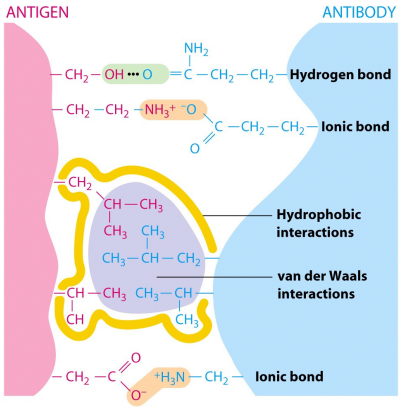
# Chapter 6

**Antigen-Antibody Interactions: Principles and Applications**




## Antigen-Antibody Properties

- You must remember antibody affinity (single) VS avidity (multiple)
- High affinity: bound tightly and longer!
- Cross-reactivity: occurs when two different antigens share an identical or a very similar epitope. The antibody's affinity for the cross-reacting epitope will be _____ than for the original epitope.
- ABO blood groups and infectious diseases (Streptococcal M antigens, Jenner?)

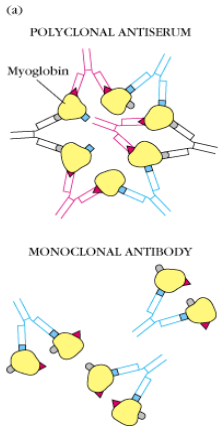
## Precipitin reactions

The interaction of antibody with antigen in solution may cause formation of an insoluble lattice that will **precipitate** out of solution.

This precipitate will only form if:

- The antibody is bivalent or polyvalent
- The antibody or antibody mixture can bind to at least two different sites on the antigen (either two different epitopes or two identical epitopes)

Monoclonal antibodies are likely to be less efficient at immunoprecipitation than polyclonal antibodies.



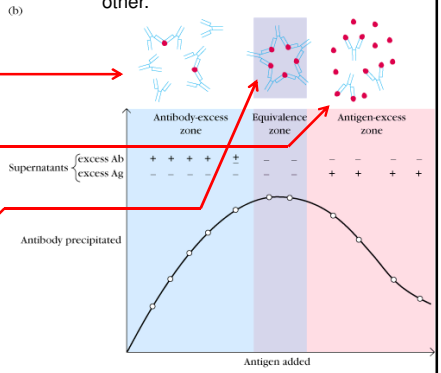

Kuby Figure 6-4a

Formation of the precipitate also requires that the antigen and antibody be present at appropriate concentrations relative to each other.

**Antibody excess** - too much antibody prevents efficient cross-linking

**Antigen excess** - too much antigen prevents efficient cross-linking

**Equivalence** - ratio of antibody to antigen is optimal and a maximum amount of precipitate is formed.




Kuby Figure 6-4b

**TABLE 6-3 Sensitivity of various immunoassays**

| Assay | Sensitivity* (μg antibody/ml) |
|---|---|
| Precipitation reaction in fluids | 20–200 |
| Precipitation reactions in gels | |
| Mancini radial immunodiffusion | 10–50 |
| Ouchterlony double immunodiffusion | 20–200 |
| Immunoelectrophoresis | 20–200 |
| Rocket electrophoresis | 2 |
| Agglutination reactions | |
| Direct | 0.3 |
| Passive agglutination | 0.006–0.06 |
| Agglutination inhibition | 0.006–0.06 |
| Radioimmunoassay (RIA) | 0.0006–0.006 |
| Enzyme-linked immunosorbent assay (ELISA) | ~0.0001–0.01 |
| ELISA using chemiluminescence | ~0.00001–0.01† |
| Immunofluorescence | 1.0 |
| Flow cytometry | 0.006–0.06 |

*The sensitivity depends on the affinity of the antibody used for the assay as well as the epitope density and distribution on the antigen.
†Note that the sensitivity of chemiluminescence-based ELISA assays can be made to match that of RIA.

SOURCE: Updated and adapted from N. R. Rose et al., eds., 1997, *Manual of Clinical Laboratory Immunology*, 5th ed., American Society for Microbiology, Washington, DC.

