Antibody-Antigen Recognition and Antibody Development

ENVIROLOGIX™

Antibodies - General

- Antibodies are proteins found in the blood and other bodily fluids of vertebrates.
- Used by the immune system to neutralize bacteria, viruses, and other foreign particles.
- Produced by B-Cells.
- Identify specific markers on foreign bodies (antigens) called epitopes. Large antigens (like bacteria) may have many unique epitopes that antibodies can be developed against.

Antibodies – General, Continued

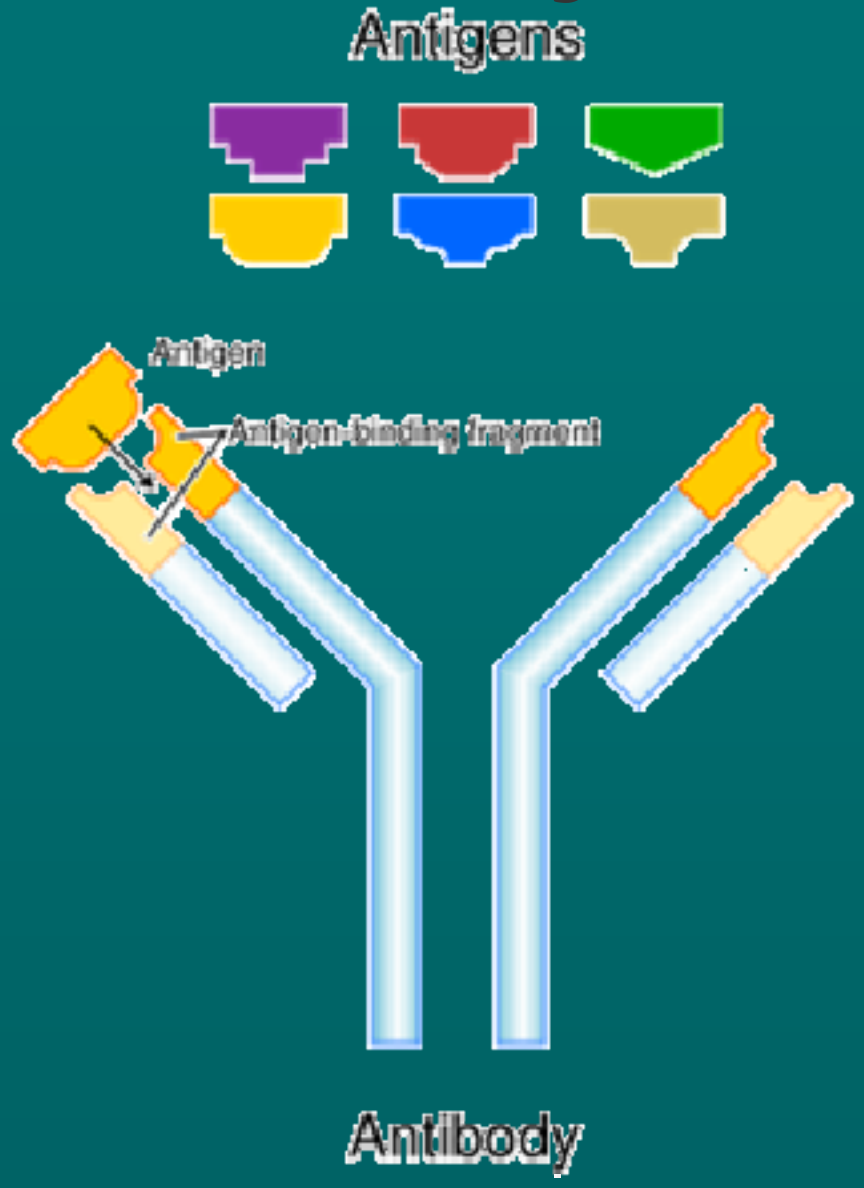
- Antibodies can be generated against proteins, nucleic acids, carbohydrates, lipids, small chemical groups, and peptides (10-15 amino acids long).
- Small molecules such as chemicals or peptides may not be immunogenic (will not usually produce antibodies if they are injected into an animal). To make antibodies against small molecules they must be coupled to a large protein to form a hapten-carrier complex.
- Antibodies are the key reagents in all immunoassays for many markets (health, environmental, veterinary, agriculture).

