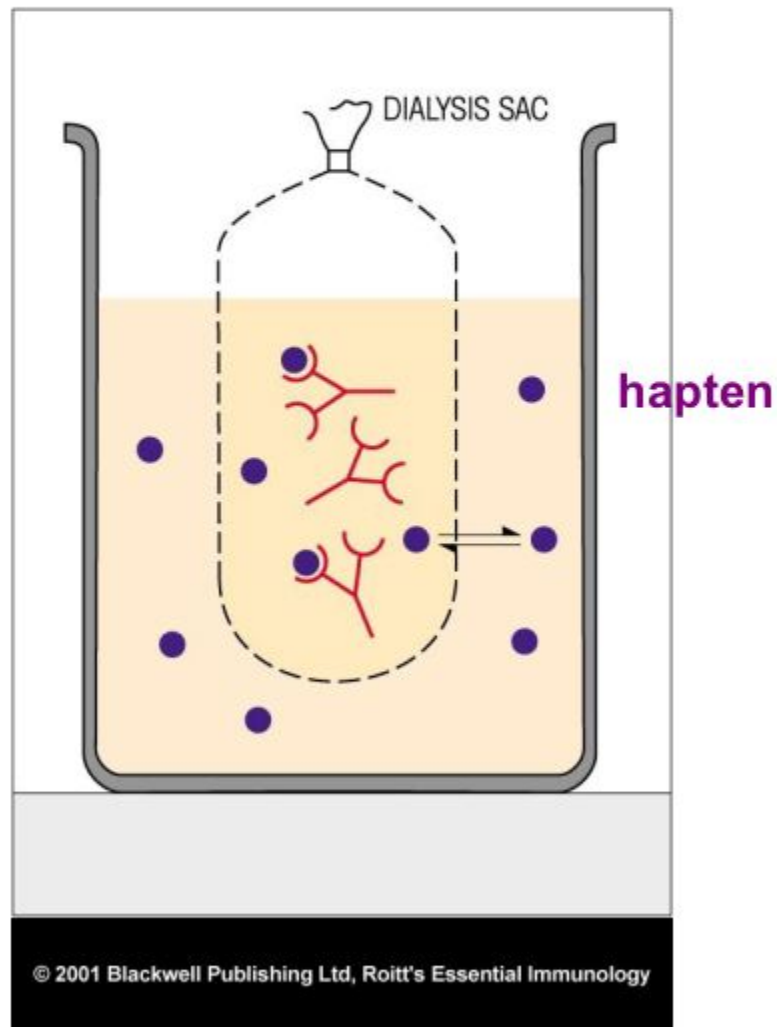


# Immunological Methods: Immunization with Haptens

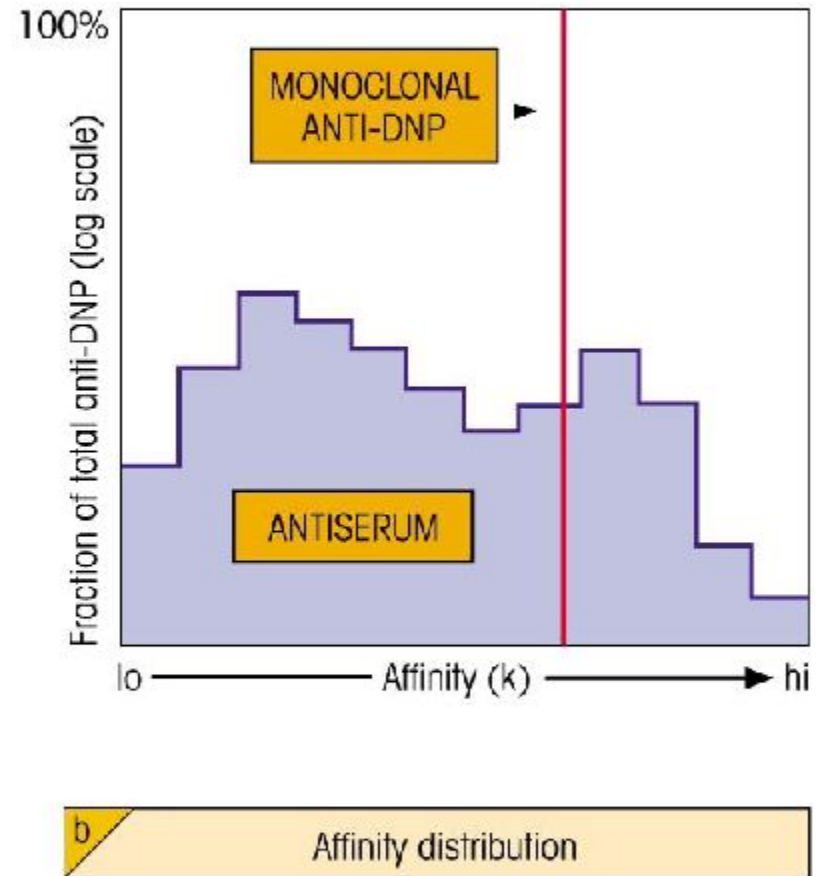
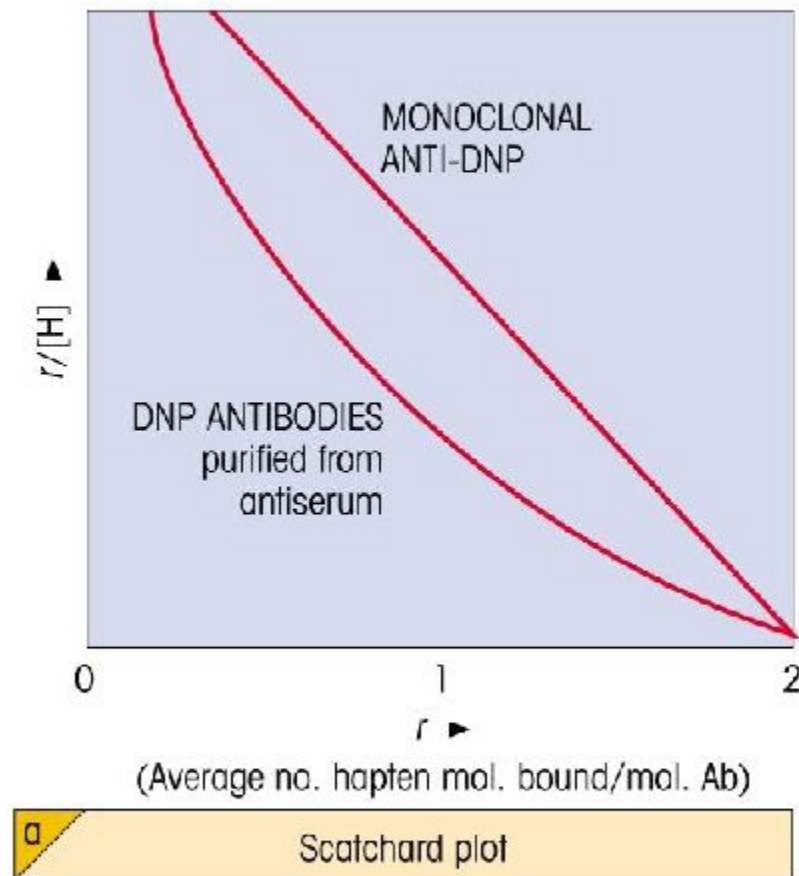