Table 6-3
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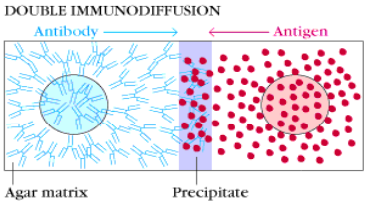
## Double Immunodiffusion (Ouchterlony)

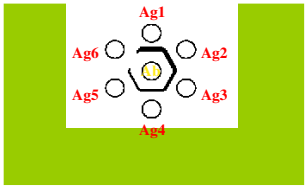
**Diffusion of antibody and antigen towards each other in an agarose gel.**

**A line of precipitate will form if the antibody binds to antigen.**

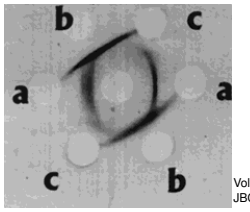
**Used to determine if an antigen or antibody is present.**




Kuby Figure 6-5




http://www.fbr.org/swksweb/immunolist.html



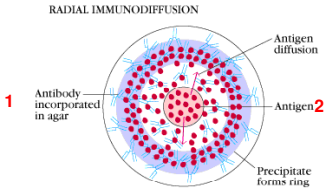

Volume 271, Number 30, Issue of July 26, 1996 pp. 18054-18060
JBC

## Radial Immunodiffusion

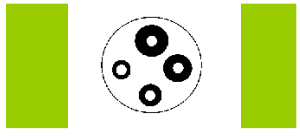
**Diffusion of antigen through an agarose gel containing antibody.**

**A precipitin ring will form. The size of the ring is proportional to the antigen concentration.**

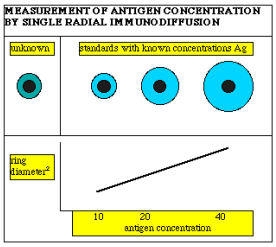
**Used to determine concentrations of specific proteins (low sensitivity)**




Kuby Figure 6-5



http://www.fbr.org/swksweb/immunolist.html




http://leahi.kcc.hawaii.edu/~johnb/micro/medmicro/medmicro.11.html

## Hemagglutination

Antibody can also cross-link cells or beads.

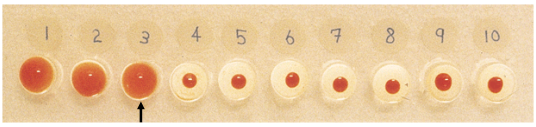
Cross-linking of red cells is called hemagglutination.

**Non-cross-linked cells settle** in a bead to the bottom of the well.

Cross-linked cells settle in a **diffuse pattern.**

Used to measure antibody presence and level **(titer).**

Used to measure antibodies to red cell antigens or to other antigens bound to the surface of red cells.




Kuby Figure 6-8

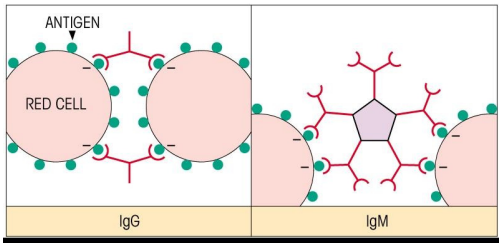



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Figure 6.7

# Immunoelectrophoresis

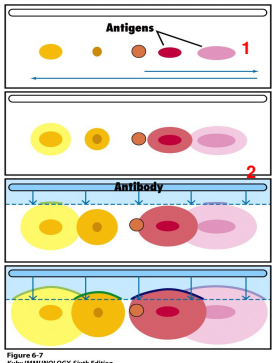


Figure 6-7
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1. Antigens are electrophoresed to separate them based on their charge
2. A trough I cut and antiserum is added.

## Enzyme-linked immunosorbent assay (ELISA)

Used to measure antigen or antibody presence and concentration.

Far more sensitive than precipitin or agglutination techniques.

Relies on the ability to covalently conjugate chemicals to the Fc region of Ig without interfering with antigen binding ("enzyme-linked") and the ability of plastic to nonspecifically bind proteins (immunosorbent).

In ELISA, an enzyme is bound to the Fc region - usually horseradish peroxidase or alkaline phosphatase. Enzyme presence can be determined by use of colorimetric substrates.

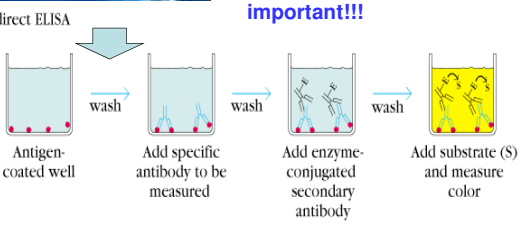
Final measurement is an absorbance.

Comparison with standard curves indicates concentration of antigen or antibody.