Antibody-Antigen Recognition

Antibodies bind to complimentary antigens by:

- Three dimensional recognition.
- Noncovalent interactions – van der Waals attraction, hydrogen bonds, salt bridges, hydrophobic interactions and electrostatic forces, and occasional ion pairs contribute to the strength of the bond between the antibody and antigen.
- Binding between antibodies and antigens is strong as is evident during an ELISA wash step.

Antigen-Antibody Recognition



ENVIROLOGIX™

Antibody Classes

- There are several types of antibodies which are grouped into isotypes.
- There are 5 different antibody isotypes or classes in mammals.
  - IgA
  - IgD
  - IgE
  - IgG
  - IgM

Ig=Immunoglobulin (another name for antibody)

Antibody Isotypes

Name	Types	Description	Antibody Complexes
IgA	2	Found in mucosal areas, such as the gut, respiratory tract and urogenital tract, and prevents colonization by pathogens.[9] Also found in saliva, tears, and breast milk.	
IgD	1	Functions mainly as an antigen receptor on B cells that have not been exposed to antigens.[10] Its function is less defined than other isotypes.	
IgE	1	Binds to allergens and triggers histamine release from mast cells and basophils, and is involved in allergy. Also protects against parasitic worms.[6]	
IgG	4	In its four forms, provides the majority of antibody-based immunity against invading pathogens.[6] The only antibody capable of crossing the placenta to give passive immunity to fetus.	
IgM	1	Expressed on the surface of B cells and in a secreted form with very high avidity. Eliminates pathogens in the early stages of B cell mediated (humoral) immunity before there is sufficient IgG.[6][10]	

ENVIROLOGIX™

Antibody Development

- Antibodies to be used in immunoassays can be developed through two different methods:
  - Polyclonal Antibodies
  - Monoclonal Antibodies
- Often immunoassays use both antibody types in the same assay.
- Immunoassays often use antibodies from different animal species in the same assay.

ENVIROLOGIX™

Polyclonal Antibodies

- Immunize a mammal (mouse, rabbit, goat, etc.) with the antigen that you would like the antibodies to be produced against (ex. Bt Cry1Ab protein).
- Large animals are preferred as amount of serum collected is greater.
- Animal elicits an immune response to the antigen (Bt Cry1Ab protein).
- Antigen induces B-cells to produce IgG antibodies specific to antigen (antibodies specific to many epitopes of the Cry1Ab protein).

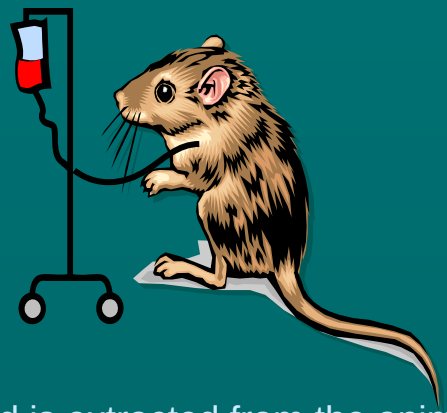
Polyclonal Antibodies, Cont.

- The primary goal is to have a high antibody titer and high antibody affinity (binds strongly to antigen).
- Many different types of antibodies will be generated with different specificities that detect different epitopes on the antigen. These antibodies have originated from different cell lines, thus the prefix “poly”.
- IgG is the most desirable antibody because of its binding properties, its high concentration in serum, and because it is simple to purify and stable.
- First serum samples with antibodies available at 6-8 weeks.

