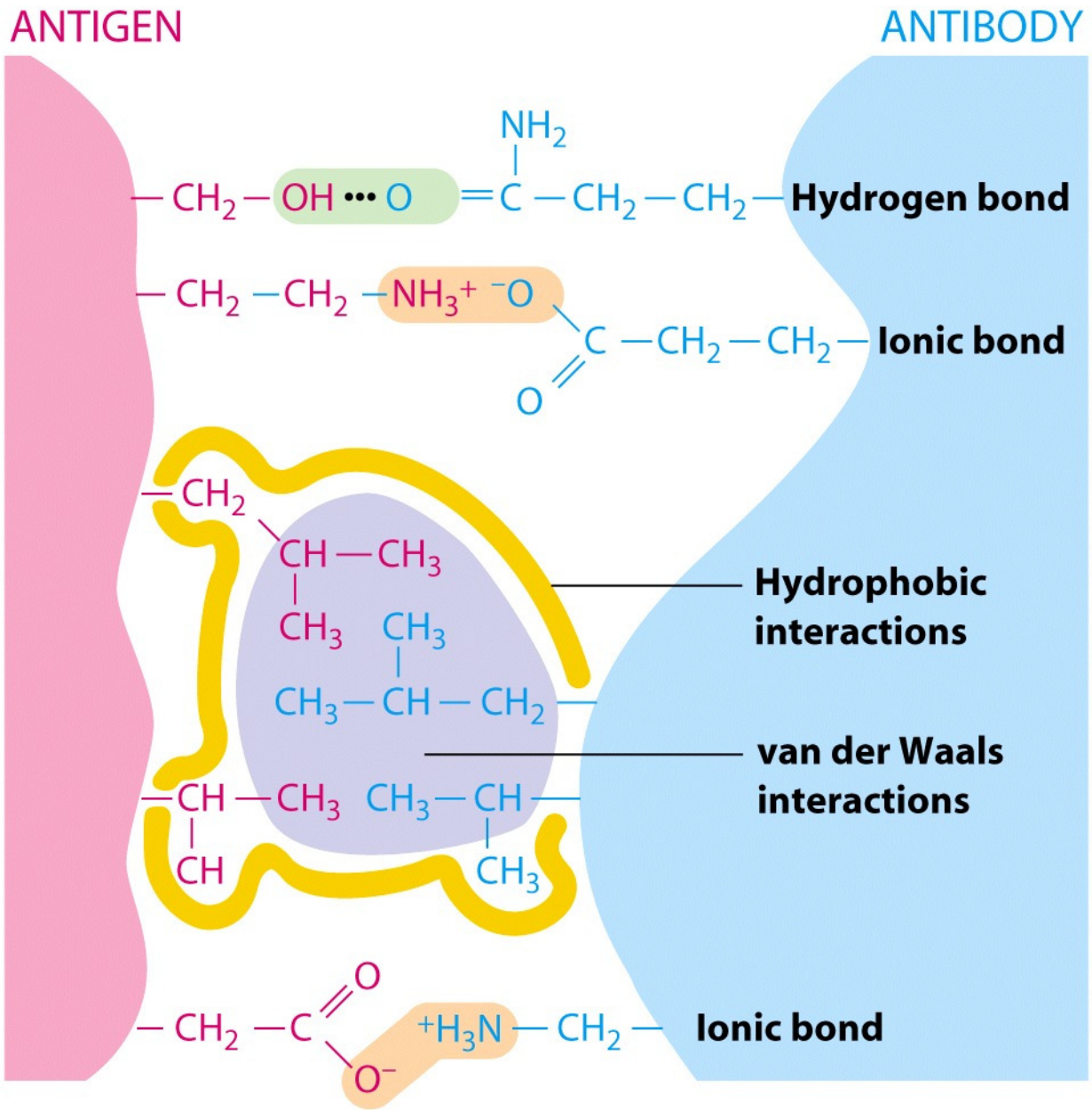

ANTIGEN
ANTIBODY
Hydrogen bond
Ionic bond
Hydrophobic interactions
van der Waals interactions
Ionic bond

Antigen-Antibody Properties

- You must remember Antibody affinity (single) VS avidity (multiple)
- Cross-reactivity: occurs when two different antigens share an identical or 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 (Jenner?)

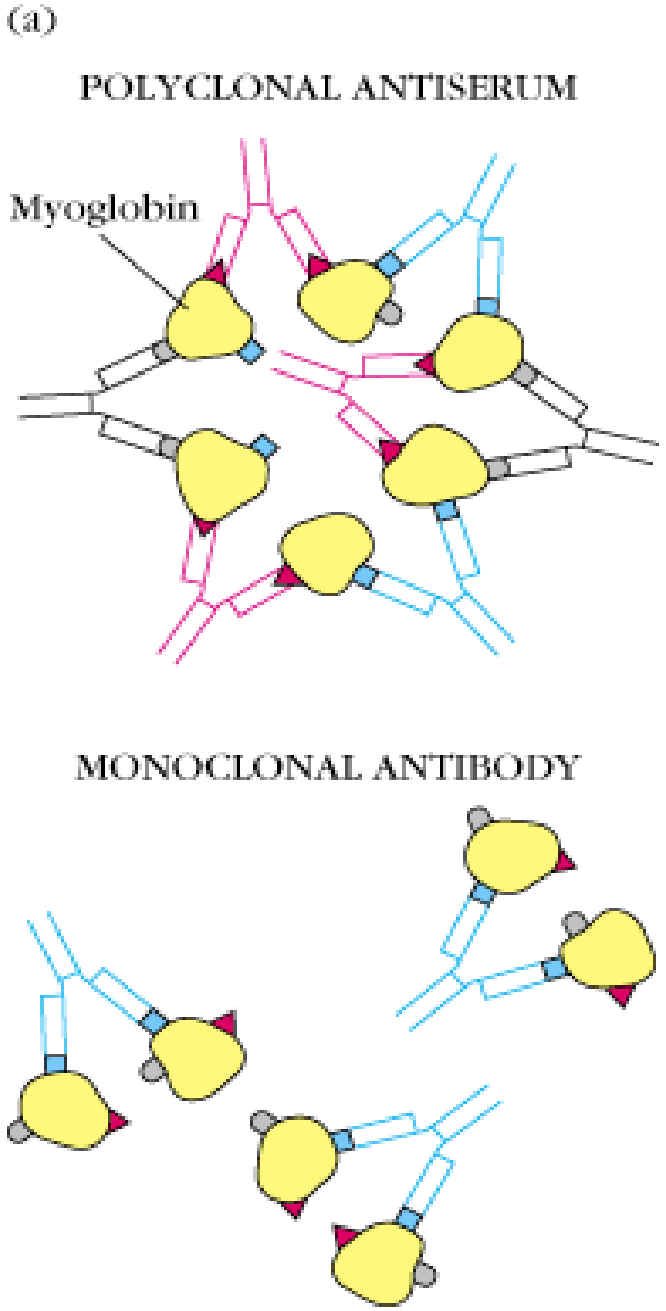
Precipitin reactions

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.