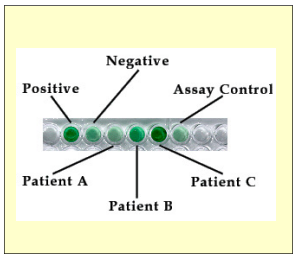
Various assay formats are possible.

(a) Indirect ELISA

**BLOCKING is very important!!!**



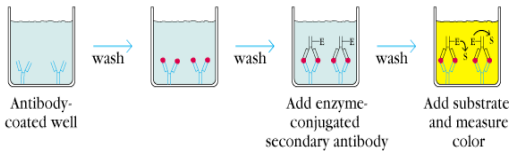

http://www.supercolostrum.com/colostrum/Information/information9.htm

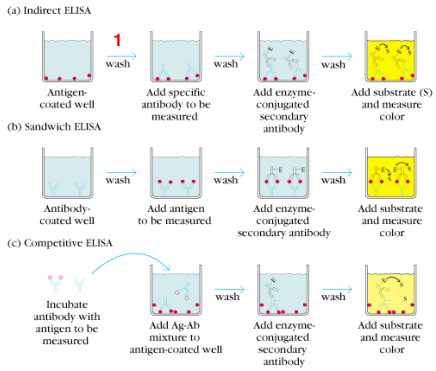



| Sample | Absorbance |
|---|---|
| Positive control | 1.689 |
| Negative control | 0.153 |
| Assay control | 0.123 |
| Patient A | 0.055 |
| Patient B | 0.412 |
| Patient C | 1.999 |

http://www.supercolostrum.com/colostrum/Information/information9.htm

(b) Sandwich ELISA






Kuby Figure 6-11

**Relative sensitivities:**

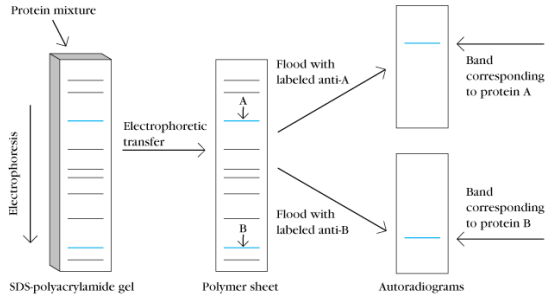
**Precipitin reactions < Agglutination reactions < ELISA**

**Western blotting - Used to identify presence of a specific antigen or antibody**

**1) Proteins (Ag0 are separated by polyacrylamide gel electrophoresis (SDS-PAGE) and transferred to a nitrocellulose-style sheet (or other).**

**2) Sheet is incubated with anti-Ag antibody**

**3) Band appears wherever the Ag is present.**



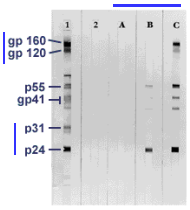

Kuby Figure 6-13

## HIV Western Blot

No bands present……..Negative

Bands at either **p31 OR p24** AND bands present at either **gp160 OR gp120**……..Positive

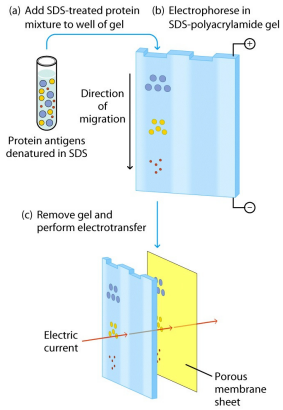
Bands present, but pattern does not Meet criteria for positivity……..Indeterminate

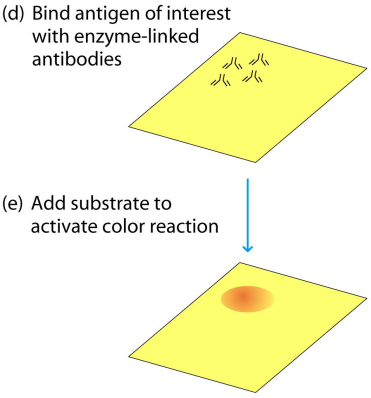



1.Lane 1, HIV+ serum (positive control)
2.Lane 2, HIV- serum (negative control)

