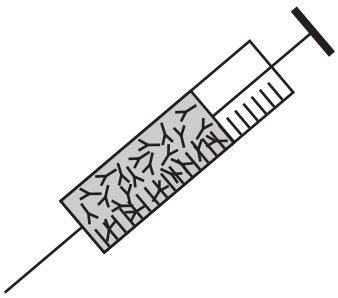
Immunological Applications

A pictorial guide to

Vaccine and Antisera Production

and

Enzyme-Linked Immunosorbent Assay (ELISA)

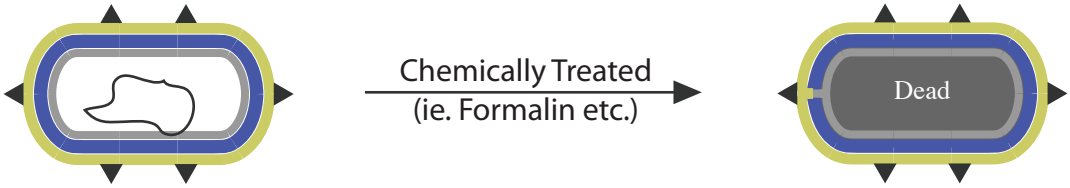


By Noel Ways

Inactivated Vaccine Classifications

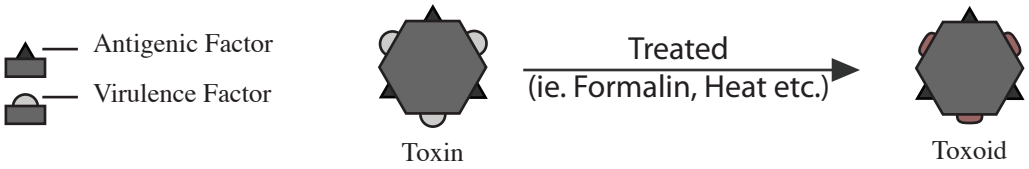
Inactivated Whole Agent Vaccines

Example: Rabies



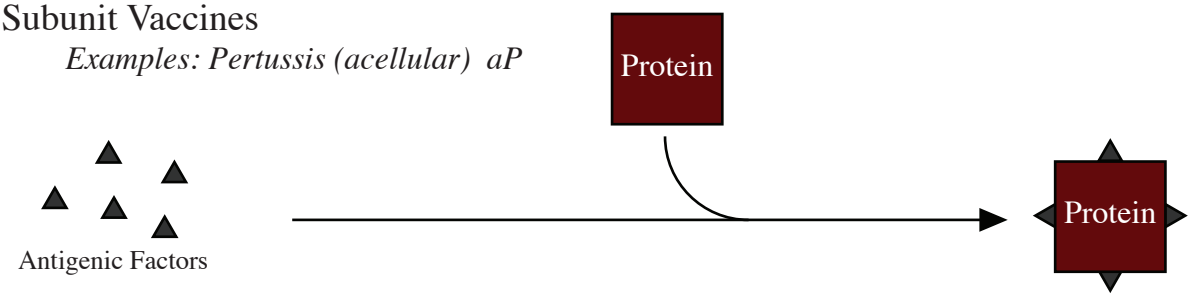
Toxoid Vaccines

Eamples: Tetanus, Diphtheria



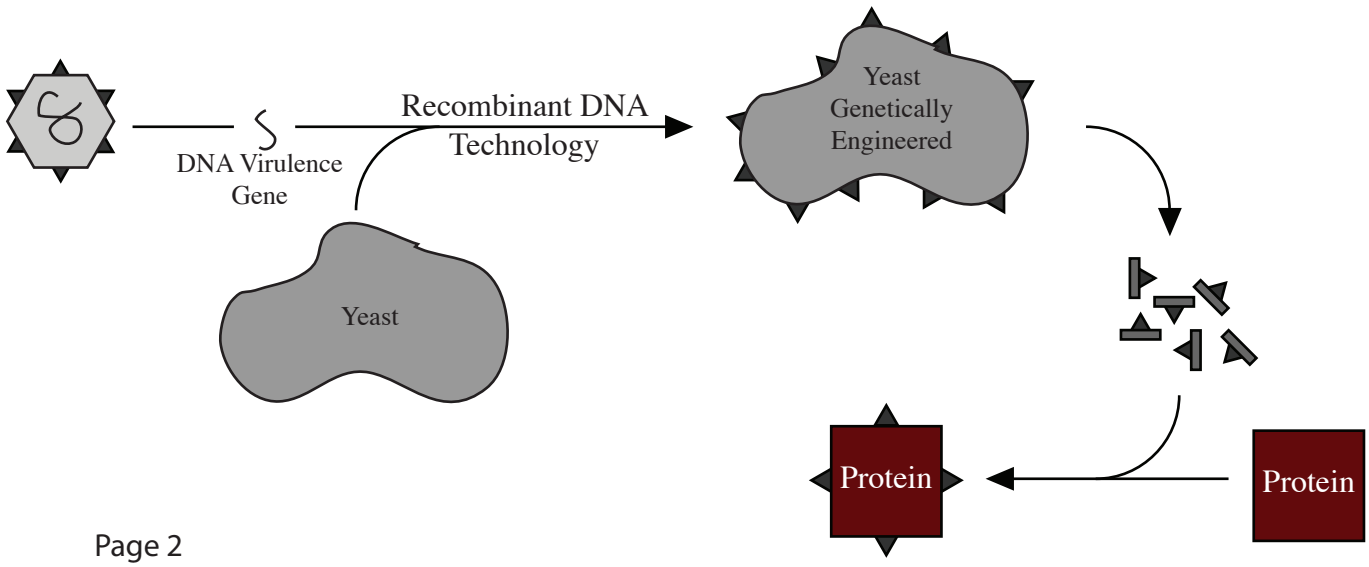
Subunit Vaccines

Examples: Pertussis (acellular) aP



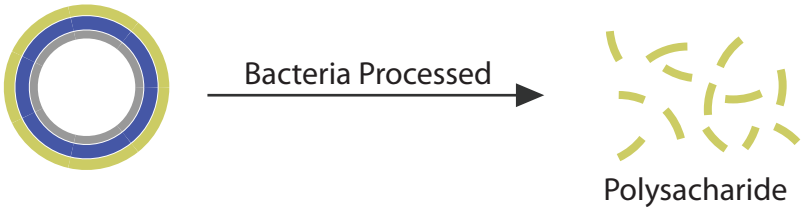
Subunit Vaccines Using Recombinant DNA Technology