**Application: Creation of peptide antisera**

# 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

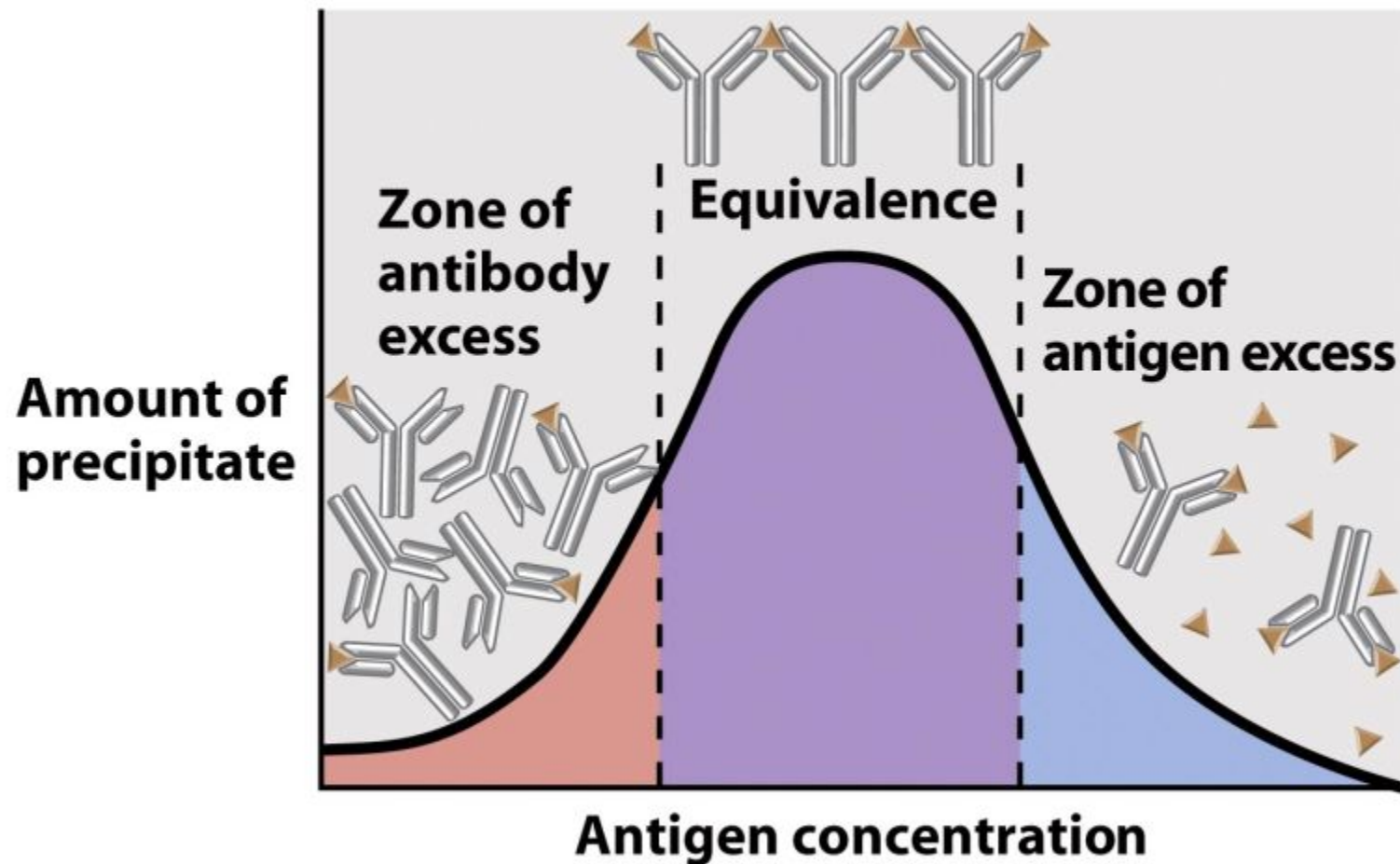


Figure 24-14a Brock Biology of Microorganisms 11/e  