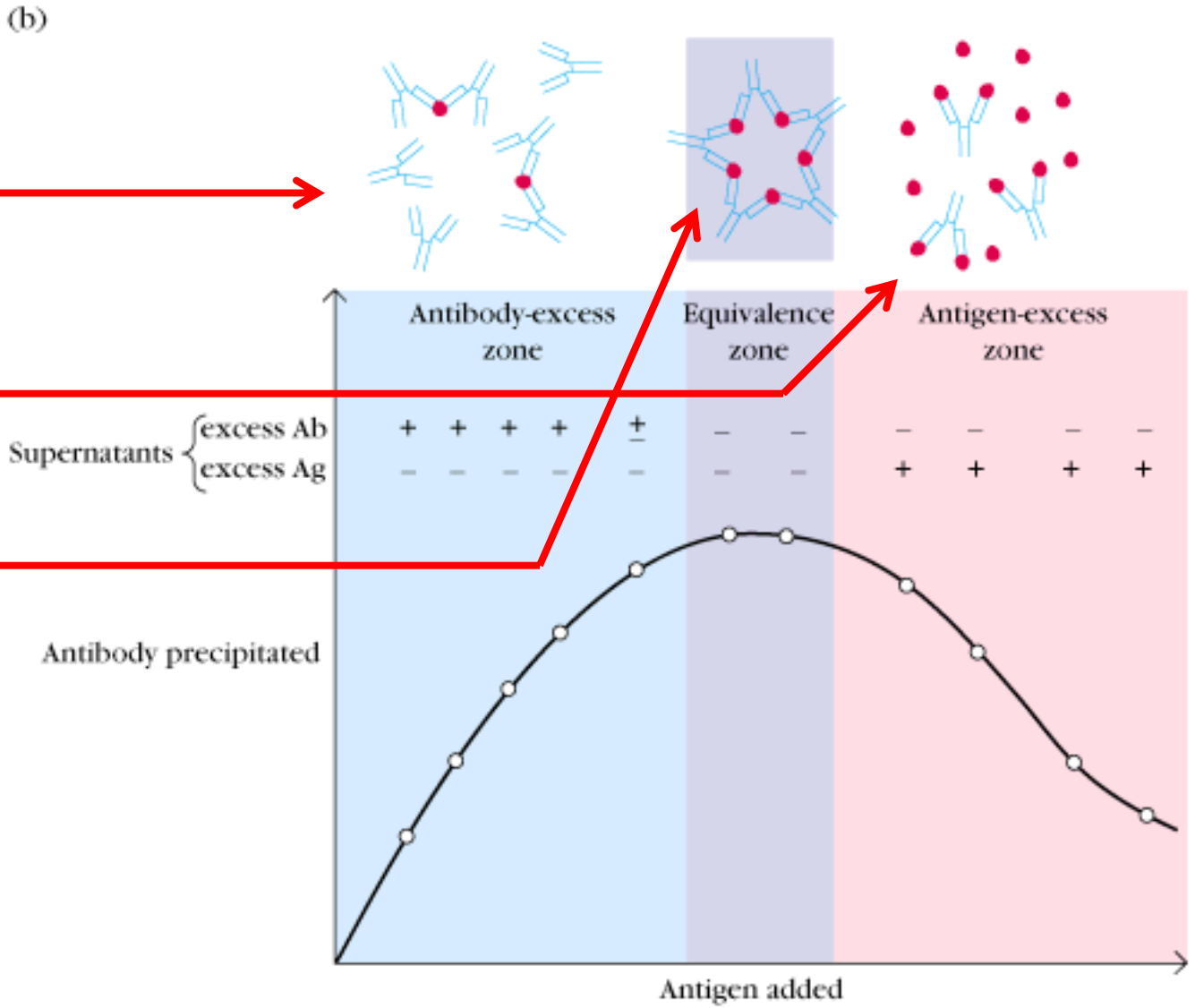
Kuby Figure 6-4a

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

Surface Plasmon Resonance (SPR)

Ab_1 and Ab_2 bind different epitopes of the same antigen.