Examples: Hepatitis B



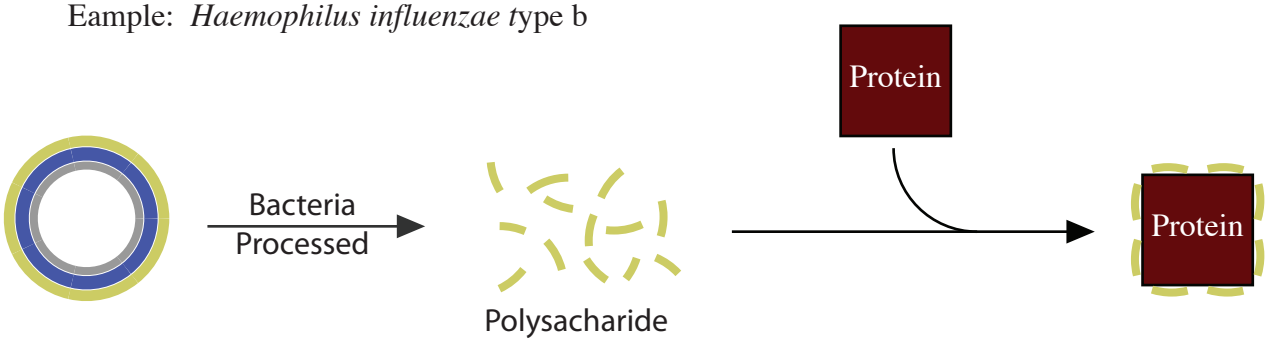
Polysaccharide Vaccines

Example: *Streptococcus pneumoniae*

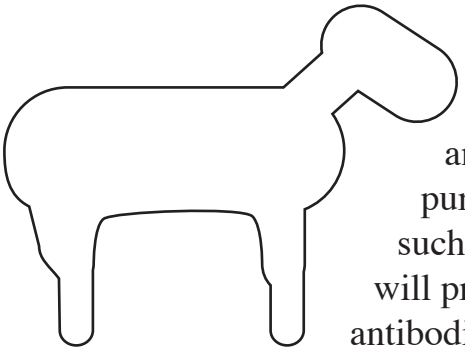


Conjugate Vaccines

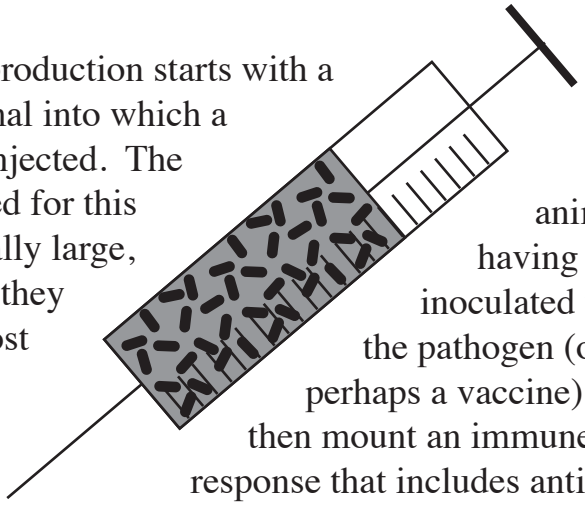
Eample: *Haemophilus influenzae* type b



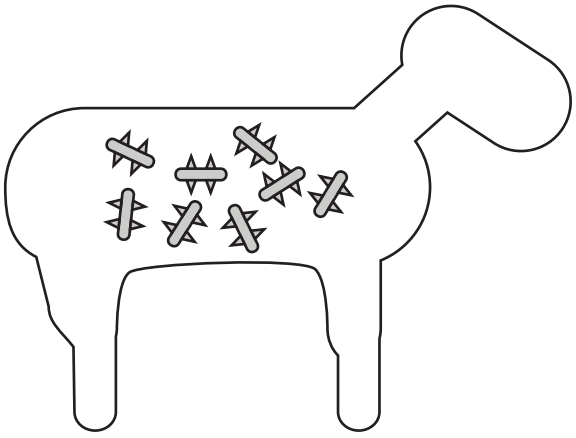
Antisera Production

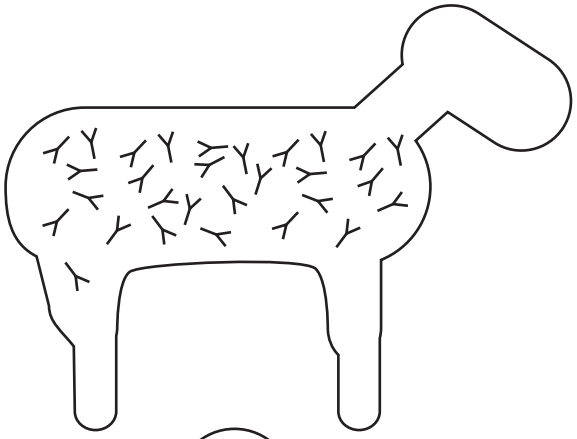


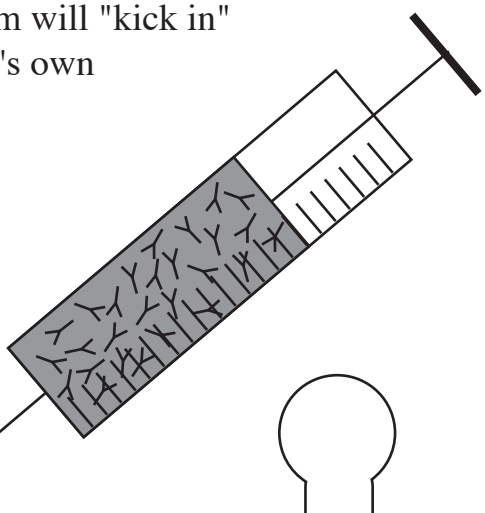
Antisera production starts with a heathy animal into which a pathogen is injected. The animals selected for this purpose are usually large, such as horses, as they will produce the most antibodies.

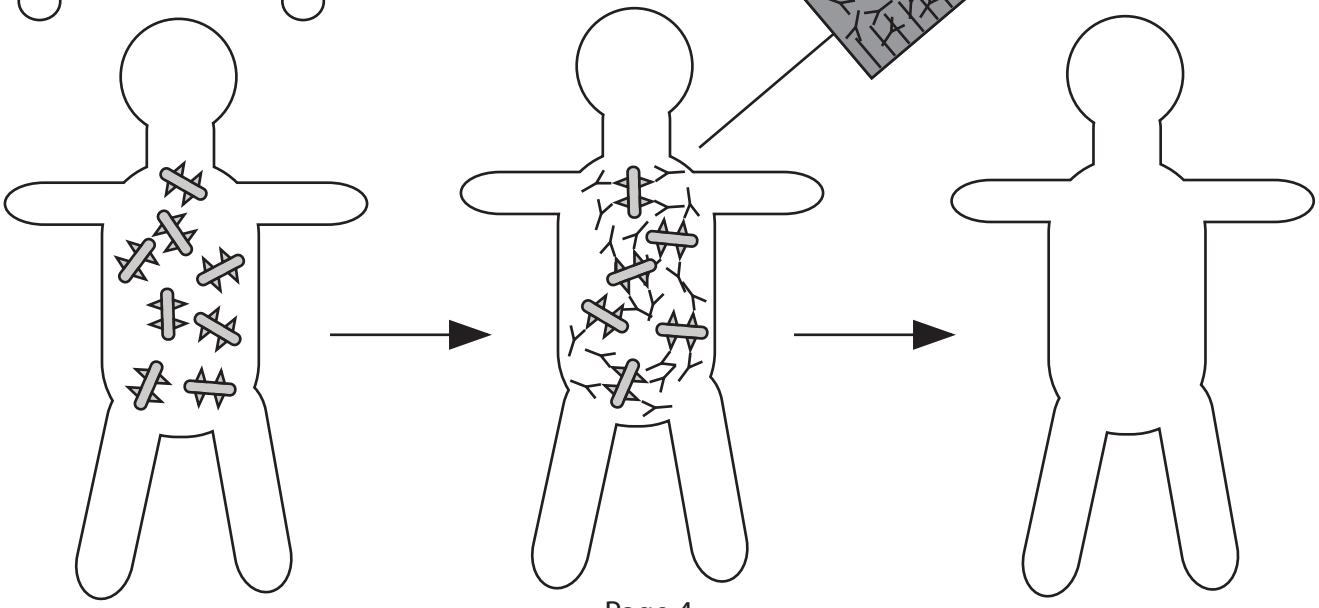


The animals having been inoculated with the pathogen (or perhaps a vaccine), will then mount an immune response that includes antibody production. Afterwards, the antibodies will then be harvested and purified in such a way as to minimize potential adverse reactions to the recipient. Once the antiserum is readied, it can then be injected into a person to provide brief but quick and effective protection. Often it is this brief window of time that health professionals are providing so that the patients own immune system will "kick in" and produce it's own antibodies.

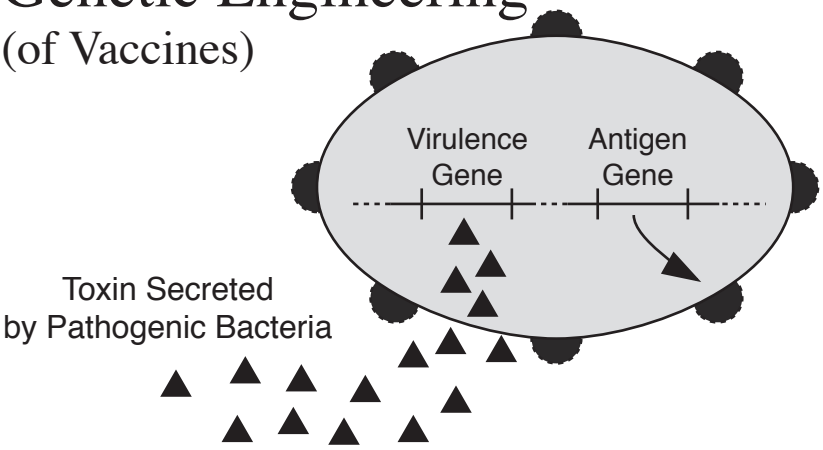






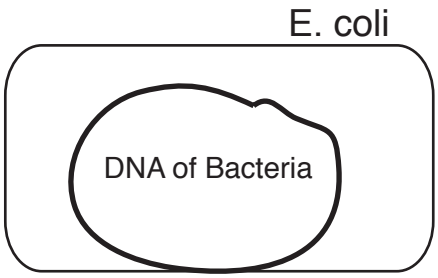


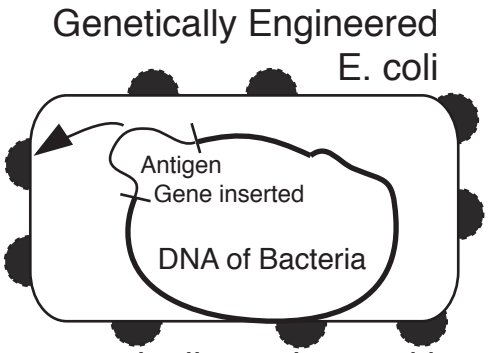
Genetic Engineering
(of Vaccines)



