(9/17) 9: Immune-Based Diagnostics

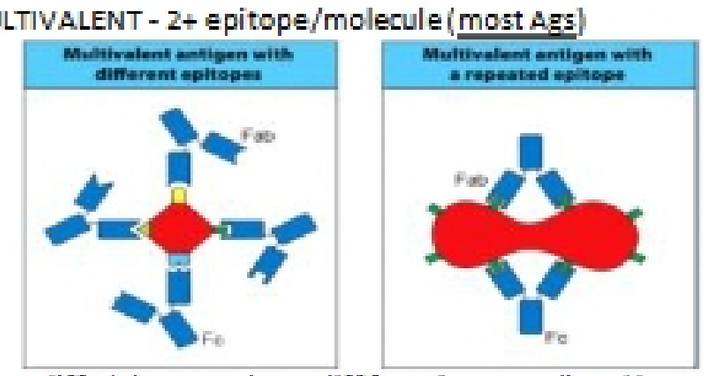
Wednesday, September 17, 2014
1:31 PM

Lecture Outline:
- Principles of Immunoassays
- Characteristics of Ag-Ab Interactions
- Uses of Ag-Ab Interactions
    1. Detection & Purification of Proteins
    2. Detection and Purification of Cells
- Examples of Immunoassays
- Commercial Immunodiagnostic Kits

Objectives:
- To review the characteristics of Ags & Abs
- To describe the basics of Ag-Ab Interactions
- To understand the principles behind immunoassays
- To understand & interpret the results of immunoassays
- To follow the rationale for the essential steps in different immunoassays

Principles of Immunoassays:
- based on specific Ab binding to Ag
- separating bound & unbound Ab conjugates to detect only Ag-bound Abs
- the specific Abs are labeled with an indicator (enzyme, fluorescent, radioisotope, etc) for detection

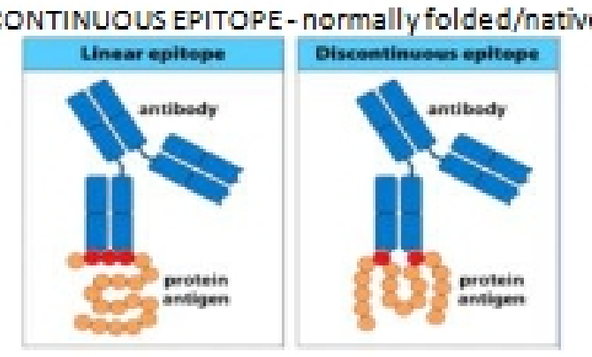
Ab Characteristics:
- SPECIFICITY - ability to discriminate between diff molecules
- AFFINITY
    - MONOVALENT - 1 epitope/molecule
        Strong: $K_A = 10^{13} M^{-1}$
        Normal: $K_A = 10^{7-10} M^{-1}$
        Weak: $K_A = 10^{4-5} M^{-1}$
    - MULTIVALENT - 2+ epitope/molecule (most Ags)

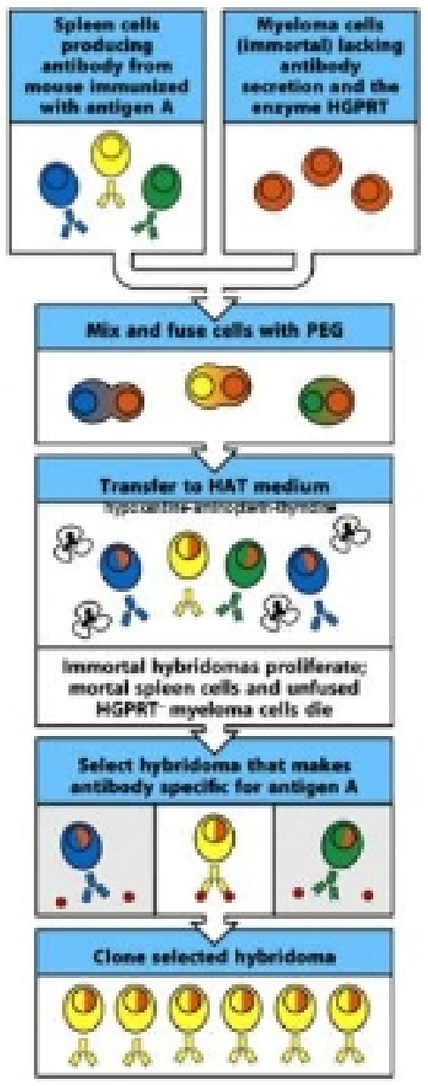


- FUNCTION - diff Ab isotypes have diff functions, mediated by Fc & Ig portions

---

LINEAR EPITOPE - denatured/processed Ags
DISCONTINUOUS EPITOPE - normally folded/native Ags