© 2006 Pearson Prentice Hall, Inc.

## Immunological Methods: IP Application - Immunodiffusion

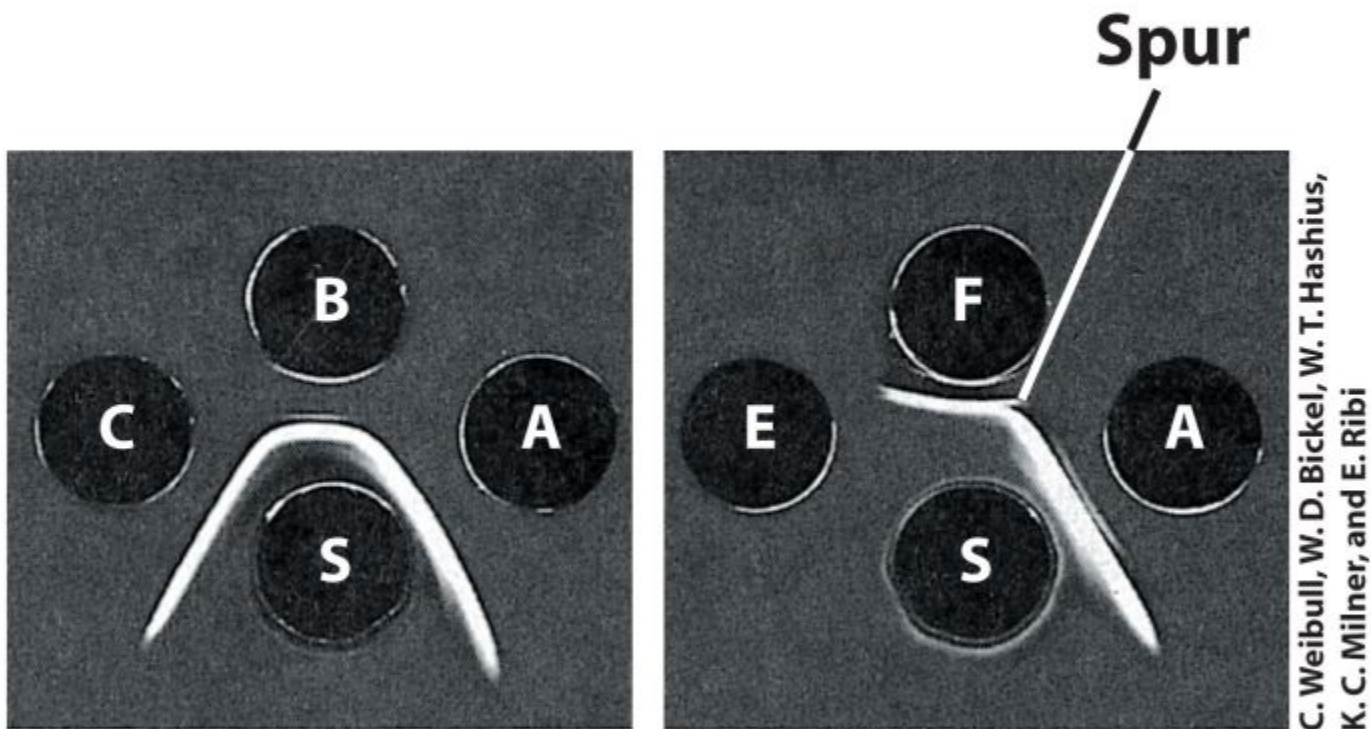
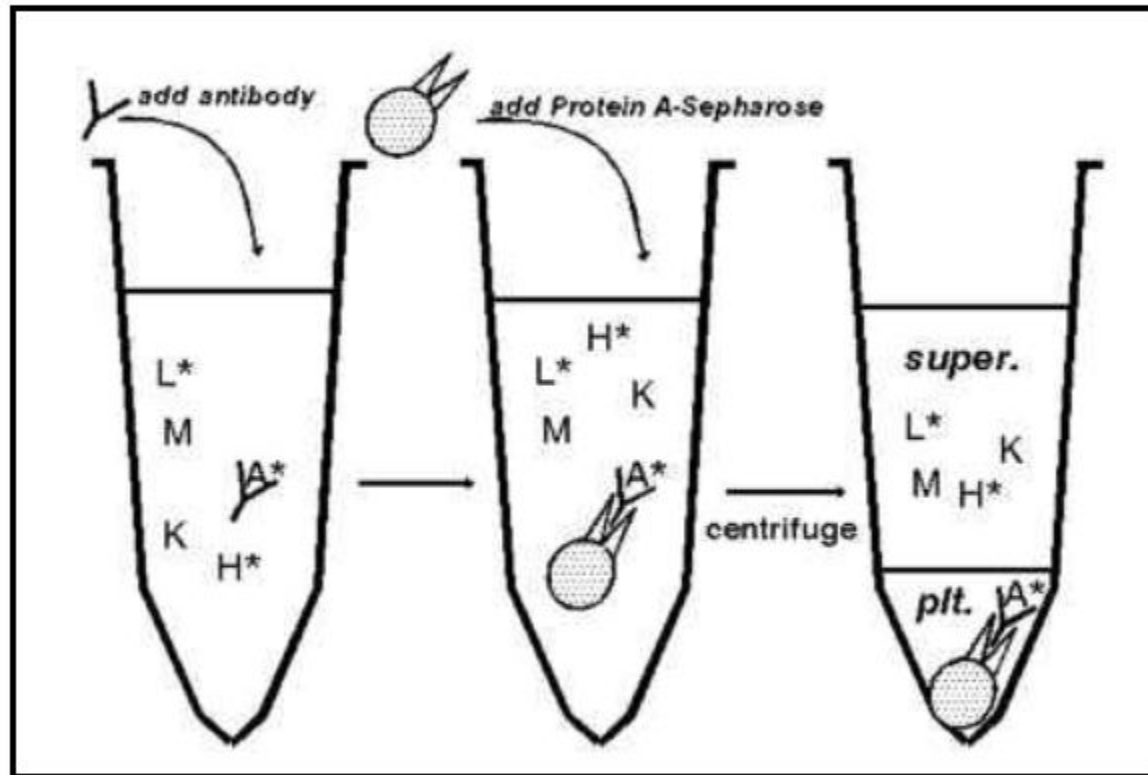