The bacterium at left is both pathogenic and antigenic. It is pathogenic because it produces a toxin. It is also antigenic because it produces molecules our immune system recognizes as foreign. Both toxin and antigen production are coded by two different genes

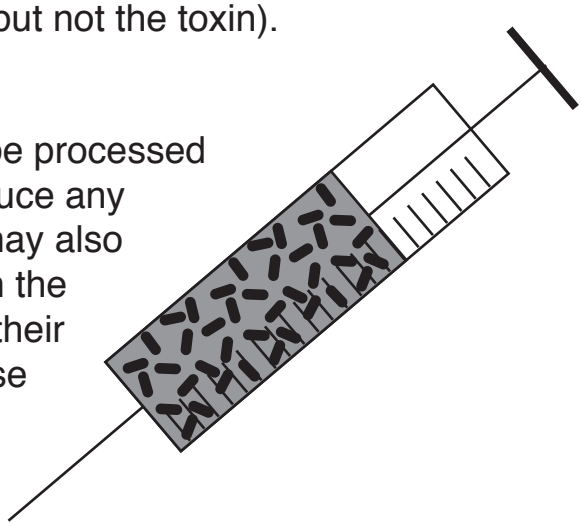
The bacterium, E. coli, is a member of out normal flora. As such, it does not harm us, but provides benefits in several ways.

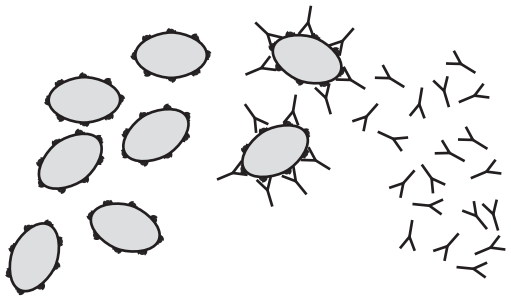




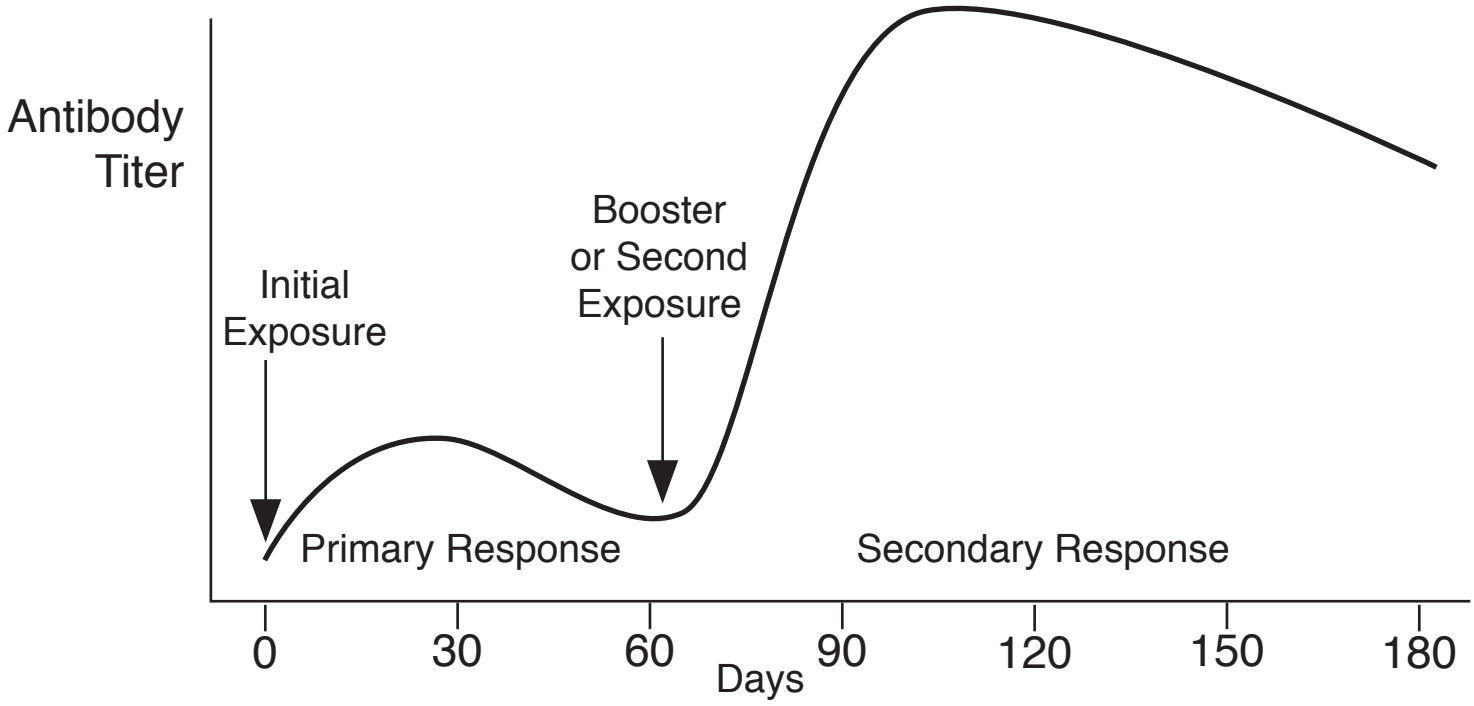
To produce a genetically engineered vaccine, the gene that codes for the antigen in the pathogenic bacterium is removed and inserted into E. coli. The bacterium will now express the antigen (but not the toxin).

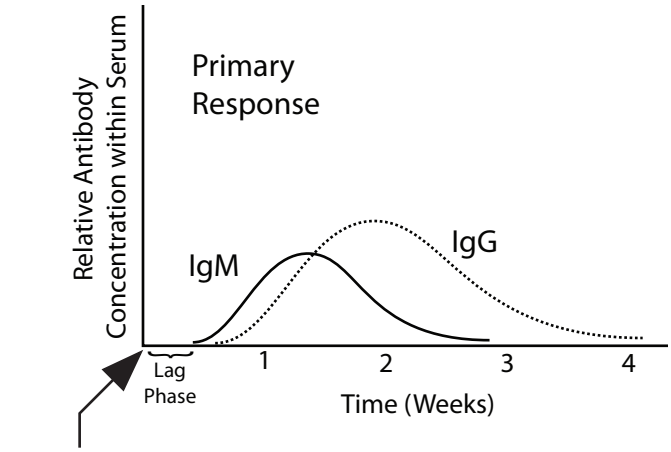
The genetically engineered bacteria can now be processed and used as a vaccine. Since it does not produce any toxins, it may be weakened, but not killed. It may also be refined so that only the antigen is present in the vaccine preparation. Once inside the person, their immune system will mount an immune response against the antigen and neutralize the vaccine, but leaving antibodies and memory cells behind in case the perceived threat should return.



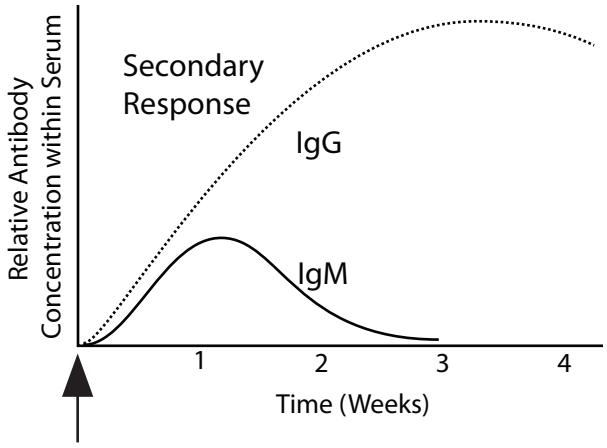


Having been vaccinated, should the original pathogenic bacterium under consideration enter the body, antibodies will bind to it and initiate it's destruction. The person is immune to the disease-causing agent. Furthermore, memory cells may also be activated, which will further boost the body's own defenses.





