Chapter 17: Immunization & Immune Testing

1. Immunization
2. Diagnostic Immunology
1. Immunization

Chapter Reading – pp. 505-511
What is Immunization?

A method of inducing artificial immunity by exposing the individual to some portion or form of the pathogen (aka “vaccination”):

• triggers an adaptive immune response resulting in the production of memory T and B cells specific for antigens from the pathogen

• a secondary exposure will result in a potent and immediate immune response to the specific pathogen due to the memory cells

**the vaccination itself should NOT cause an infection**
Different Types of Vaccines

1) **Attenuated** whole agent vaccines:
   - live but “weakened” pathogen
     - genetically modified
     - mutant or less virulent strains of the pathogen

2) **Inactivated** whole agent vaccines:
   - pathogen that has been killed in some way
     - usually by chemical treatment, also by heat

**whole agents are generally more effective due to containing multiple antigens, but also carry more risk of infection**
3) **Toxoid vaccines:**
   - chemically inactivated protein exotoxins
   - inactivated toxins are referred to as **toxoids**

4) **Subunit vaccines:**
   - a specific protein or protein fragment from pathogen
     - purified from pathogen directly OR
     - produced as a **recombinant** vaccine in other organism

5) **Conjugated vaccines:**
   - small or non-protein antigens attached to a “carrier”
     - necessary to enhance immune response

**these “molecular vaccines” tend to be less effective however they are safer than whole agents**
Methods of Vaccine Production

1) growth & purification of pathogen itself
   • e.g., culturing bacteria, growing viruses in eggs
     • treated and packaged after purification

2) production of recombinant antigen
   • typically in yeast or bacteria
     • gene encoding protein antigen placed in plasmid
     • expressed in bacterial or yeast host cells
     • protein is purified & used in vaccine
2. **Diagnostic Immunology**

Chapter Reading – pp. 512-520
Polyclonal Antibodies

Collection of antibodies (Ab’s) produced by many B cells specific for the same antigen (i.e., from many B cell “clones”)

1) immunize animal (usu. rabbit, goat, chicken) w/desired antigen (protein or whole pathogen)

2) collect blood serum from immunized animal (full of Ab’s that bind various epitopes on antigen)

3) use for testing
Monoclonal Antibodies

A monoclonal Ab (mAb) is a collection of identical Ab’s from a single B cell clone.

Sometimes monoclonal Ab’s are preferable to polyclonal Ab’s

- much “cleaner” than polyclonal serum, can give cleaner, more precise results
  - recognize only 1 epitope on the antigen
- preferable when greater target specificity is needed
Techniques in Diagnostic Immunology

Diagnostic immunology uses antibodies to acquire clinical data via techniques that involve:

**Immunoprecipitation**
- the formation of insoluble Ab:Ag complexes

**Agglutination**
- the formation of visible Ab:Ag aggregates

**Viral Hemagglutination**
- tests involving viruses that bind to & agglutinate RBCs

**Fluorescent Antibody Staining**
- reveal the presence of specific pathogens

**ELISA**
- automated technique revealing presence of Ab or Ag
**Immunoprecipitation**

*Soluble* protein antigen (Ag) and antibody (Ab) will form insoluble complexes when mixed in the right proportions:

- Excess antibody or antigen will result in no insoluble material.
- ~Equal proportions of Ab & Ag results in an insoluble complex or precipitate.

<table>
<thead>
<tr>
<th>Amount of antibody precipitated</th>
<th>No precipitate</th>
<th>Precipitate</th>
<th>No precipitate</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Increasing amount of antigen</strong></td>
<td><img src="image" alt="Diagram" /></td>
<td></td>
<td><img src="image" alt="Diagram" /></td>
</tr>
</tbody>
</table>

(a) Antibody excess  
(b) Optimal proportions  
(c) Antigen excess

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Immunodiffusion Tests

- Sources of antigen & antibody diffuse into agar.

- Precipitation will occur where they meet provided they bind to each other.
Agglutination Tests

Large, complex antigens (e.g., bacteria) can be agglutinated by specific antibody:

- does not require precise proportions as with immuno-precipitation
- allows detection of antibodies to specific antigens as well as the determination of “antibody titer” by serial dilution
Antibody Titration

Serum added in increasing dilutions

1:1  1:10  1:100  1:1000  1:10,000

Control (no specimen added)

Antigen (identical in each well)

++++  +++  ++  +  –  Control

Very strong agglutination  No agglutination
Many viruses such as *Influenzavirus* can stick to and agglutinate red blood cells in a process called **viral hemagglutination**: 

- does not involve any antibodies yet works in the same manner
Neutralization of Viral Hemagglutination

This type of diagnostic test reveals the presence of specific viral antibodies in serum (i.e., due to exposure to the virus) due to the prevention of viral hemagglutination:

- Antibodies to the virus in serum (if present) will inhibit hemagglutination by binding to virus
**Fluorescent Antibody Labeling**

It is generally more practical to label a secondary antibody (2° Ab) to reveal the binding of unlabeled primary antibody (1° Ab)

- 2° Ab is specific for constant region (F_C) of 1° Ab

1. Antigen is attached to slide and flooded with patient’s serum.

2. Fluorescent-labeled anti-Ig antiglobulin is added.

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ELISA

ELISA stands for Enzyme-Linked Immunosorbent Assay and has the following features:

- it involves the use of multi-well plates and automated plate readers
  - allows the rapid analysis of large numbers of samples
- uses antibodies labeled with a specific enzyme
  - addition of enzyme substrate results in colored product that is visible and measurable by plate reader
- can be used to detect the presence of Ab or Ag
  - antigen is fixed to a surface to detect antibody
  - antibody is fixed to a surface to detect antigen
Antigen attached to well in plate.

A protein such as gelatin is added to block the uncoated surface.

Patient serum is added; complementary antibody binds to antigen.

• with indirect ELISA a labeled 2° Ab is used to detect Ab in a patient’s serum
Enzyme-linked anti-antibody is added and binds to bound antibody.

Enzyme’s substrate is added, and reaction produces a visible color change.
Direct ELISA

- with direct ELISA a labeled 1° Ab is used to detect antigen in a sample
- also referred to as an “antibody sandwich ELISA”
Key Terms for Chapter 17

• attenuated vs inactivated whole agent vaccines
• toxoid, subunit & conjugated vaccines
• monoclonal vs polyclonal antibody
• immunoprecipitation, immunodiffusion test
• agglutination, antibody titration
• viral hemagglutination
• direct vs indirect ELISA

Relevant Chapter Questions
MC: 1, 4, 5, 8, 9, 11-14