Figure 24-14b Brock Biology of Microorganisms 11/e  
© 2006 Pearson Prentice Hall, Inc.

C. Weibull, W. D. Bickel, W. T. Hashius,  
K. C. Milner, and E. Ribi

## 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

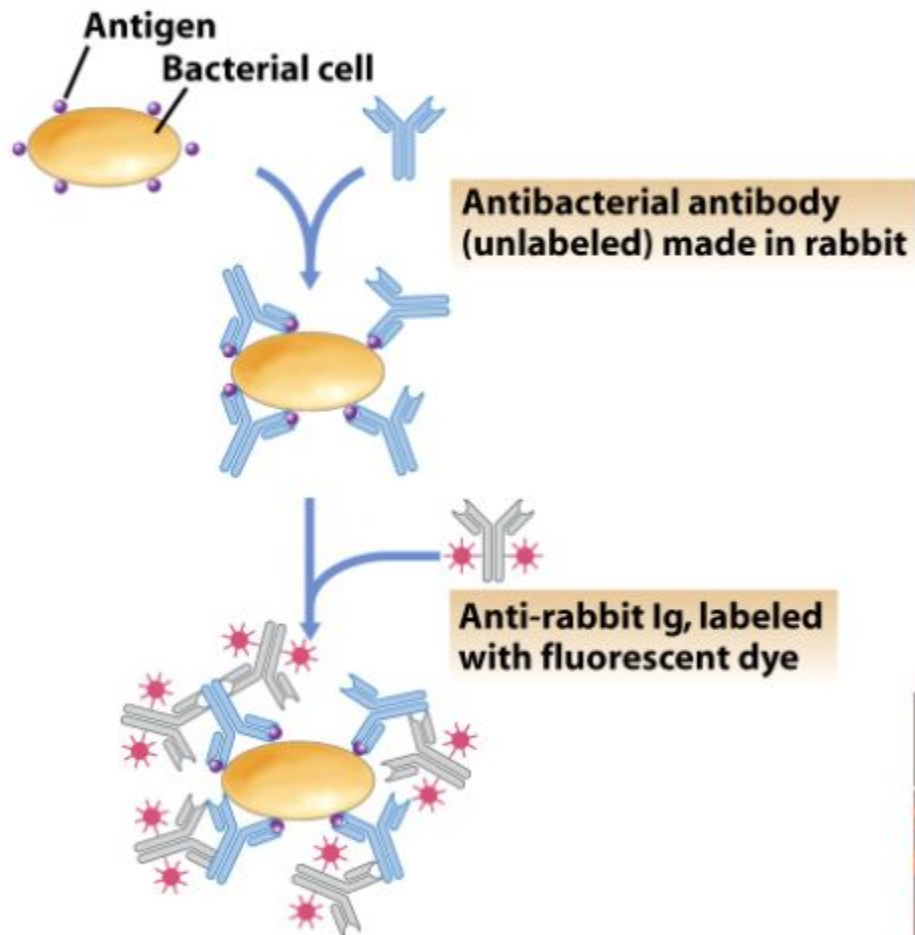


Figure 24-18b Brock Biology of Microorganisms 11/e  
© 2006 Pearson Prentice Hall, Inc.