Initial Exposure



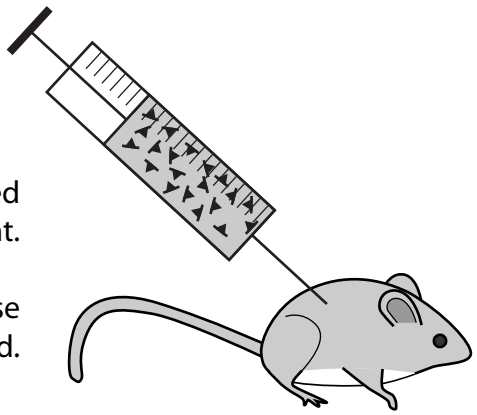
Booster or Second Exposure

(Graphs adapted from Anatomy and Physiology by Frederic H. Martini, 2004)

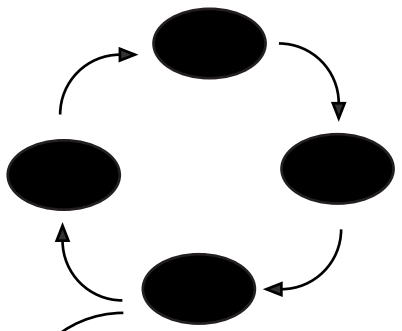
Monoclonal Antibody Production

Antigen injected into recipient.

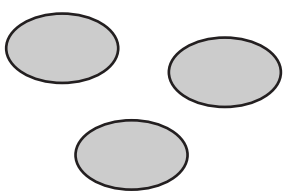
Immune response induced.



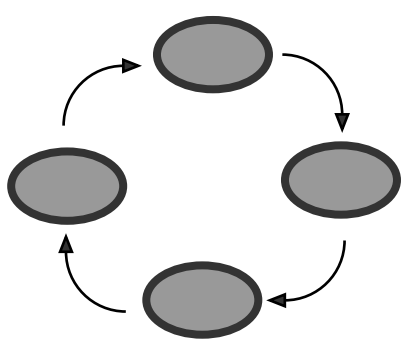
Cancerous B cell isolated from myloma. Note, uncontrolled cell division. The cell line is long lived.



Antibody producing B cells isolated from spleen. Note, normal B cells are short lived



A hybrid is formed by the fusion of a normal antibody producing B cell and a cell from a myloma. This new cell is called a hybridoma. Hybridomas will continually divide and make antibodies associated with the original B cell.

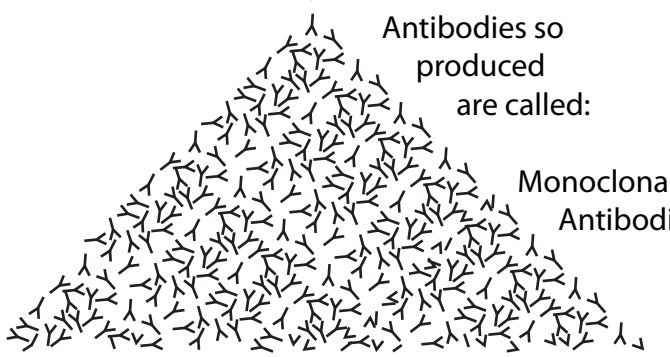


Antibodies from a hybridoma will be:

- identical
- made in large quantities
- specific

Antibodies so produced are called:

Monoclonal Antibodies

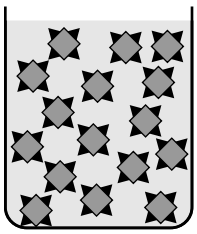


References:

Microbiology
by Tortora, Fiunke, and case

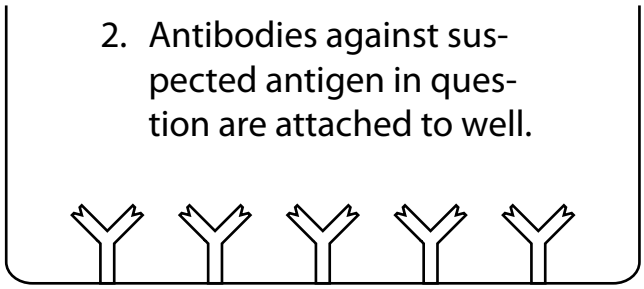
Biology of Microorganisms
by Madigan, Martinko, and Parker

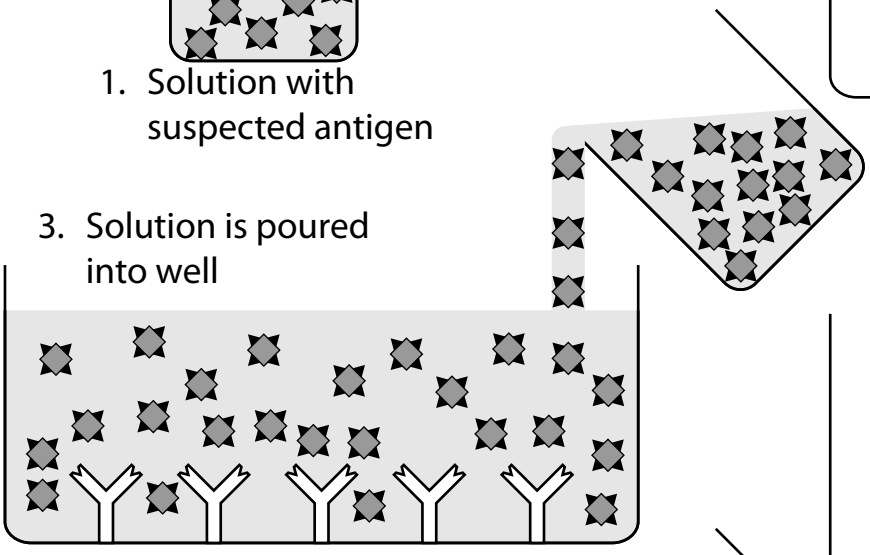
Direct ELISA



1. Solution with suspected antigen

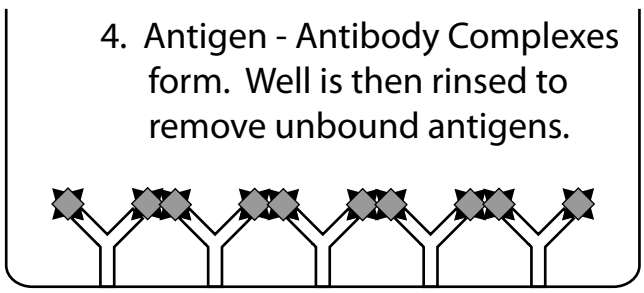
2. Antibodies against suspected antigen in question are attached to well.

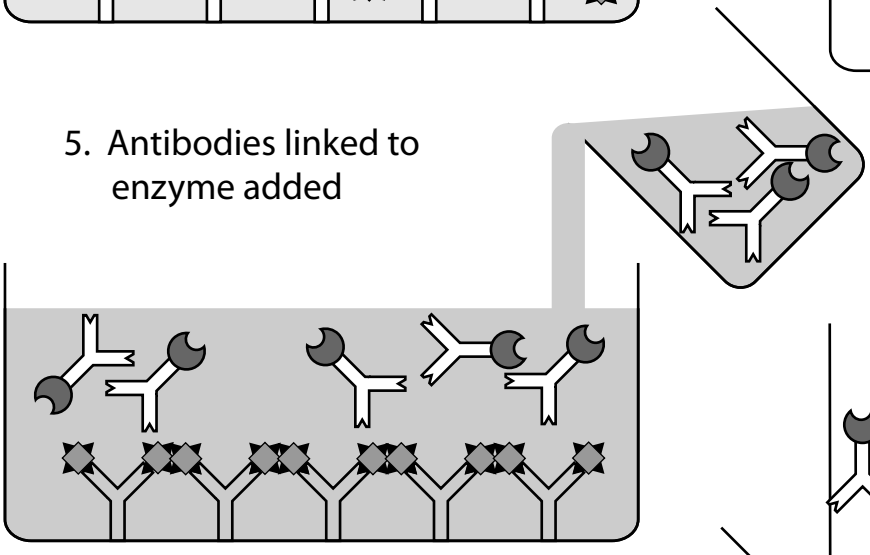




3. Solution is poured into well

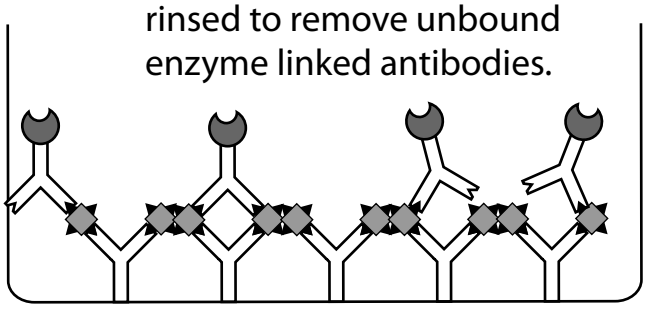
4. Antigen - Antibody Complexes form. Well is then rinsed to remove unbound antigens.