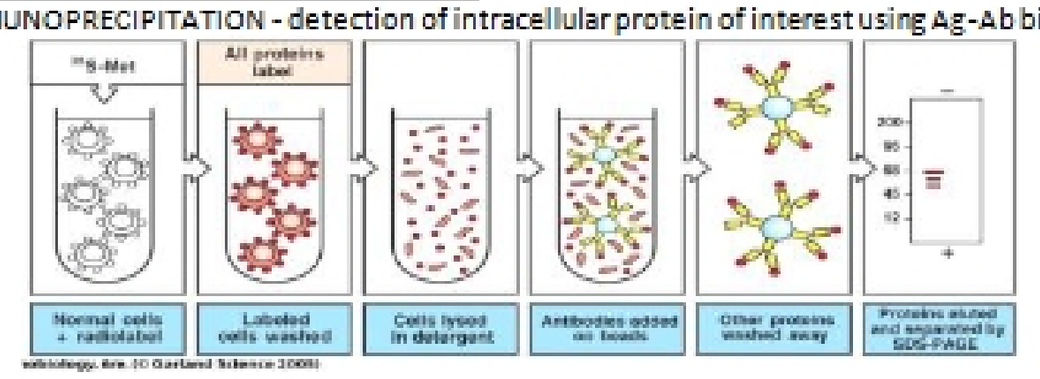


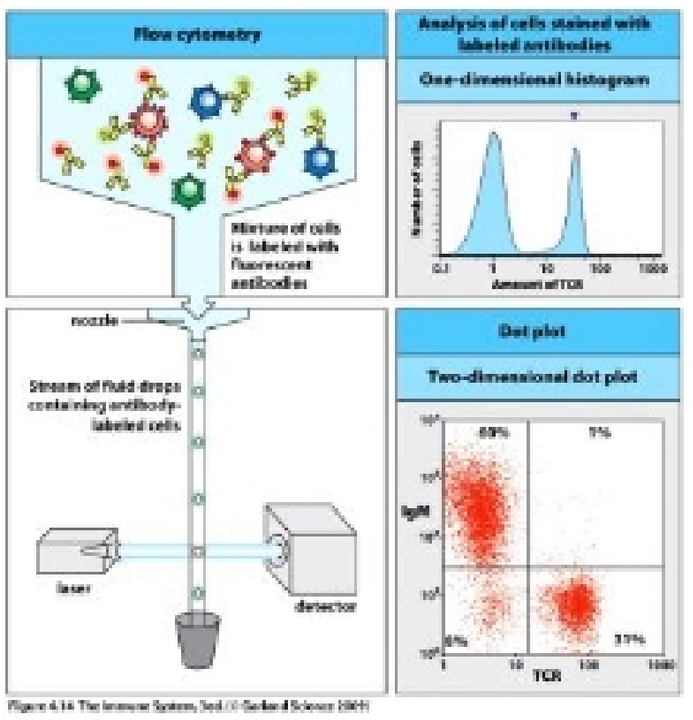


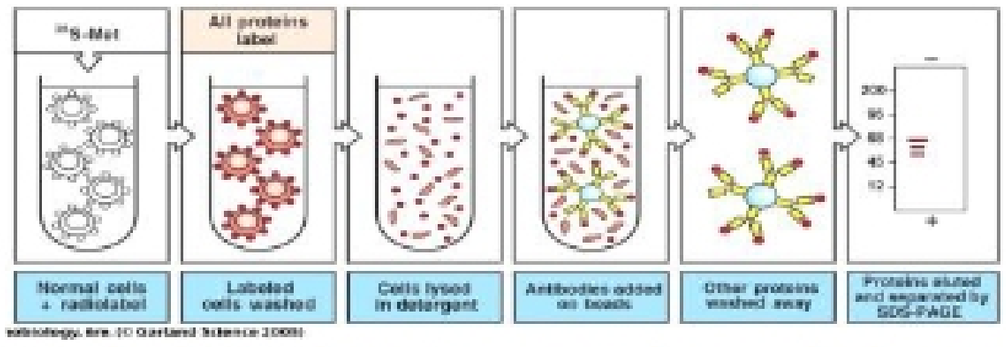
MONOCLONAL - Abs immunized with Ag from mouse spleen are fused with myeloma cells (immortal), and the resulting hybridomas proliferate