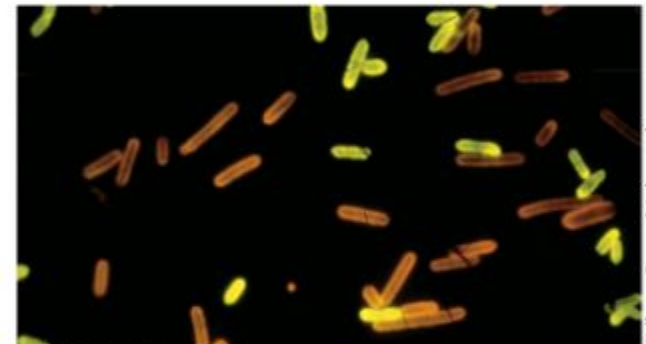
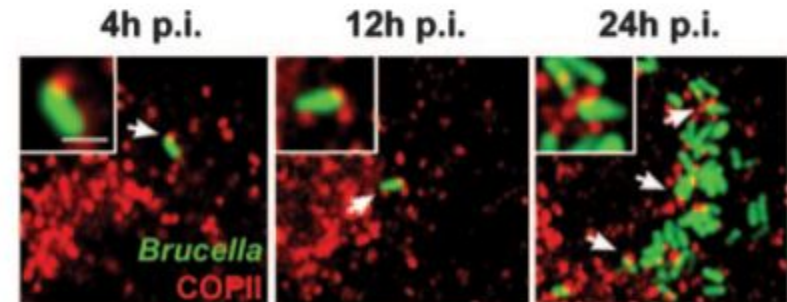


Figure 24-17 Brock Biology of Microorganisms 11/e  
© 2006 Pearson Prentice Hall, Inc.

Wellcome Research Laboratories



# Immunological Methods: Fluorescence-Activated Cell Sorter - FACS

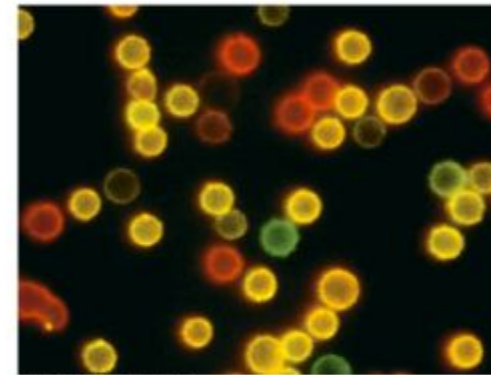
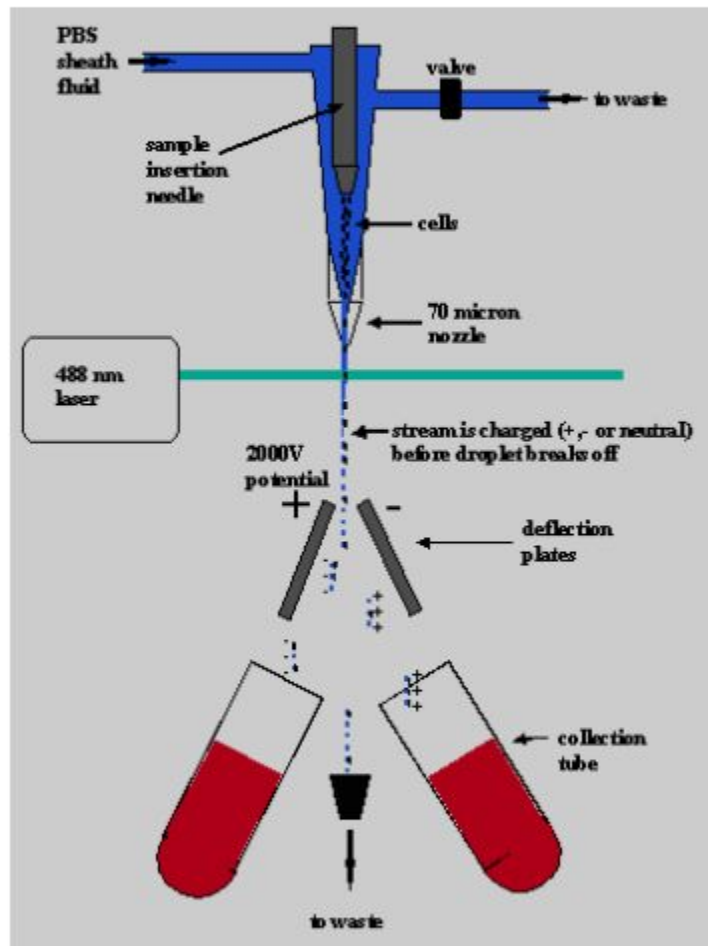


Figure 24-21 Brock Biology of Microorganisms 11/e  
© 2004 Pearson Prentice Hall, Inc.