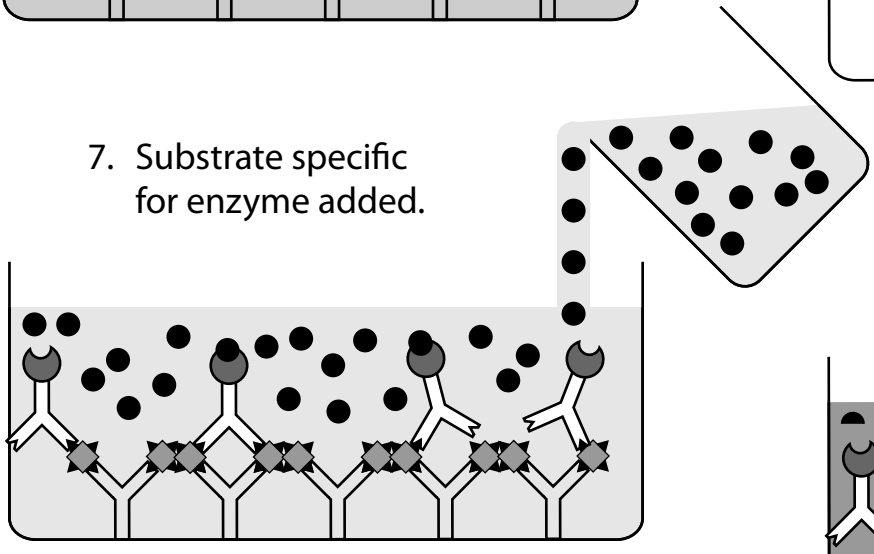




5. Antibodies linked to enzyme added

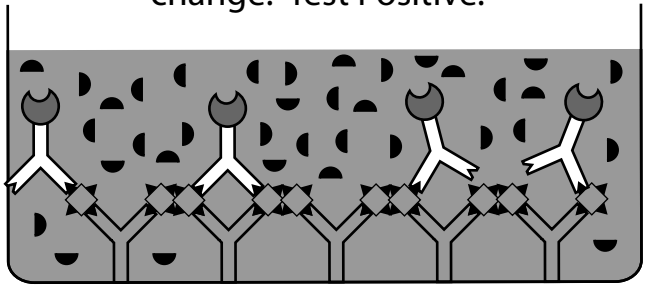
6. Antibodies linked to enzyme bind to antigen. Well is then rinsed to remove unbound enzyme linked antibodies.



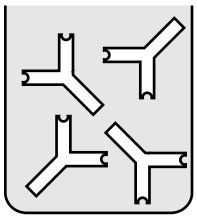


7. Substrate specific for enzyme added.

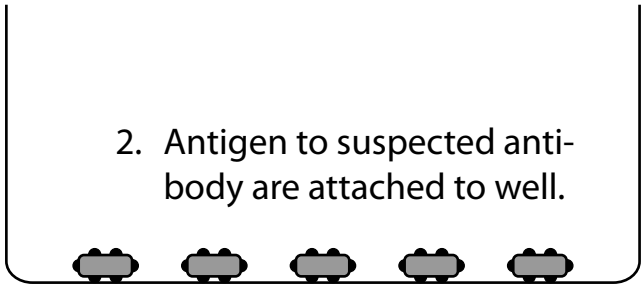
8. Enzyme hydrolizes substrate. New product results in color change. Test Positive.



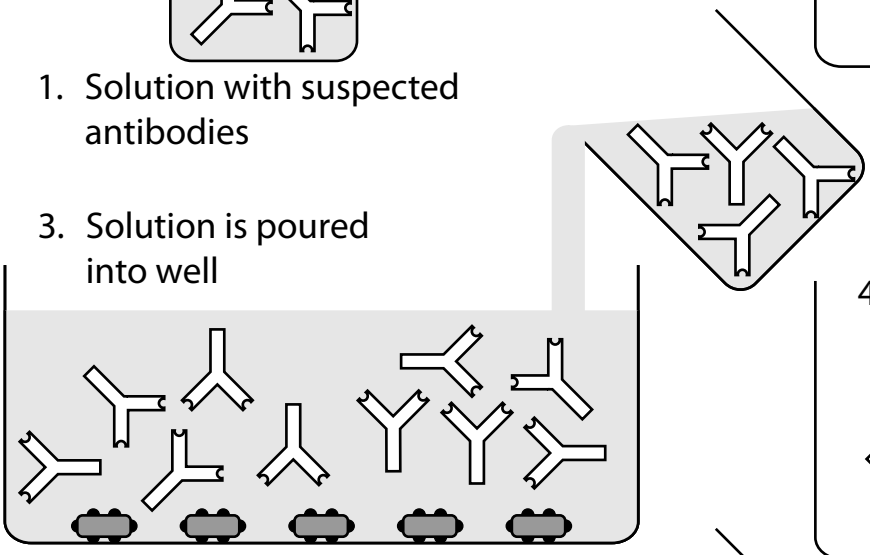
Indirect ELISA



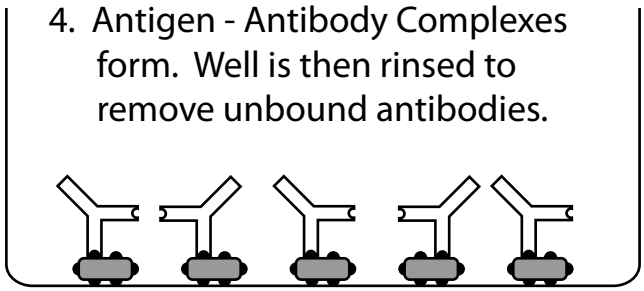
1. Solution with suspected antibodies



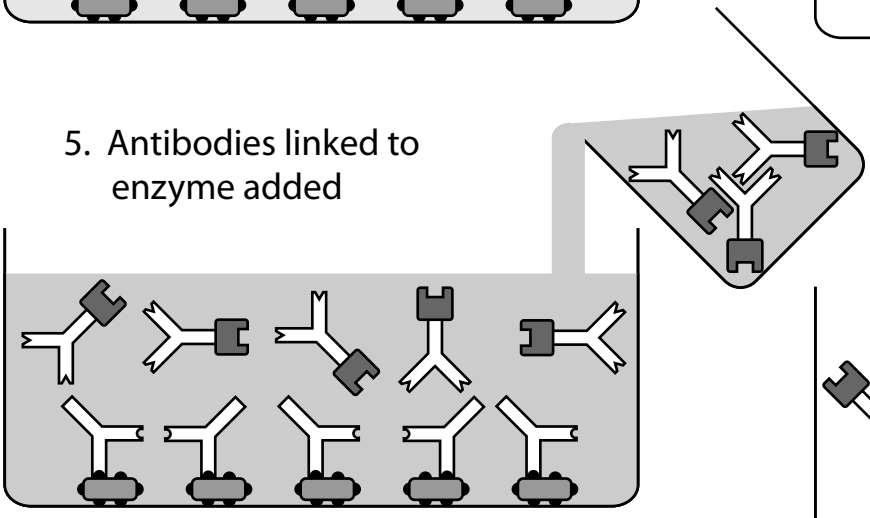
2. Antigen to suspected antibody are attached to well.



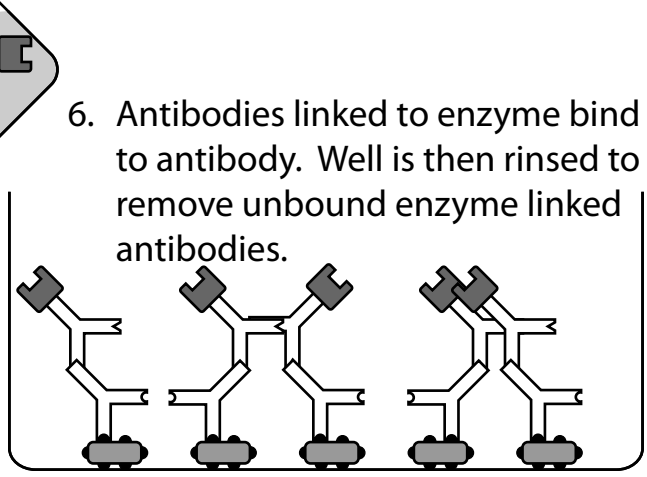
3. Solution is poured into well



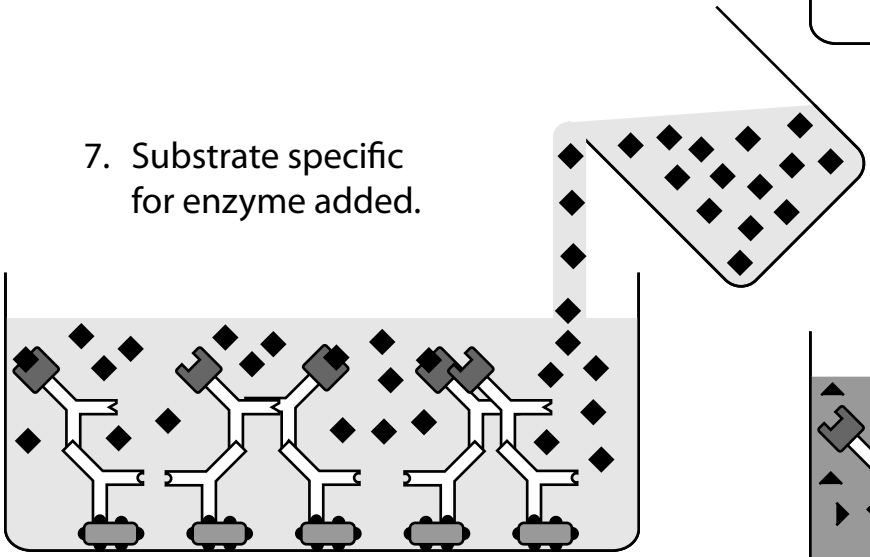
4. Antigen - Antibody Complexes form. Well is then rinsed to remove unbound antibodies.