Polyclonal Antibody Development

An animal is injected with the target antigen (ex: Bt protein)

The animal creates antibodies specific to this antigen



Blood is extracted from the animal, the antibodies are harvested and purified

The purified antibodies are applied to a test medium

ENVIROLOGIX™

Monoclonal Antibodies

- All the antibodies that are produced are identical because they are produced by the same type of immune cell that are all clones of a single parent (thus the prefix “mono”).
- Monoclonal Antibodies are created by fusing two types of cells:
  - A B-cell that produces the desired antibodies (but has a defined lifespan).
  - A myeloma cell that is cancerous and can live and divide forever given sufficient environment.
- The fused cell is called a hybridoma cell and it possess the antibody producing ability of the B-cell with the infinite life of the myeloma cell.

ENVIROLOGIX™

Monoclonal Antibodies, Cont.

- The hybridoma cells can be grown in a nutrient media and the antibodies are secreted into the supernatant.
- The supernatant is harvested and the antibodies are purified.
- Cells can be frozen in liquid nitrogen and resurrected and grown when additional antibody is needed.
- Monoclonal antibodies insure a consistent antibody with theoretically endless supply.
- Antibodies take 6-9 months to develop if the fusion of an appropriate antibody producing B-Cell is successful.

Collection of Antibodies

- Polyclonal Antibodies – Serum samples are drawn from the animal. Facilities are monitored by the USDA to insure humane treatment of the animals. They regulate the animal environment and volumes of serum that can be drawn.
- Monoclonal Antibodies -The cell supernatant is collected with a pipette from the incubation flask.