Richard Lewis

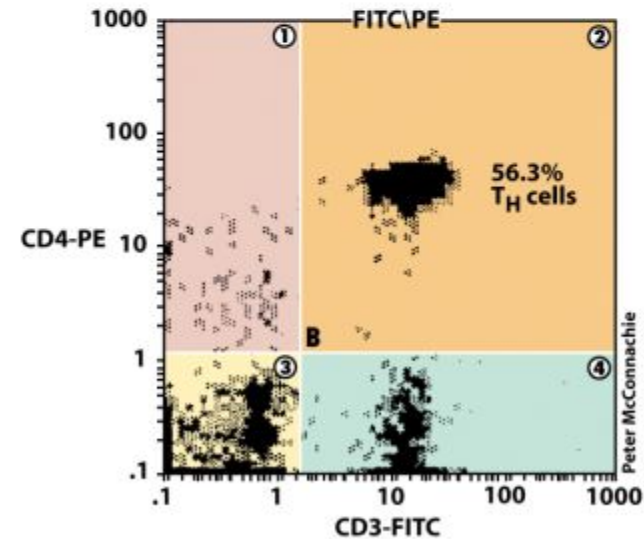


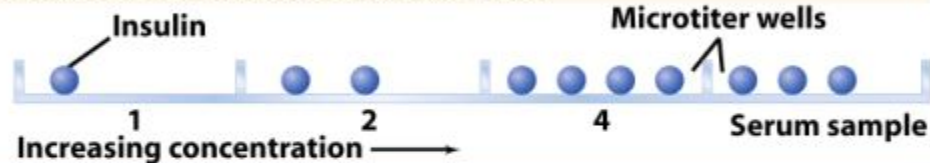
Figure 24-22a Brock Biology of Microorganisms 11/e  
© 2004 Pearson Prentice Hall, Inc.

Peter McConnachie

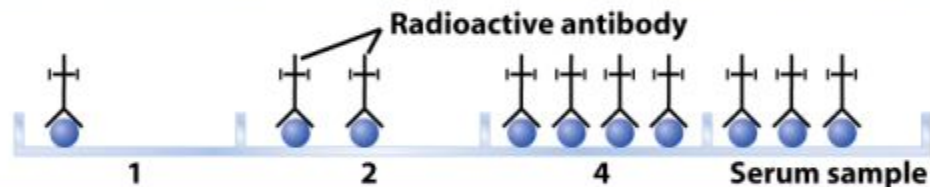
Courtesy Vanderbilt Medical Center  
[www.mae.wmich.edu/faculty/liou/wp\\_MEmicro04p2\\_ManikK.ppt](http://www.mae.wmich.edu/faculty/liou/wp_MEmicro04p2_ManikK.ppt)

# Immunological Methods: Radioimmunoassay - RIA

1. Bind insulin to wells of microtiter plate



2. Add excess anti-insulin antibodies that are labeled with  $^{125}\text{I}$ ; wash to remove unbound antibody



3. Count radioactivity in gamma radiation counter. Wells labeled 1, 2, and 4 establish a standard curve with known amounts of antigen (insulin). The radioactivity in the last well indicates, by comparison to the standard curve, how much insulin is present in a known amount of serum.

Figure 24-26 part 1 Brock Biology of Microorganisms 11/e  
© 2006 Pearson Prentice Hall, Inc.

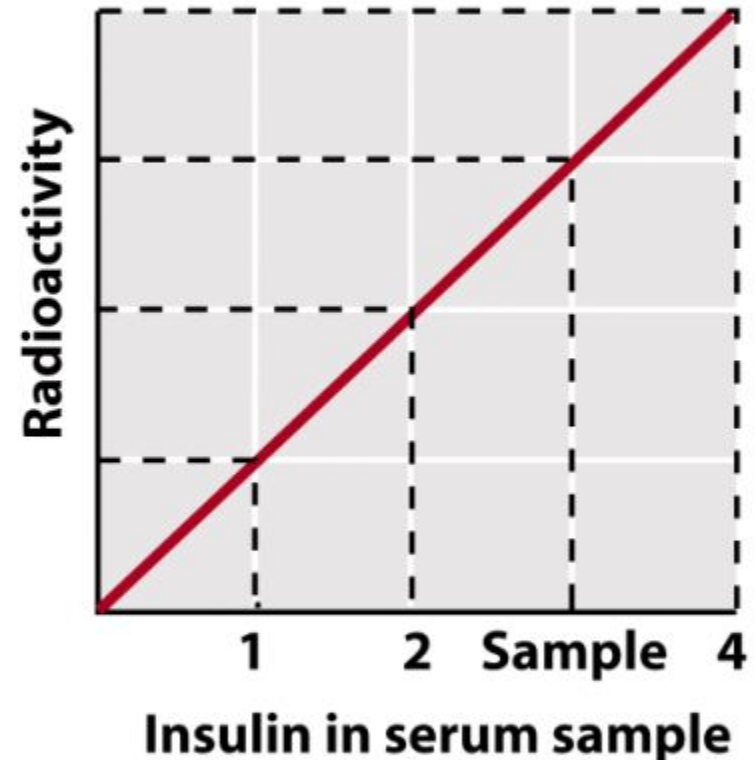


Figure 24-26 part 2 Brock Biology of Microorganisms 11/e  
© 2006 Pearson Prentice Hall, Inc.