3.Lane A, Patient A
4.Lane B, Patient B
5.Lane C, Patient C

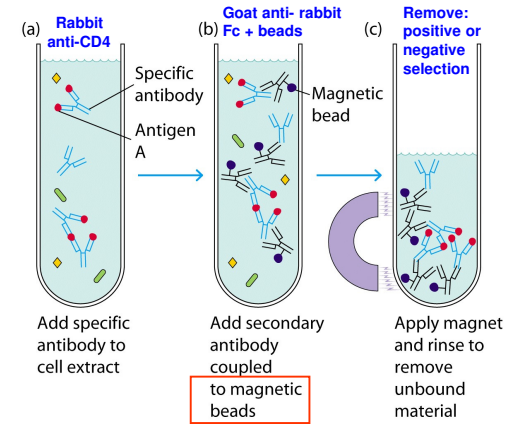
http://www.biology.arizona.edu/immunology/activities/western_blot/west2.html

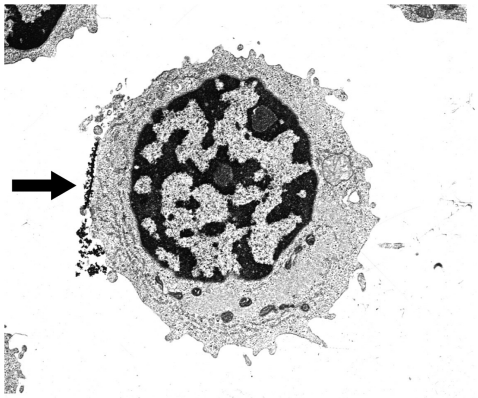


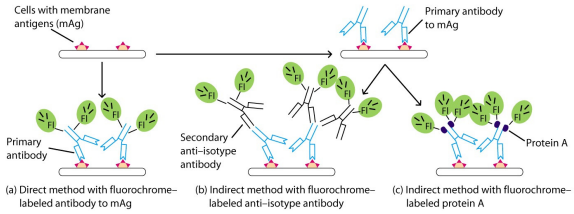
## Immuno-magnetic separation (IMAS)

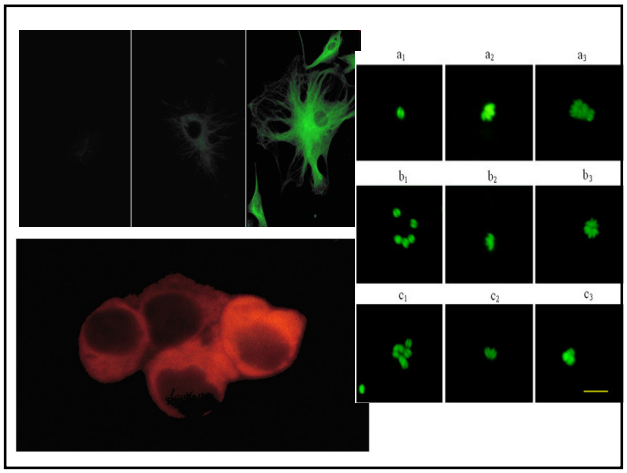
- Technique used to separate cells, proteins, nucleic acids using antibodies or ligands-bound to magnetic beads.
- Removed out from mix suspension using a magnet.



