Applications of Immunology
1. Vaccines
2. Diagnostic tests

Vaccines
Chinese were first to “vaccinate”: variolation
- ground up small pox scabs, rub into wound or inhale
- some people got mildly ill and then were immune to small pox
- some died
Jenner developed first Western vaccine:
- observed that milk maids that got cow pox never got small pox
- he inoculated people with cow pox to prevent small pox infection
- set stage for vaccine development
(vacca = cow: name given by Pasteur later)
vaccine = suspension of organisms or fraction of organisms used to induce immunity

- only way to deal with deadly viral diseases
(bacterial diseases can be treated after with antibiotics)
Mechanism of action:
- exposure (injection) induces primary immune response:
  - antibodies and long term memory cells are formed
  - slow, takes 1-2 weeks
- natural exposure later induces secondary immune response:
  - memory cells stimulated to act
  - rapid and intense response
  - pathogen destroyed before it causes disease
Herd immunity = having enough of the population vaccinated to prevent spread of disease
Types of vaccines: on handout

Diagnostic Immunology
- use purified antibody solutions (antiserum) to diagnose disease
Diagnostic antibodies can be produced to detect particular microbes:
A. In animals (mixed antiserum)
1. inject animal with microbe or antigenic fragments
2. allow immune response (1-2 weeks)
3. harvest blood
4. purify antibodies from serum to make antiserum = purified antibody solution to one particular antigen
- these preparations will produce multiple antibody types that recognize different epitopes on the antigen
B. Monoclonal antibodies
- Isolate one immortalized B cell
- Clone in culture
- Produce cheap, pure, antiserum with one type of antibody that recognizes only one epitope on the antigen
- Requires cell culture, but no need for animal husbandry and blood purification

-Diagnostic immunology is the future of medical diagnosis for infectious disease: no more biochemical tests

---

A. Direct agglutination tests
-To detect if patient has antibodies to particular antigen
  (Antibodies present = exposure or infection by the agent)
-Known infectious agents are bound to a microtiter dish
-Patient serum is added
-If patient has antibodies to agent agglutination will occur

---

B. Indirect agglutination tests
-Indirect = uses latex particles
-Two ways:
  1. Known antigen is bound to latex particles
     - Assay for patient sample for antibody
  2. Known antibodies are bound to latex particles
     - Assay for antigenic agent

---

Types of Diagnostic Immunology Assays

1. Agglutination Reactions
-To detect particulate antigens in solution
-Antibodies cause clumping (agglutination) of their specific antigens
  (e.g., Hemagglutination for blood typing: detects surface antigens on RBCs)
2. Fluorescent Antibody Labeling
-use antibody chemically linked to fluorescent dye that is visible with UV light
A. Direct FA Test
-identify or visualize microbes in clinical specimens
-probe specimen with fluorescent antibodies

A. Indirect FA Test
-detect the presence of antibodies in serum
-fix known antigen to slide, probe with patient serum, tag with fluorescent antihuman immune serum globin (αHISG)

-αHISG antigen binding sites are specific for any human IgG (or IgM) molecules as their epitope
-Fc region of αHISG will have fluorescent molecules or enzymes attached to allow detection/visualization of binding

3. ELISA
(Enzyme Linked ImmunoSorbant Assay)
-used to detect either antibodies or antigens in patient sample
-performed in microtiter plate
-positive reaction produces color change
-process can be automated: computer readout of many samples at once
-risk of false positives
B. Indirect ELISA
- antigen bound to dish
- assay for presence of antibody in sample

4. Western Blot / Immunoblotting
- often used to confirm ELISA positive result
- blot assays both size of protein antigen and specific reaction with antibody
- size confirmation proves/disproves possible cross reaction (false positive)
method:
1) proteins collected from patient sample and separated by size on gel electrophoresis

- electric field drives proteins through gel: large stay near top, small move toward bottom

2) separated proteins are transferred (blotted) to a nylon membrane
3) membrane exposed to antiserum for suspect pathogen
4) antibodies in antiserum specifically bind to their epitope
5) a colored substrate is added: reacts with the Fc region of bound antibodies thus coloring location of antigen with bound antibody
6) pathogen is confirmed by:
   - binding of specific antiserum
   - size of the epitope