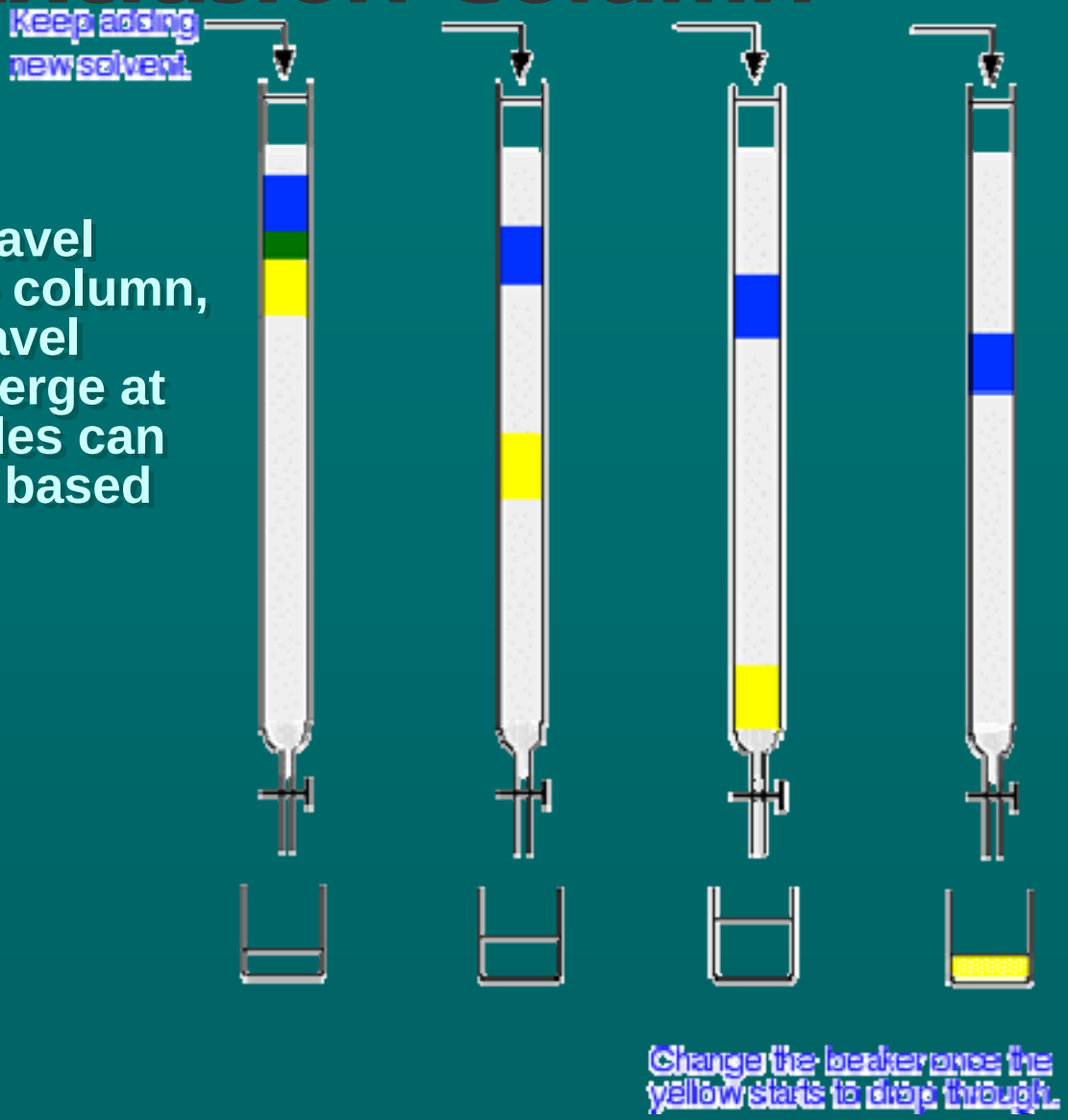
ENVIROLOGIX™

Antibody Purification

- Antibodies can be purified by passing them over columns or via precipitation:
  - Antibodies are affinity purified with a protein A or protein G column that binds to the Fc portion of most immunoglobulins.
  - SAS (Saturated Ammonium Sulfate) precipitation can also capture all proteins larger than 100,000kd (capturing Antibody). Antibodies are resuspended in PBS.
  - Purification of specific antibodies can be achieved by immobilizing the antigen to a solid support surface in a way that the important epitopes are available for antibody binding.
- Columns are then washed with PBS to wash away all non-specific proteins and contaminants.

Antibody Purification – Size Exclusion Column

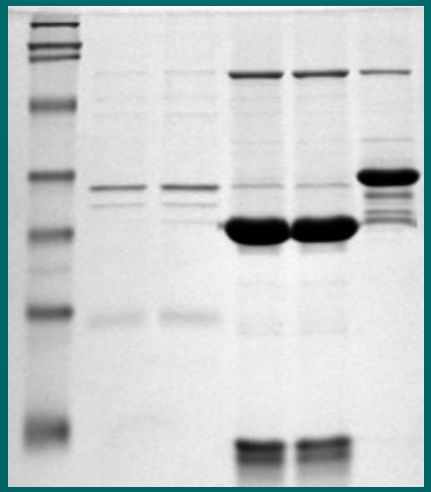
- Small molecules travel slowly through the column, large molecules travel quickly (first to emerge at the bottom). Samples can be purified by size based on flow rates.



ENVIROLOGIX™

Antibody Purification, Cont.

- A low pH or high salt solution is passed over the column and the antibodies relax their bond to column and are washed through and collected. The pH is then returned to neutral and the antibodies regain their original confirmation.
- Purified antibody is then dialyzed into the appropriate storage buffer and is concentrated for use in larger batches.
- Antibodies are run on an SDS PAGE gel (SDS Polyacrylamide Gel Electrophoresis) to make sure there are no contaminants.