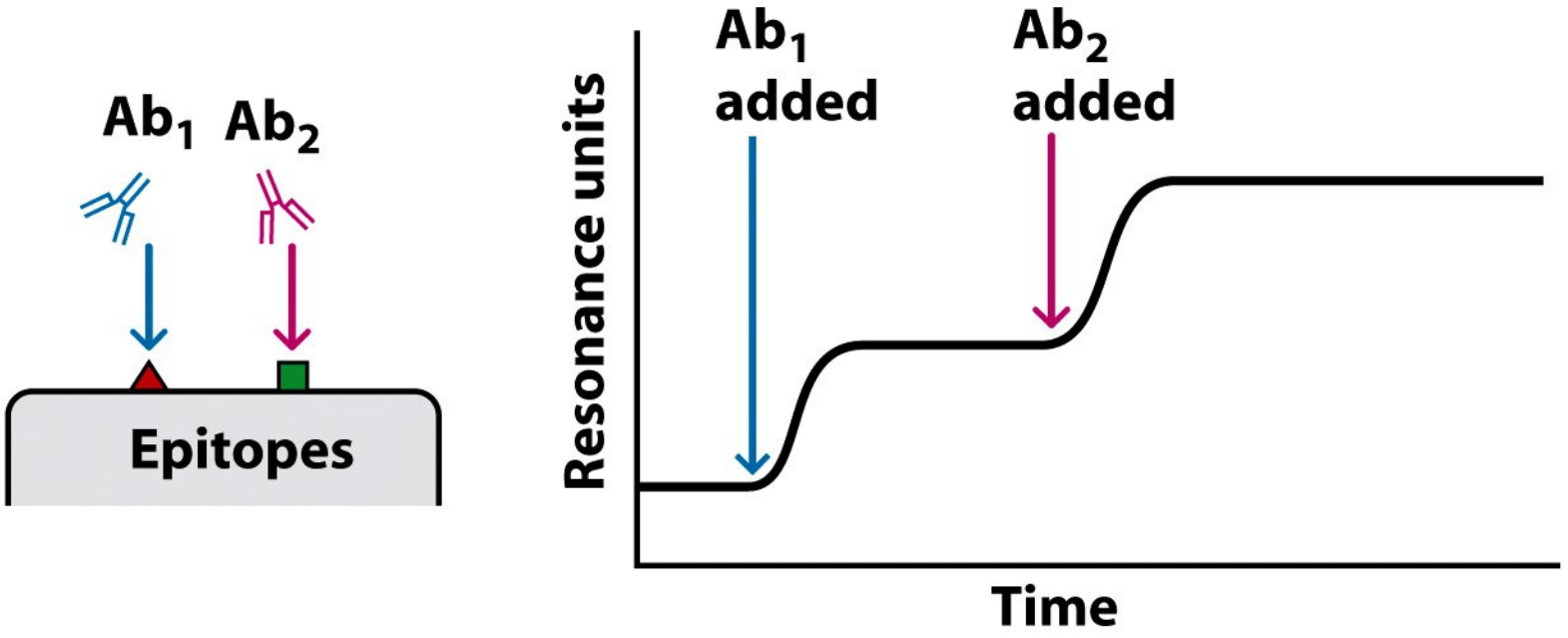


Figure 6-5a
Kuby IMMUNOLOGY, Sixth Edition

Ab_1 and Ab_2 bind to the same epitope.

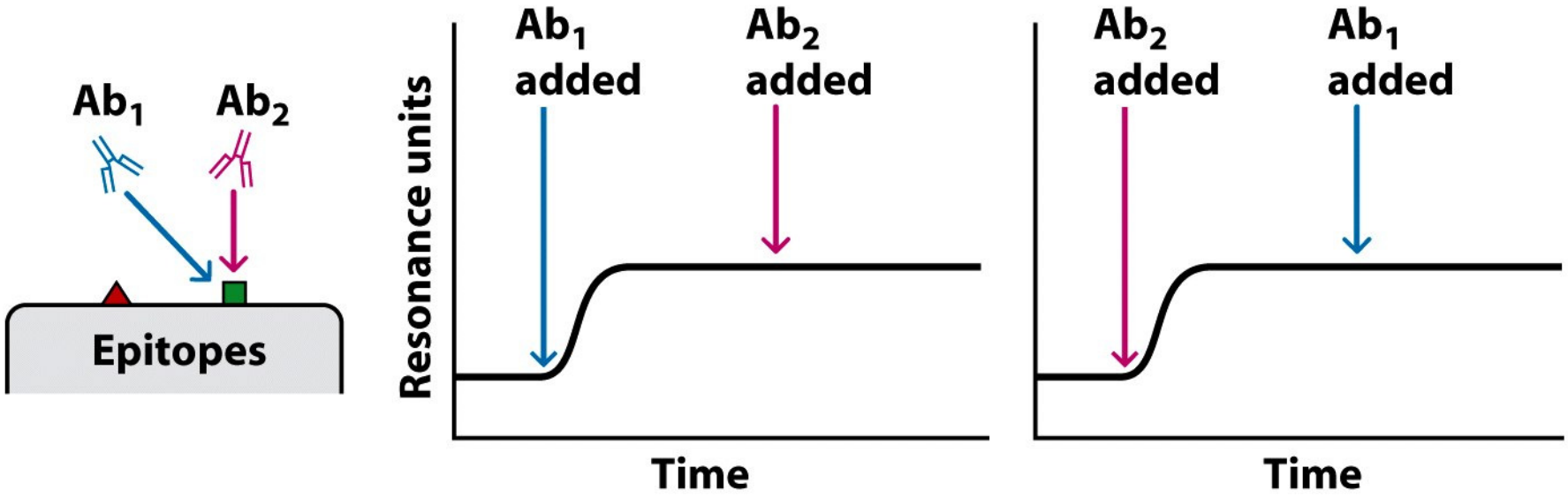


Figure 6-5b
Kuby IMMUNOLOGY, Sixth Edition

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.

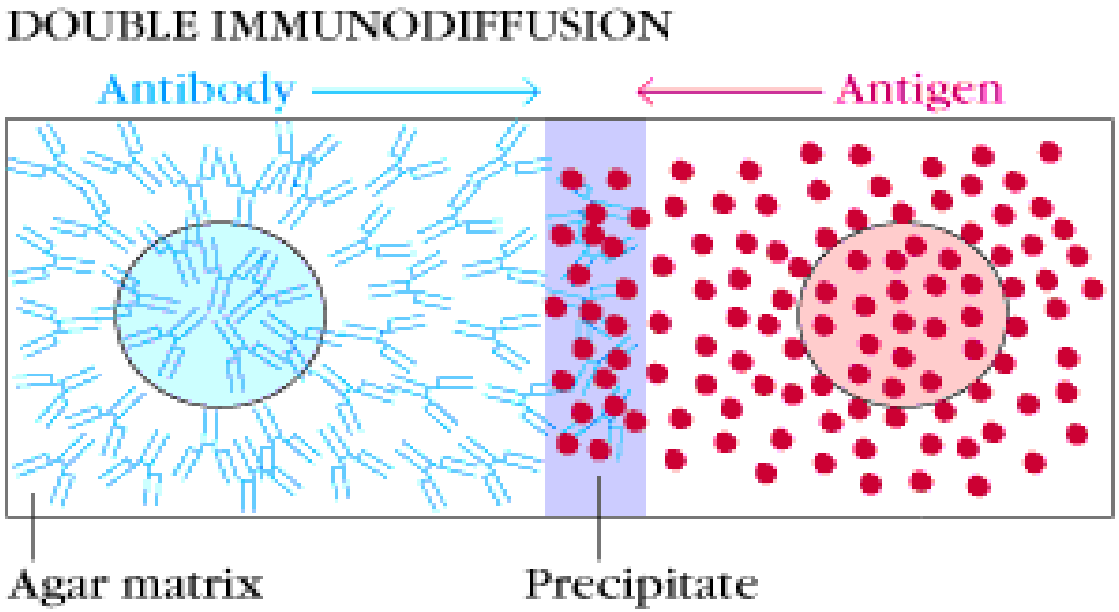
Table 6-3
Kuby IMMUNOLOGY, Sixth Edition