# Immunological Methods: Enzyme-Linked Immunosorbant Assay ELISA

## Procedure

1. Antibodies (Y) to virus (★) bound to wells of microtiter plate

2. Add patient sample (secretions, serum, and so on) suspected of containing virus particles or virus antigens and wash wells with buffer

3. Add antivirus antibody containing conjugated enzyme (E-Y-E)

4. Wash with buffer

5. Add substrate for enzyme and measure amount of colored product (•).

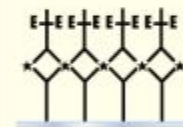
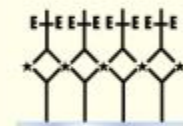
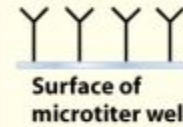
## Results

Colored product

## Quantitation

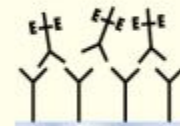
Colored product produced is proportional to amount of antigen.

## Positive test



++

## Negative test



-

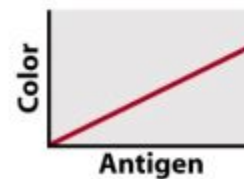
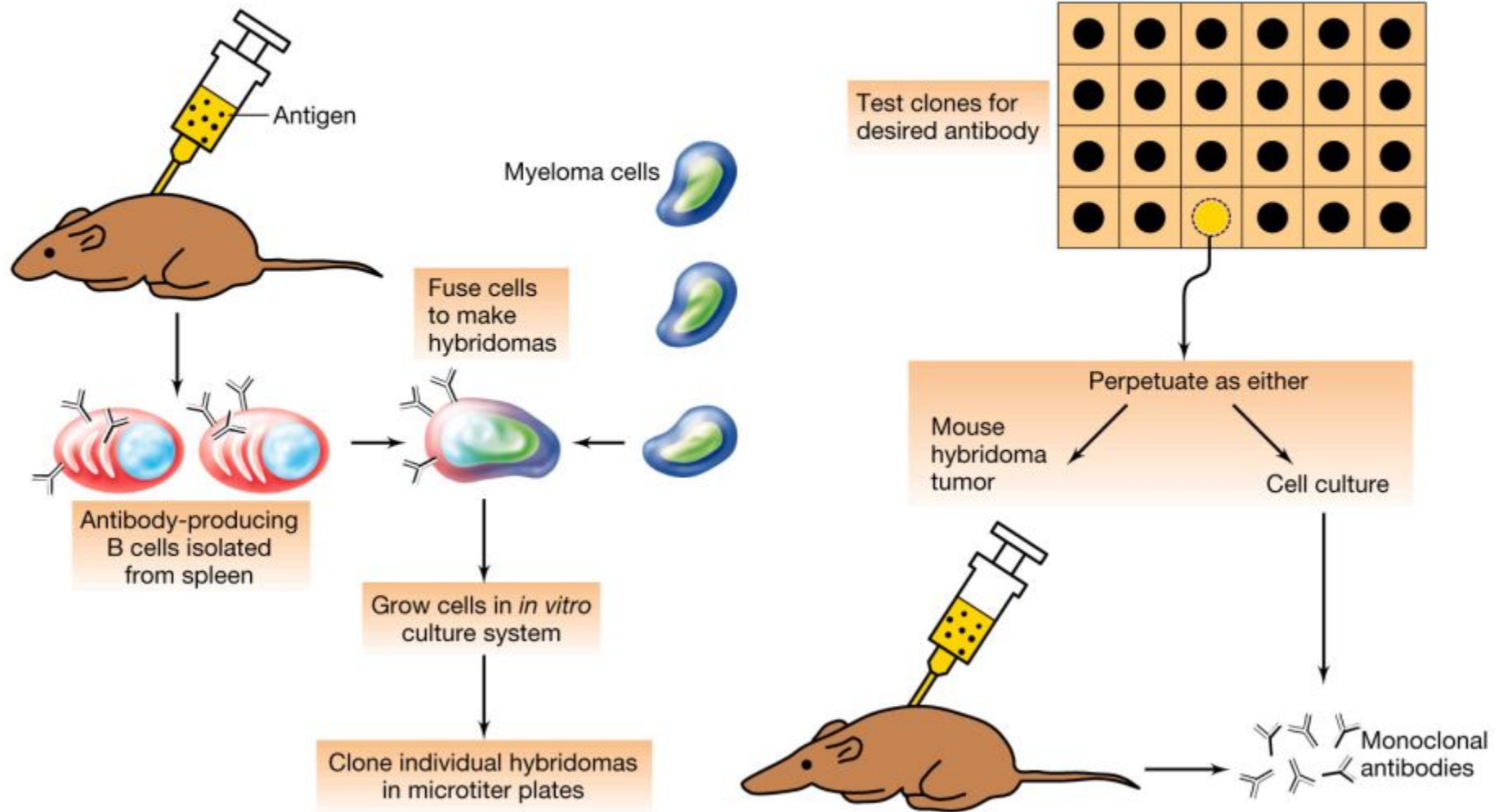