ENVIROLOGIX™

Antibody Screening and Selection

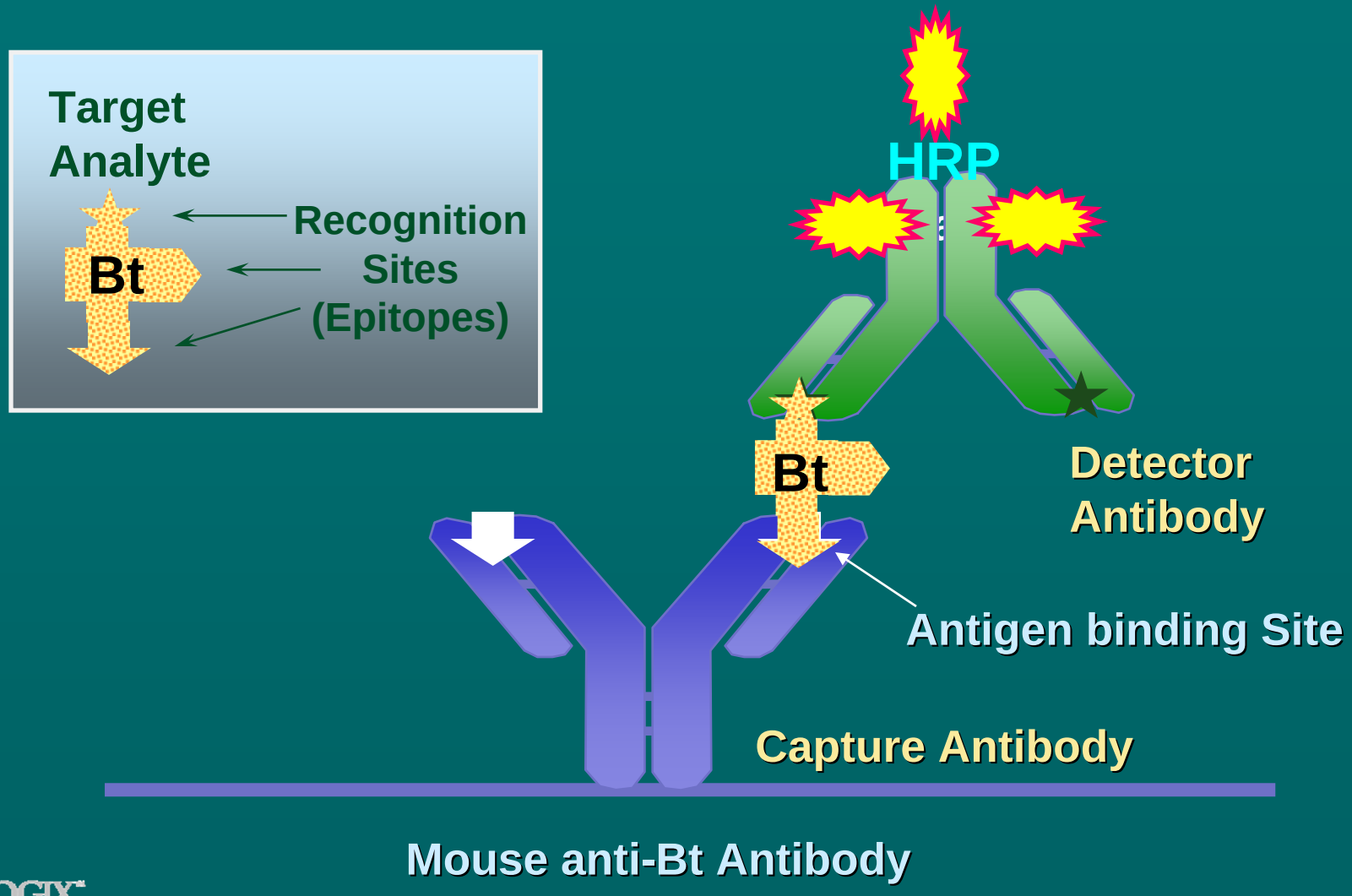
- Antibody lots are screened with an ELISA plate to determine which are most sensitive and specific.
- The affinity of the antibody is also evaluated (which antibody binds fastest).