Double Immunodiffusion

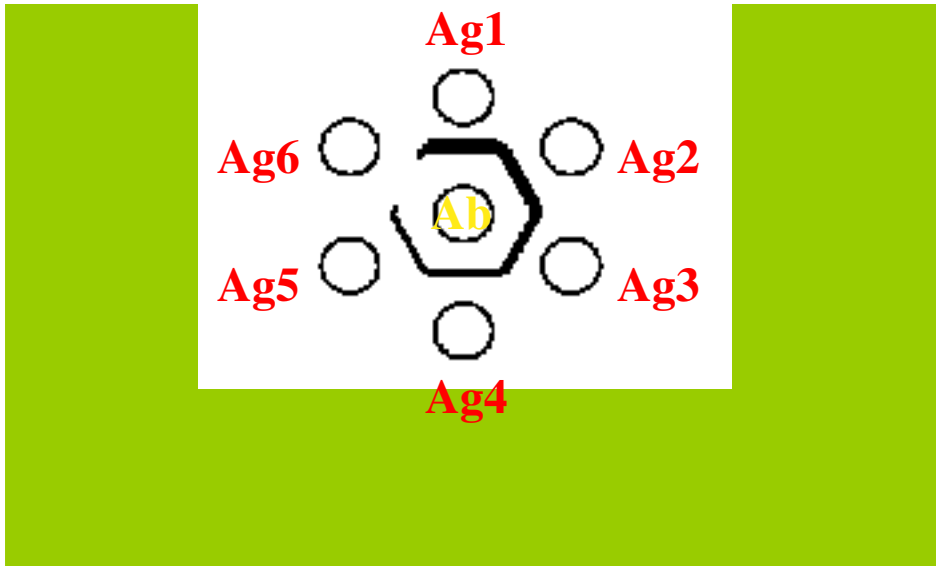
Diffusion of antibody and antigen towards each other in an agarose gel.

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

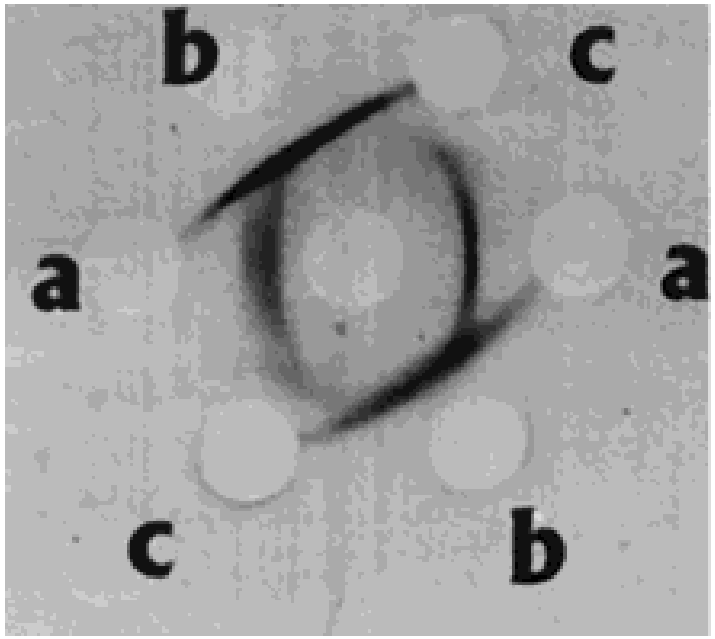
Used to determine if an antigen or antibody is present.



Kuby Figure 6-5



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



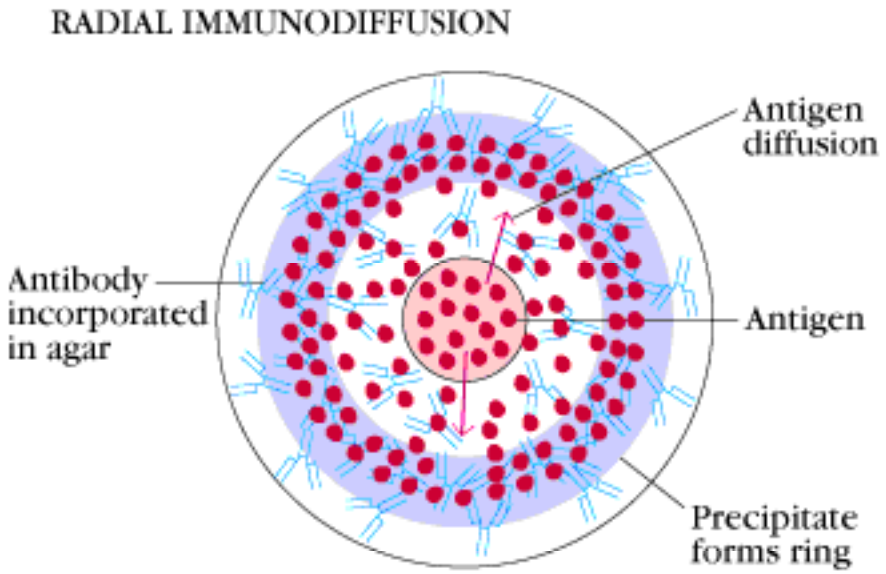
Volume 271, Number 30, Issue of July 26, 1996 pp. 18054-18060
JBC

Radial Immunodiffusion

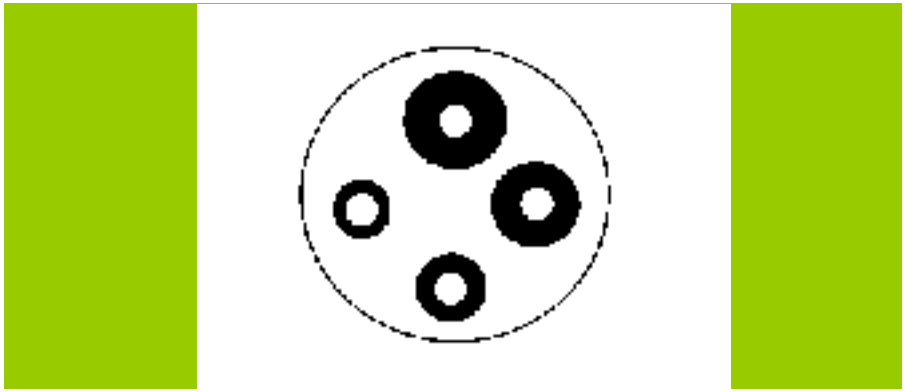
Diffusion of antigen through an agarose gel containing antibody.