Figure 24-23 Brock Biology of Microorganisms 11/e  
© 2006 Pearson Prentice Hall, Inc.

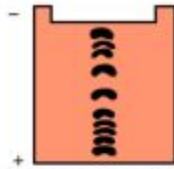
# Immunological Methods: Generation of Monoclonal Antibodies



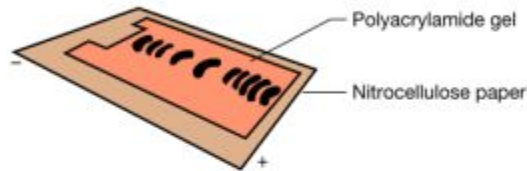


# Immunological Methods: Western Blot as Diagnostic Tool

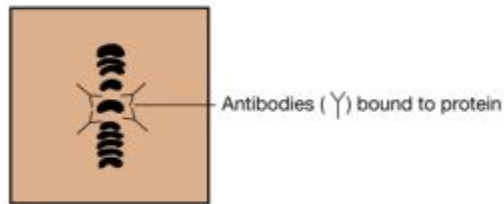
1. Denature proteins by boiling in detergent



2. Subject to electrophoresis; proteins separate by molecular weight

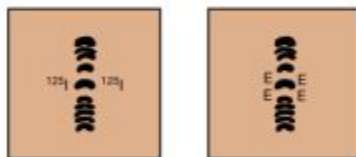


3. Blot the separated proteins from the gel to nitrocellulose paper

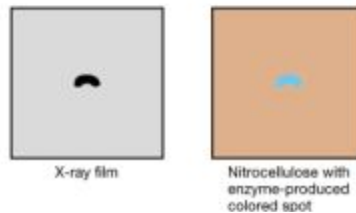


(a)

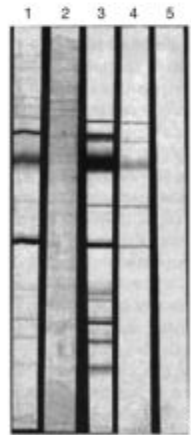
4. Treat nitrocellulose paper containing blotted proteins with antibodies; each antibody recognizes and binds to a specific protein, labeling the protein for detection



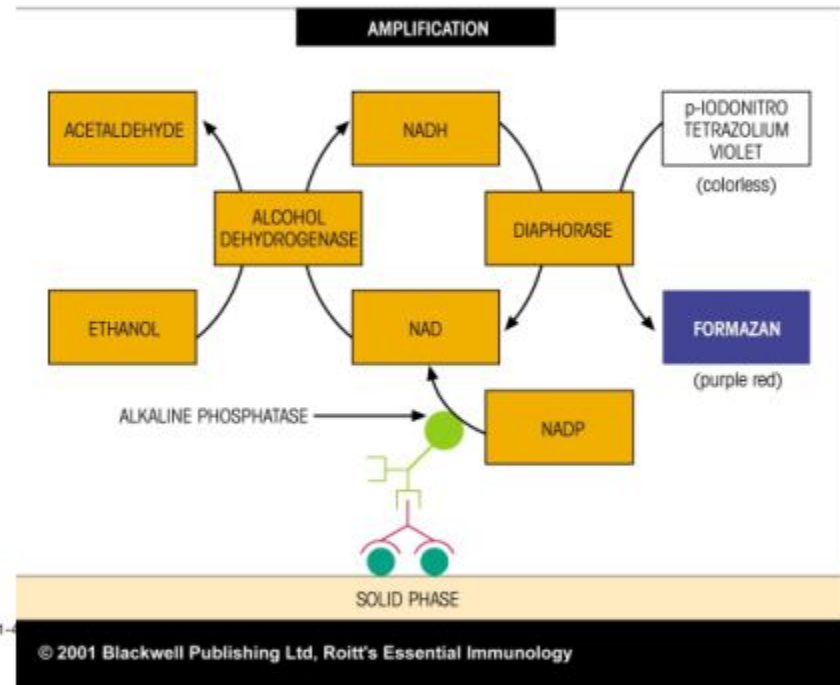
5. Add marker to bind to antigen-antibody complexes, either (left) radioactive *Staphylococcus* protein A-<sup>125</sup>I, or (right) antibody containing conjugated enzyme



(a)



(b)



**Alternative used in the course:  
Chemoluminescent reaction**