ENVIROLOGIX™

Incorporation of Antibodies into an Assay

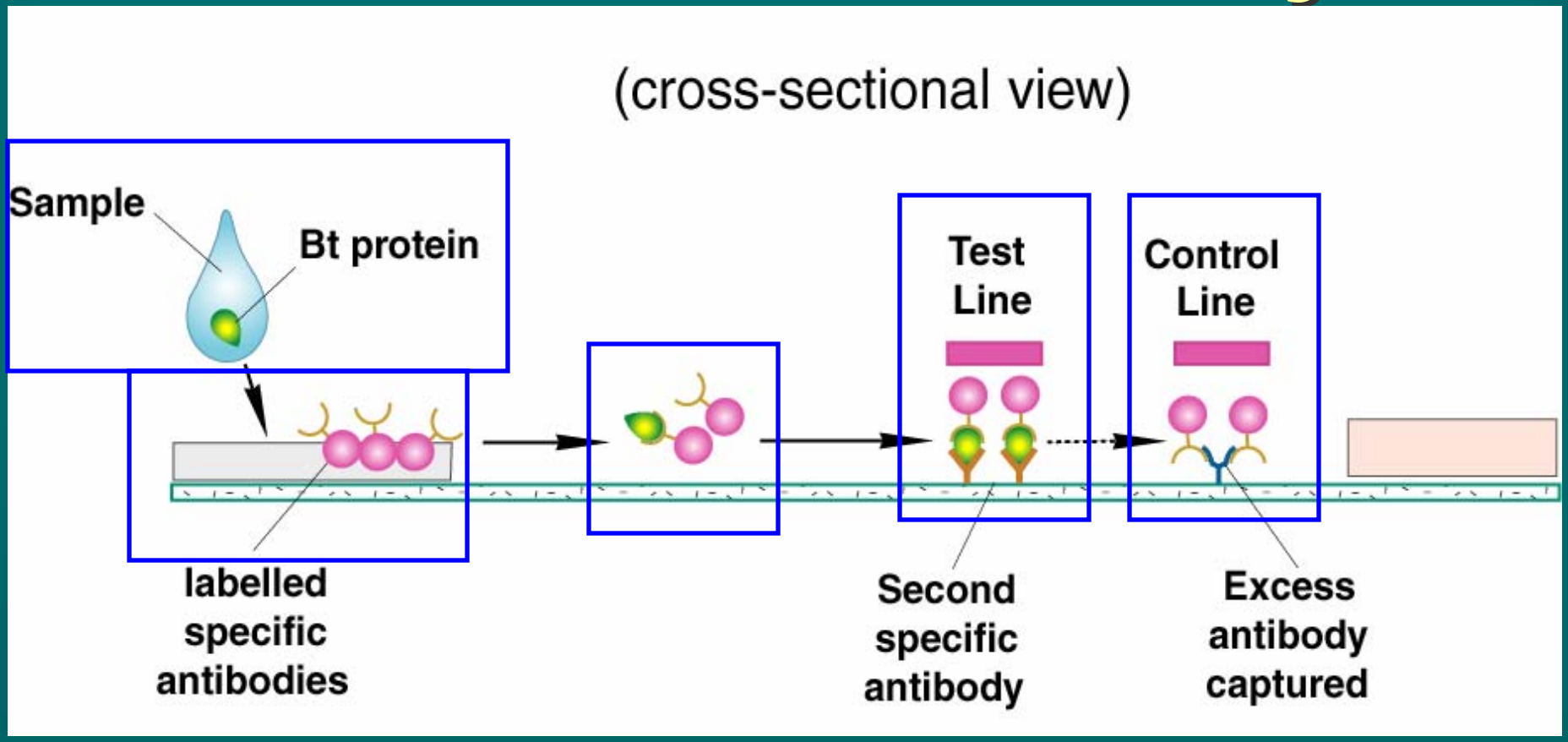
- Antibody pairings are developed in ELISA and then are transferred to a Lateral Flow Device (LFD).
  - When the target antigen has multiple repeating epitopes the same monoclonal antibody can be used for the capture and conjugate.
  - When the target antigen does not have multiple repeating epitopes the assay may require a combination of monoclonal and polyclonal antibodies (one as the capture and one as the conjugate).
  - Polyclonal antibodies are notoriously "stickier" resulting in potential false positives. As a result these antibodies are excellent in ELISA tests where there is a wash step.
- Antibodies must be conjugated to a marker in assays:
  - LFD: Conjugate antibody to a gold particle (latex beads are also an option)
  - ELISA conjugate the antibody to an enzyme (HRP or Alk. Phos.)

ELISA - Sandwich Immunoassay



ENVIROLOGIX

Lateral Flow Device – Sandwich Immunoassay



ENVIROLOGIX™

Antibody References

- Antibodies: A Laboratory Manual
  By: Ed Harlow and David Lane