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

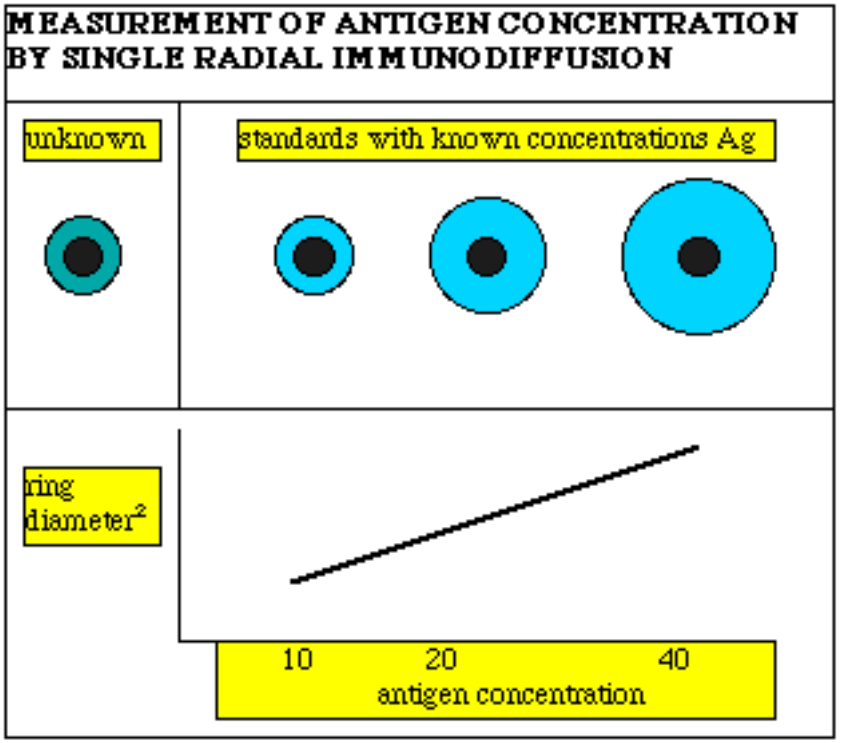
Used to determine concentrations of specific proteins (low sensitivity)



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.

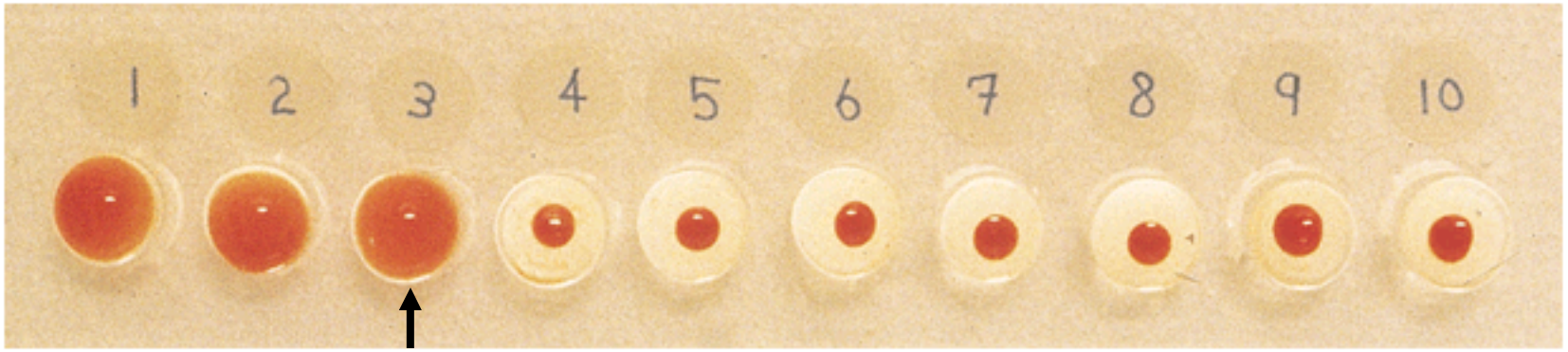
Cross-linking of red cells is called hemagglutination.

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 (titre).