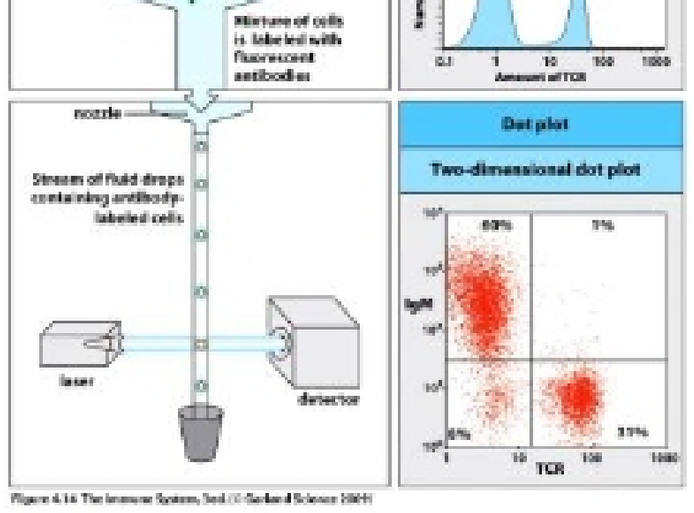
Detection of Specific Ab:
1. precipitation/agglutination
2. enzyme-linking (horseradish peroxidase, alkaline phosphatase)
3. radioisotope-linking
4. solid matrix-linking (agarose)
5. fluorophor-linking (FITC)

Detection & Purification of Proteins of Interest:
IMMUNOPRECIPITATION - detection of intracellular protein of interest using Ag-Ab binding







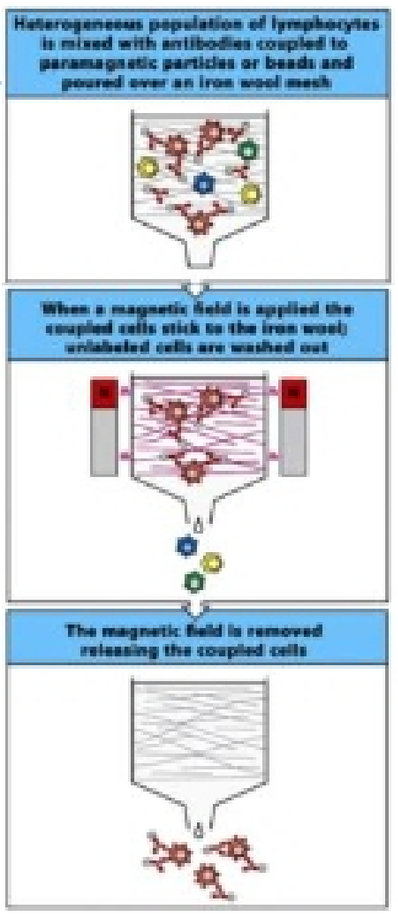


Separation & Detection of Cell Populations that Express Membrane Proteins of Interest:

FLOW CYTOMETRY - cells labeled w/red fluorophore are deflected to a collection tube (other cells = green)

Cell Separation by Magnetic Separation:

RADIOISOTOPES - Abs coupled w/paramagnetic particles (beads) are filtered via magnetic attraction to iron wool mesh ----



Protein Samples: (serum, urine, etc)

- WHOLE CELL EXTRACTS - lyse whole cells in detergent-containing buffer (cold); (may need homogenization /sonication)
- CELLULAR FRACTIONS - nuclear extracts, membrane fraction, mitochondria
- chromatography-fractionated cellular proteins
- in vitro expressed proteins

Cellular Samples:

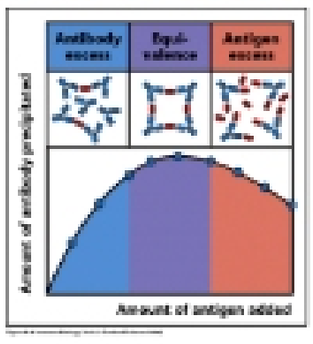
- cell surface molecules
- intracellular molecules

Examples of Immunoassays for Detection & Quantification of Specific Molecules or Cell Types:

- (assay dependent upon detection tag)
- IMMUNOFLUORESCENCE MICROSCOPY:

Label	Ab specificity	Stains __ of Pancreatic Islets
FITC	GAD	β cells
Rhodamine	glucagon	α cells