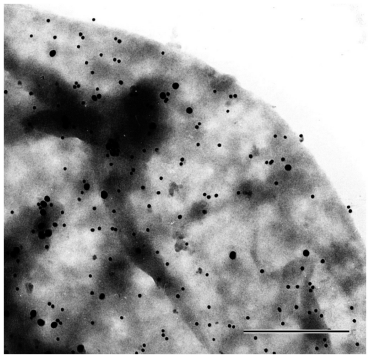



## Immunofluorescence



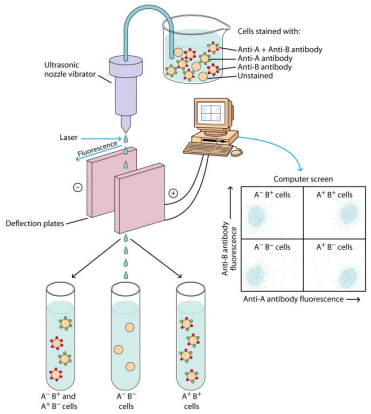



## Immunogold labeling

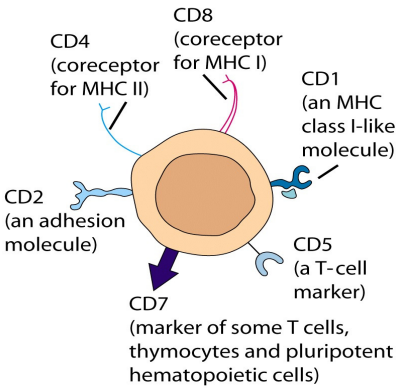


## Fluorescence Activated Cell Sorter (FACS)

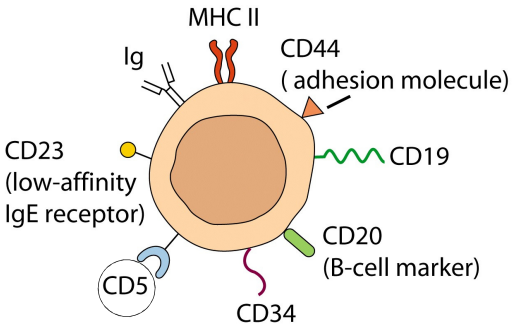
- Mixed cell population
- Requires two different fluorochromes
- Commonly used: FITC, PE, Texas Red, etc




ALL of the T lineage

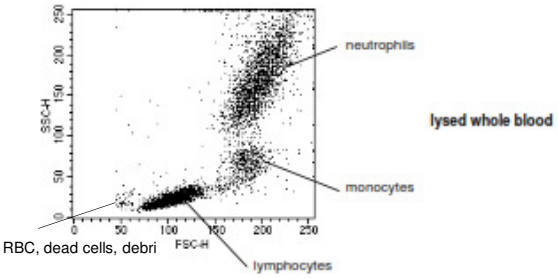



A B-lineage CLL




**SSC** – **SIDE SCATTERED LIGHT** proportional to granularity or internal complexity

**FSC** – **FORWARD SCATTERED LIGHT** proportional to size or surface area



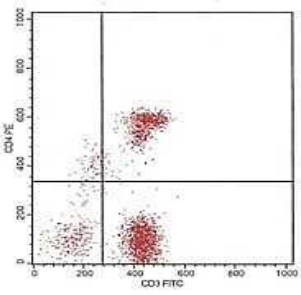

**FACS Analysis**

**Reagents:**

**CD4-PE Phycoerythrin (PE)**

**VS**

**CD3 Fluorecein (FITC)**

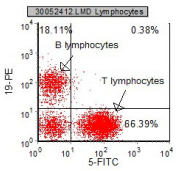


# Markers for Th cells

- **Naïve** – CD62L (L-selectin), CD45RA
- **Activated** – CD25, CD69, CD38
- **Memory** – CD45RO, CD44

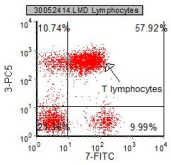
**High (hi) vs Low (lo)**

UNIQUE



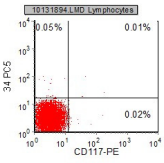

**Mutual exclusive markers** - CD19 and CD5 stain either one population on another, but never both.

EXPRESSES BOTH



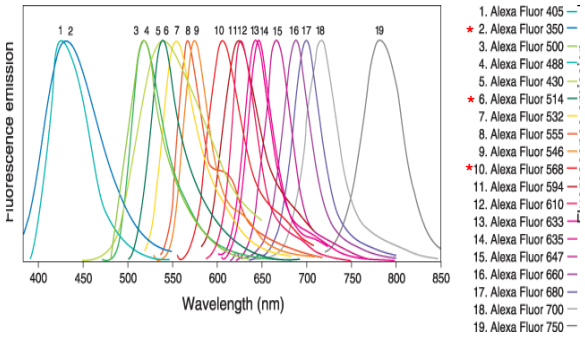

**Co-expression** - there are many cells that are both CD3+ and CD7+. It is said that CD3 and CD7 co-express on T Lymphocytes.

NO EXPRESSION




**Nonexpression** - The gated lymphocytes do not express CD34 or CD117.

# The Alexa Fluor Dye Series




## Commonly Used Fluorochromes

| Probe | Ex (nm) | Em (nm) | MW | |
|---|---|---|---|---|
| **Reactive and conjugated probes** | | | | |
| Cascade Blue | 375;400 | 423 | 596 | |
| Lucifer yellow | 425 | 528 | | |
| Phycoerythrin (PE) | 480;565 | 578 | 240 k | |
| Fluorescein (FITC) | 495 | 519 | 389 | |
| BODIPY-FL | 503 | 512 | | |
| TRITC | 547 | 572 | 444 | |
| Rhodamine | 570 | 576 | 548 | |
| Texas Red | 589 | 615 | 625 | |
| TruRed | 490,675 | 695 | | |