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



Kuby Figure 6-8

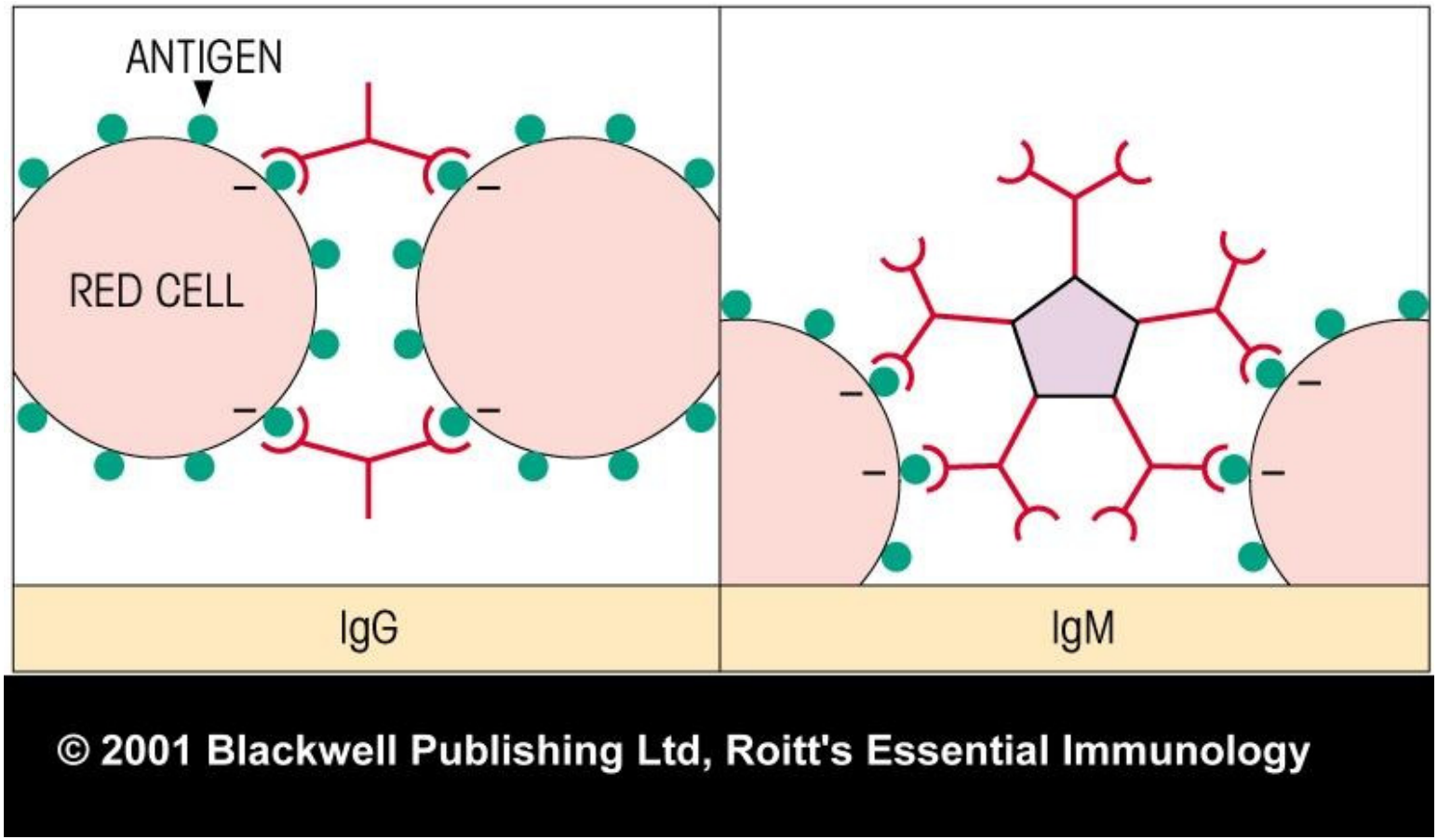


Figure 6.7

Immunoelectrophoresis

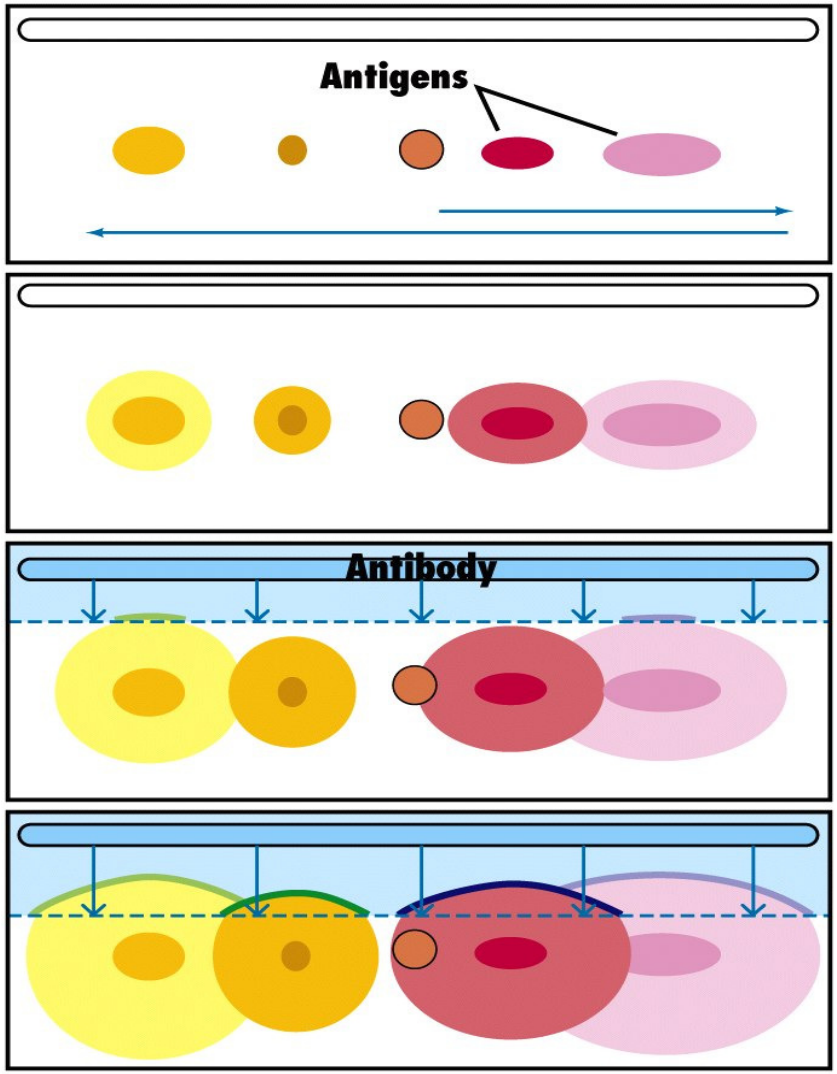


Figure 6-7
Kuby IMMUNOLOGY, Sixth Edition

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).