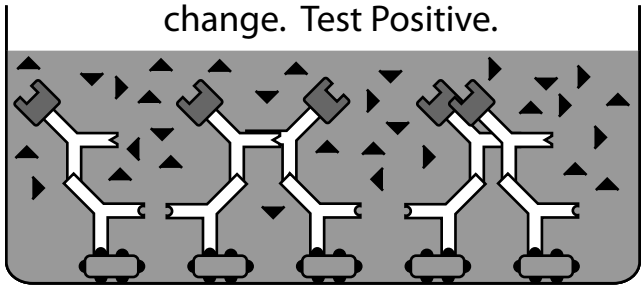
5. Antibodies linked to enzyme added



6. Antibodies linked to enzyme bind to antibody. Well is then rinsed to remove unbound enzyme linked antibodies.

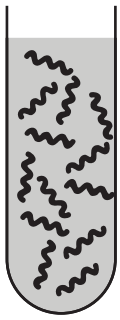


7. Substrate specific for enzyme added.

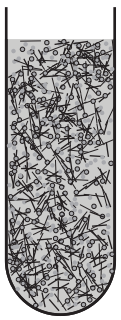


8. Enzyme hydrolizes substrate. New product results in color change. Test Positive.

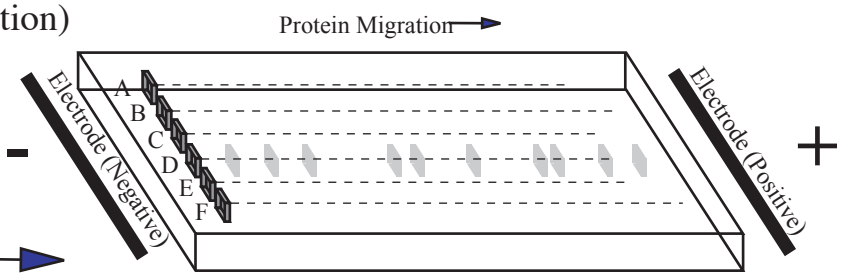
Western Blot (colorimetric detection)



1. Bacteria (such as *Borrelia burgdorferi*) grown and isolated.

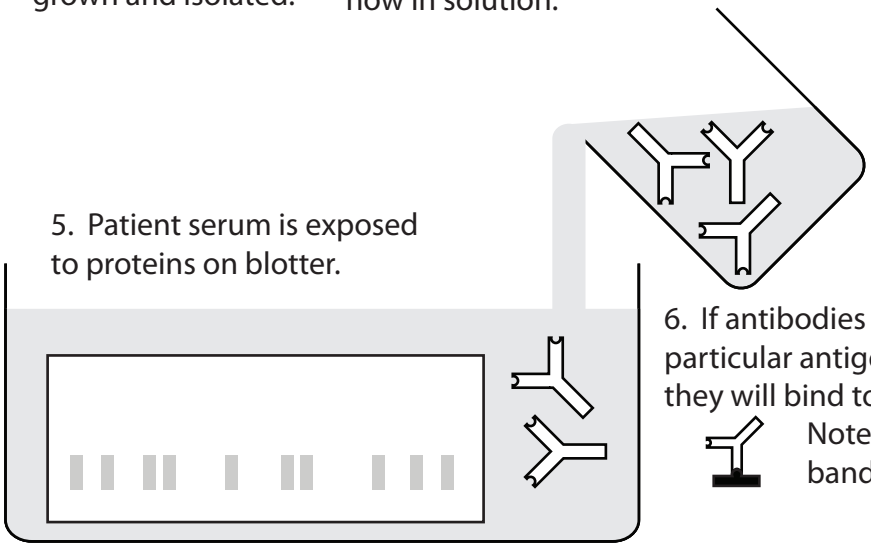


2. Bacteria processed. Proteins (▬) of bacteria now in solution.



3. Proteins separated by exposure to electric field. Small proteins will migrate farther than larger proteins.

4. Proteins of bacteria transferred to blotter

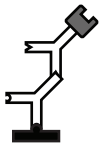


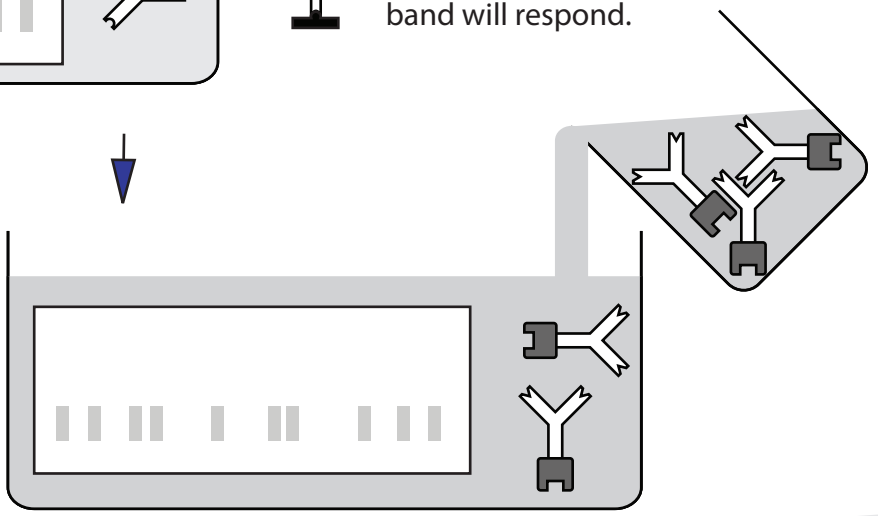
5. Patient serum is exposed to proteins on blotter.

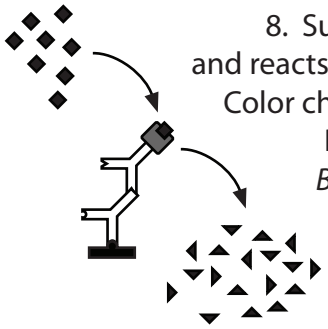
6. If antibodies specific for a particular antigen are present, they will bind to antigen.

Note, only ONE band will respond.

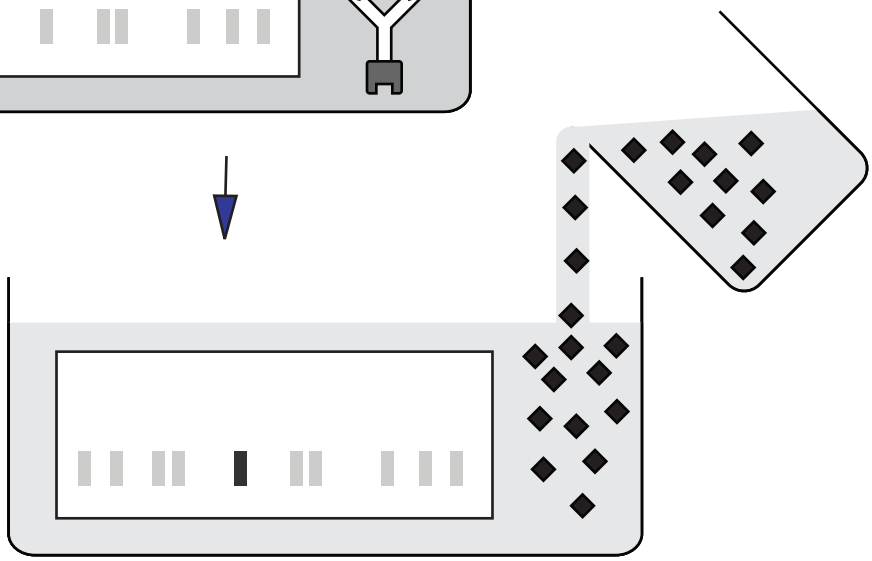
7. Antibodies against human antibodies (antihuman antibodies) linked to enzyme added. Antiantibodies react with antibodies.