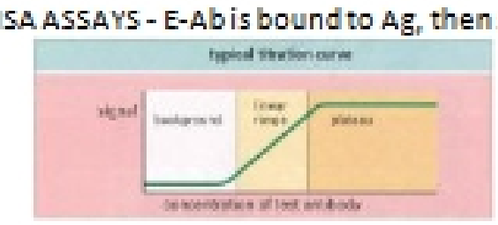
- AGGLUTINATION ASSAYS - based on maximized matrix formation which occurs when Ab ≈ Ag (basis of blood typing)

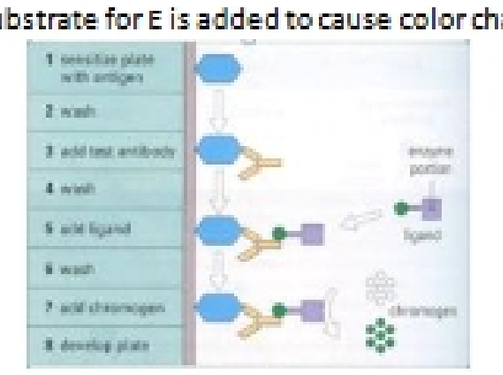


Serum from individuals of type	Red blood cells from individuals of type: O	A	B	AB
	Express the carbohydrate structures: R–GlcNAc–Gal (Fuc)	R–GlcNAc–Gal–GalNAc (Fuc)	R–GlcNAc–Gal–Gal (Fuc)	R–GlcNAc–Gal–GalNAc (Fuc) + R–GlcNAc–Gal–Gal (Fuc)
Anti-A and anti-B antibodies	no agglutination	agglutination	agglutination	agglutination
Anti-B antibodies	no agglutination	no agglutination	agglutination	agglutination
Anti-A antibodies	no agglutination	agglutination	no agglutination	agglutination
No antibodies to A or B	no agglutination	no agglutination	no agglutination	no agglutination

Figure 4-8 Immunobiology, 7ed. (© Garland Science 2008)

- ELISA ASSAYS - E-Ab is bound to Ag, then substrate for E is added to cause color change ----------





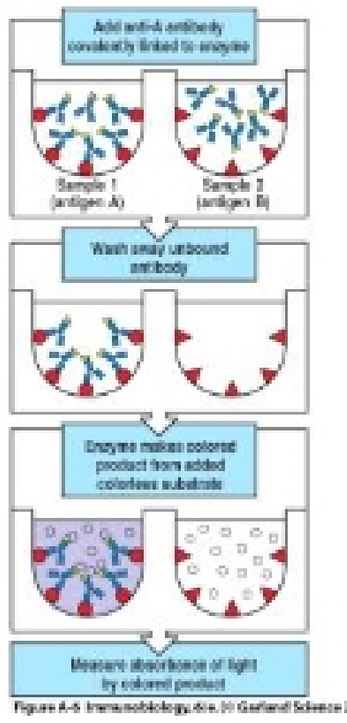
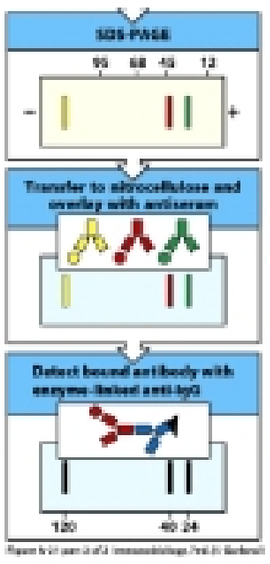


Figure 4-6 Immunobiology, 6th ed. (© Garland Science 2005)

- WESTERN BLOTTING - detection of proteins in cell lysates

*diff types of epitopes (linear/discontinuous) require diff detection Abs



Enhancing Sensitivity/Flexibility:

- do Indirect rather than Direct assays, using 2° detection Abs (ANTI-IMMUNOGLOBULINS)
- amplify the signal (biotin-stretavidin)
- set up a Competitive Inhibition ELISA

Other Immunology-Based Procedures:

- immunization w/Ag & detection of Ag-specific Abs
- In Vivo measurement of immune responses
  - skin tests (tuberculin, allergens)
  - protection from infectious organisms

Commercial Immunodiagnostic Kits:

- frequently used (pregnancy test)
- Advantages & Limitations

Advantages:	**Limitations**:
- non-toxic	- cross-reactivity
- sensitive	- presence of inhibitors
- easy/simple	- storage failure
- reliable	- human error