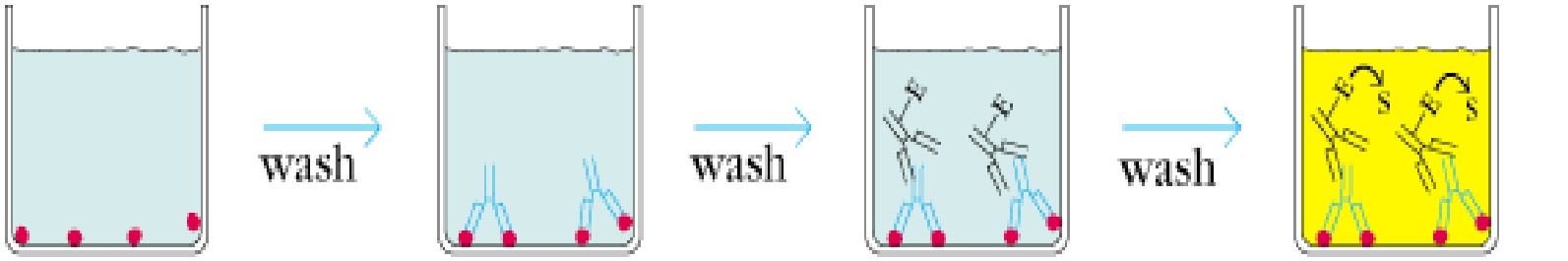
In ELISA, an enzyme is bound to the Fc region - usually horseradish peroxidase or alkaline phosphatase. Enzyme presence can be determined by use of colorimetric substrates.

Final measurement is an absorbance.

Comparison with standard curves indicates concentration of antigen or antibody.

Various assay formats are possible.

(a) Indirect ELISA



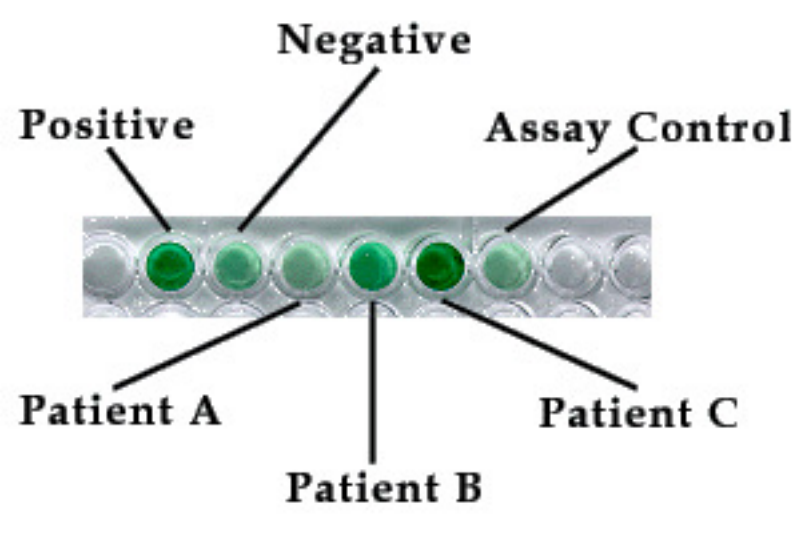
Antigen-coated well

Add specific antibody to be measured

Add enzyme-conjugated secondary antibody

Add substrate (S) and measure color

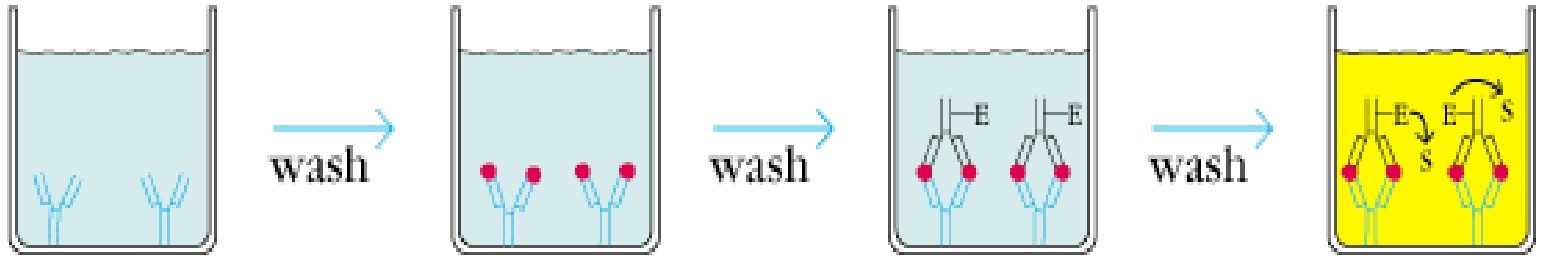
http://www.supercolostrum.com/colostrum/Information/information9.htm