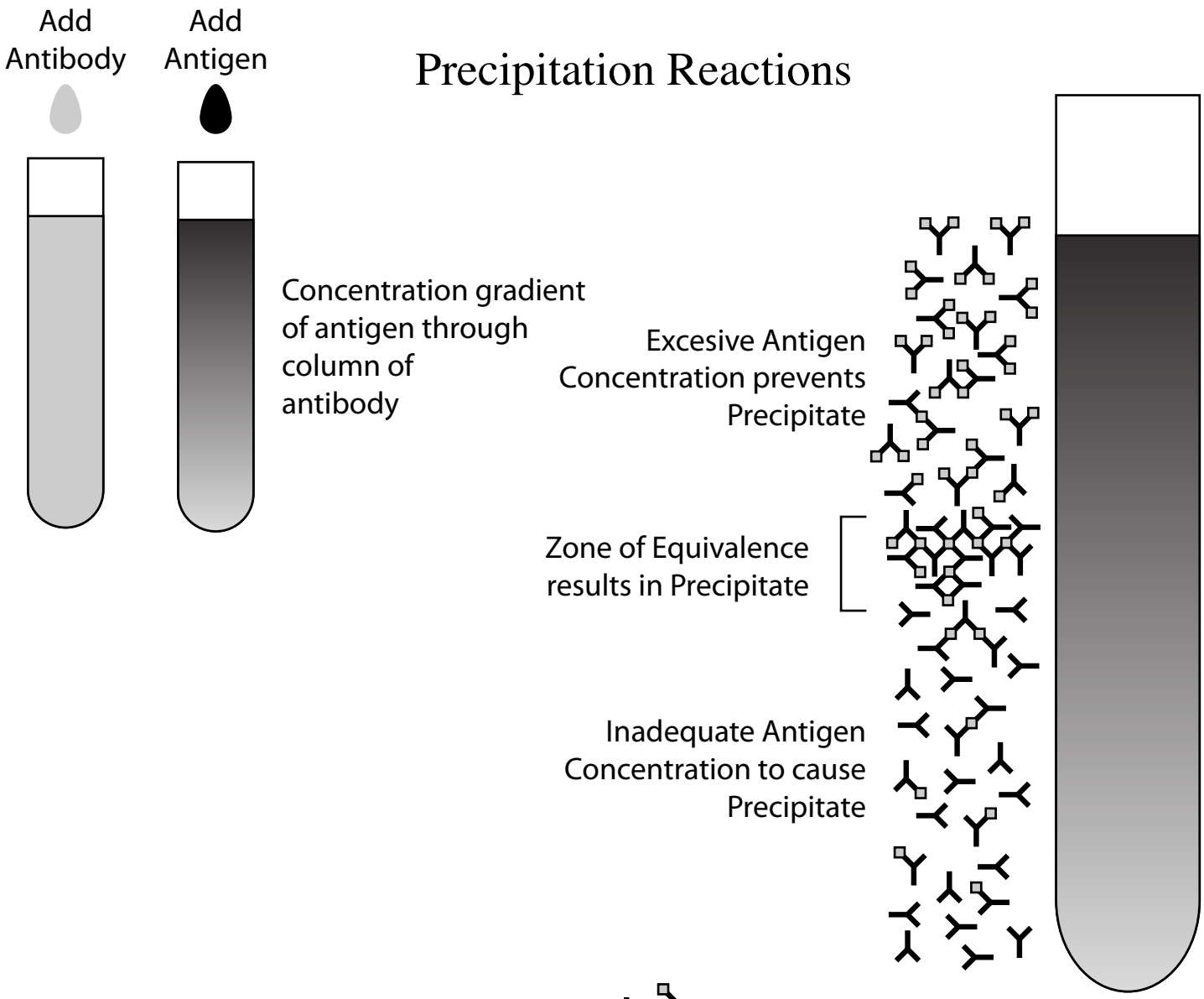


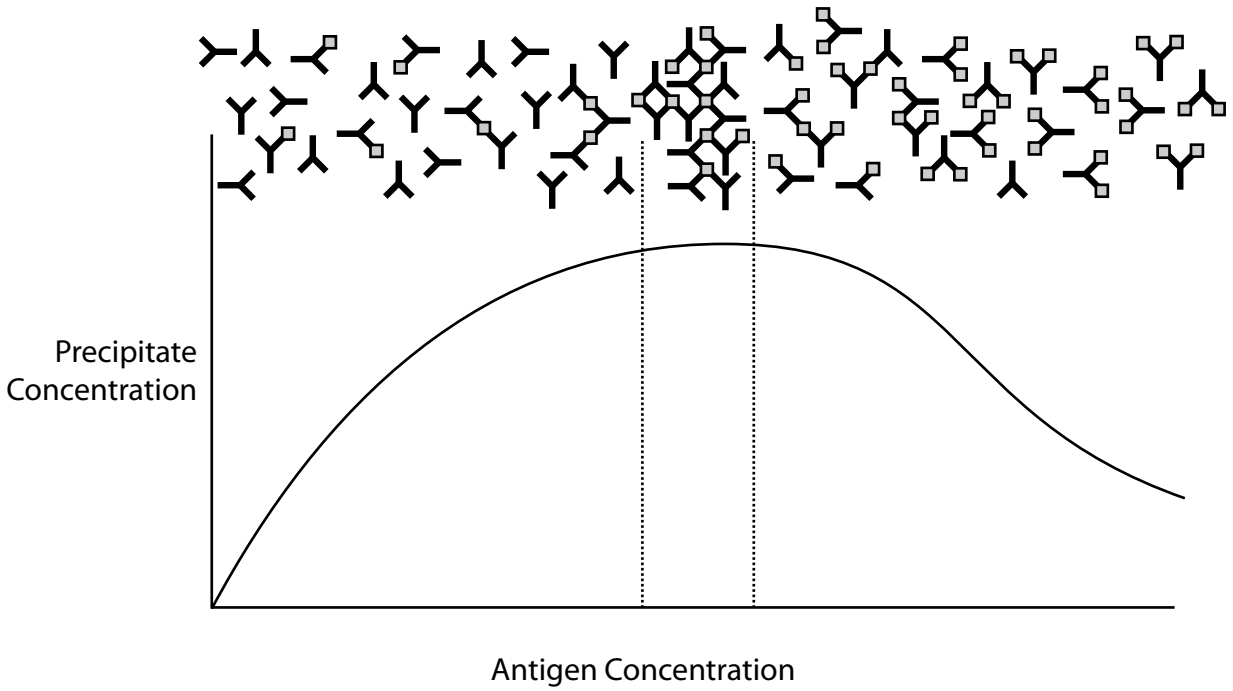


8. Substrate (◆)added and reacts with enzyme (💾). Color change occurs (◀ ▶). Patient positive for *Borrelia burgdorferi*.



Precipitation Reactions

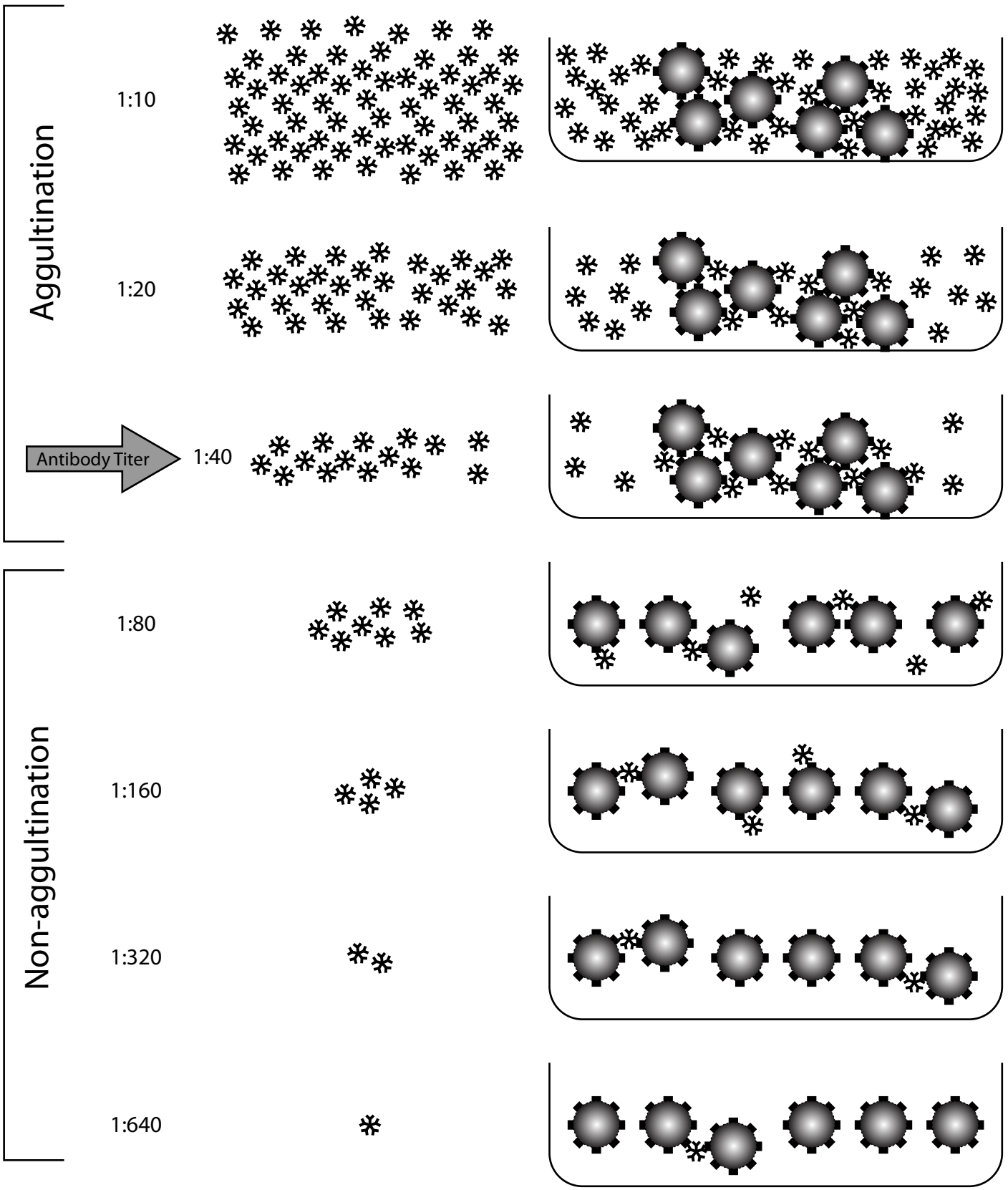




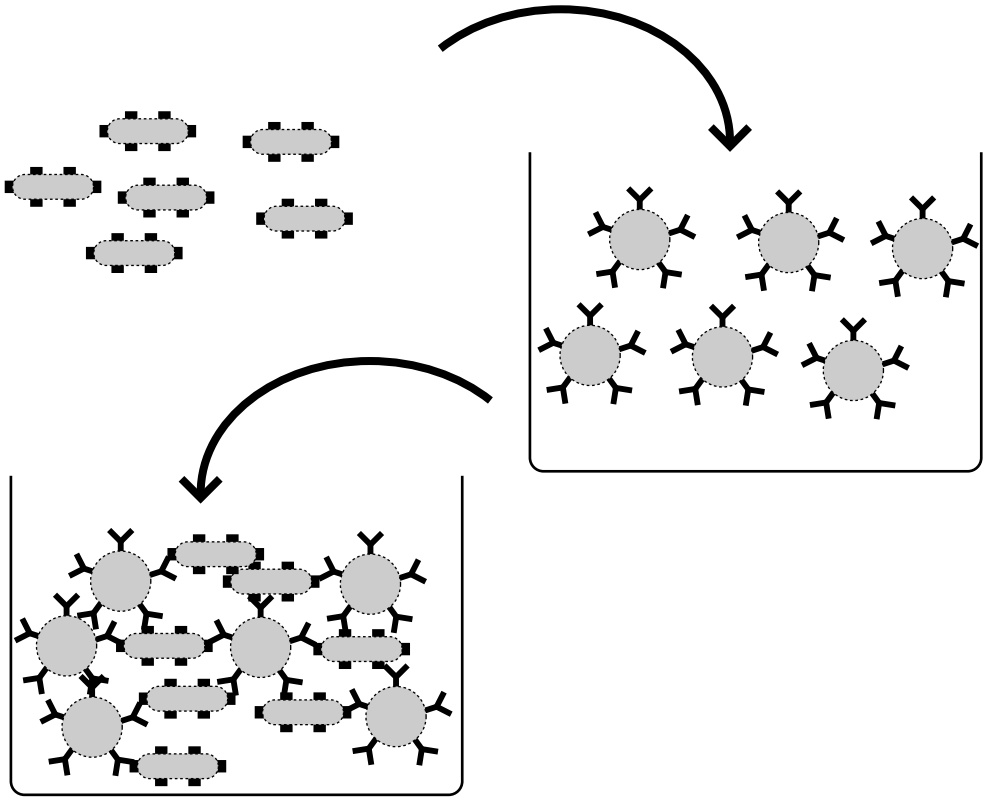
Adapted from:
http://210.36.18.48/gxujingpin/dwwswx/im/7.htm

Direct Aggutination

Antibody Serial Dilution

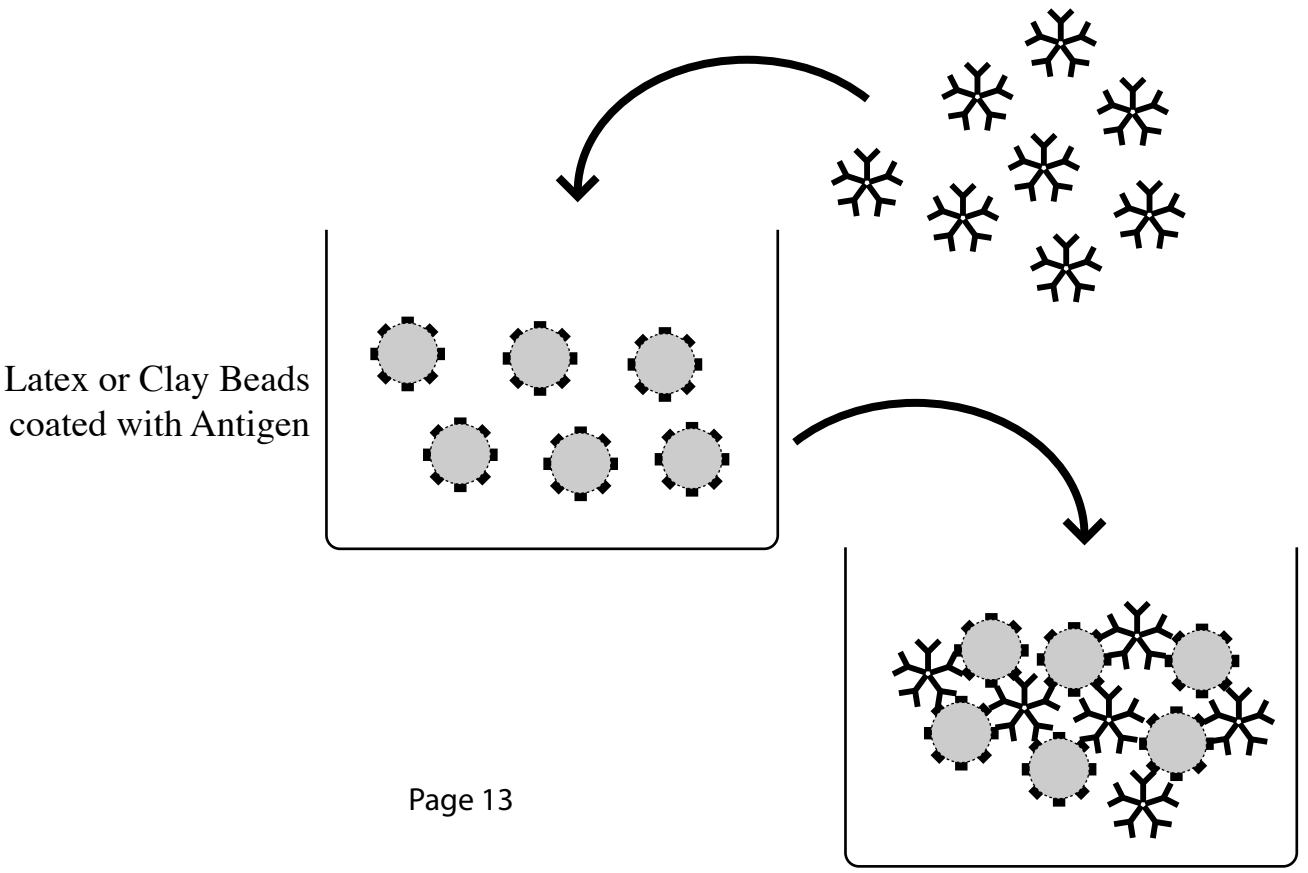


Direct Agglutination Tests



Latex or Clay Beads
coated with Antibodies

Indirect (Passive) Agglutination Tests



Latex or Clay Beads
coated with Antigen