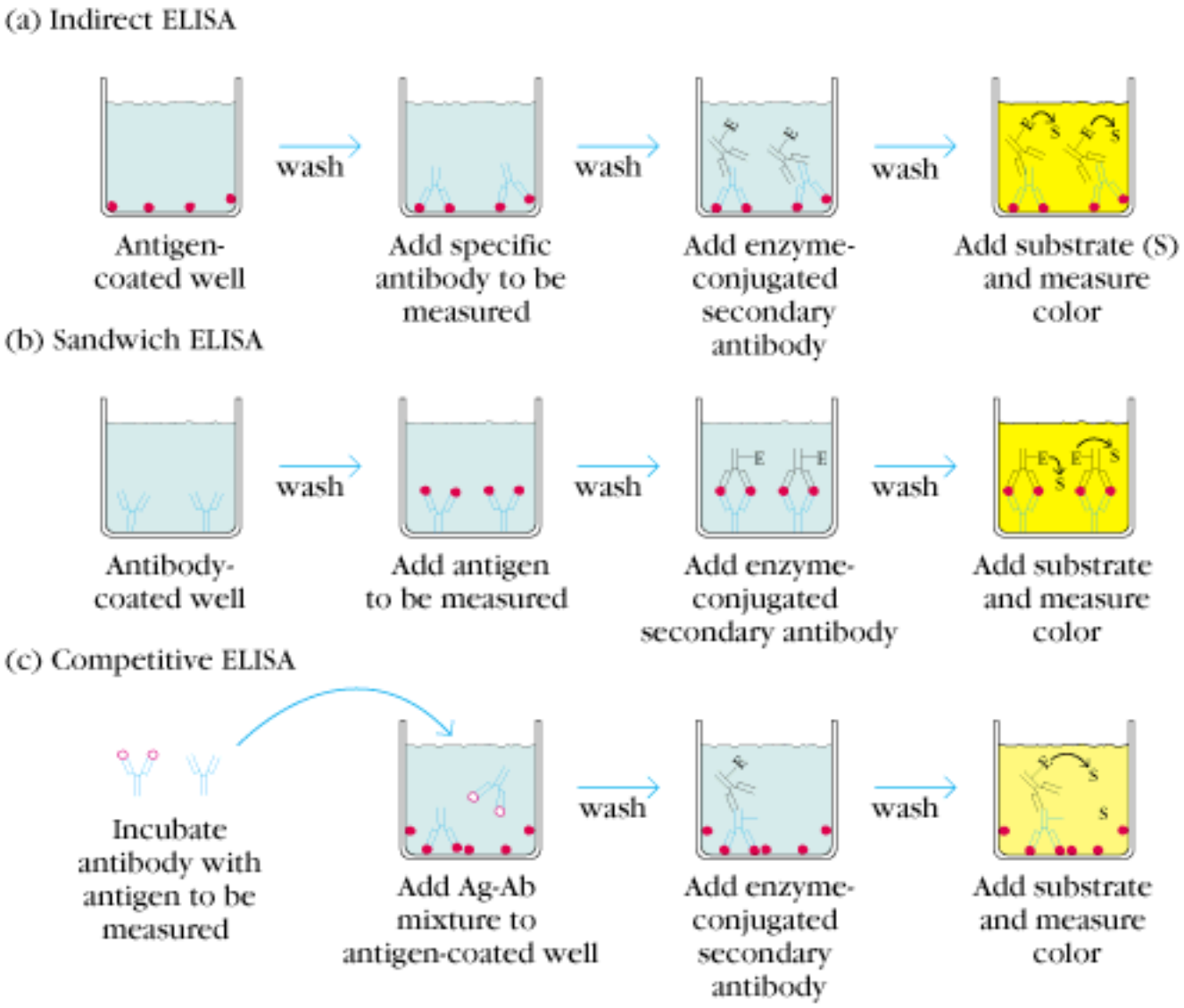


Sample	Absorbance
Positive control	1.689
Negative control	0.153
Assay control	0.123
Patient A	0.055
Patient B	0.412
Patient C	1.999

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

(b) Sandwich ELISA





Kuby Figure 6-11

Relative sensitivities:

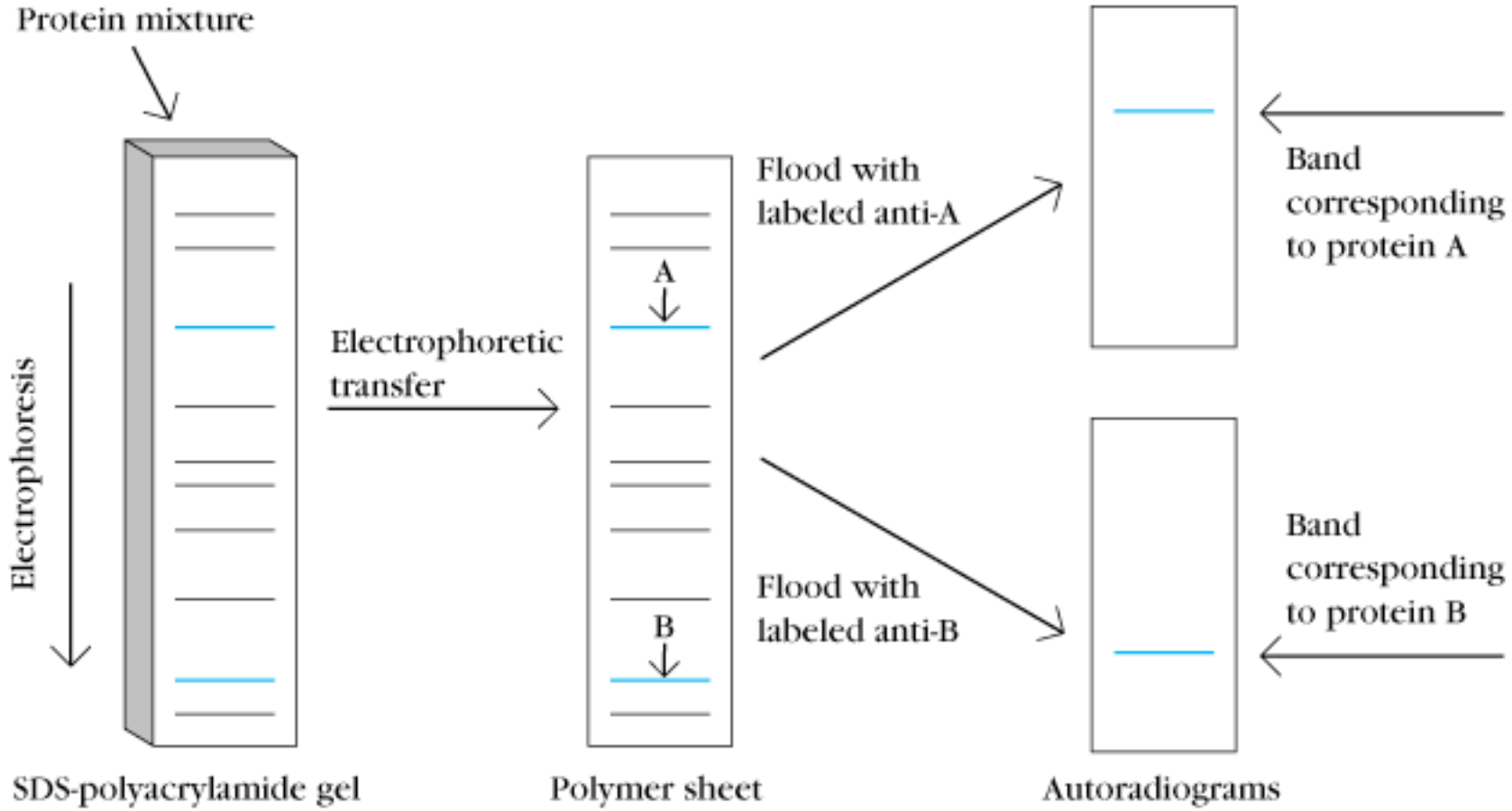
Precipitin reactions < Agglutination reactions < ELISA

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

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

Sheet is incubated with anti-Ag antibody

Band appears wherever the Ag is present.



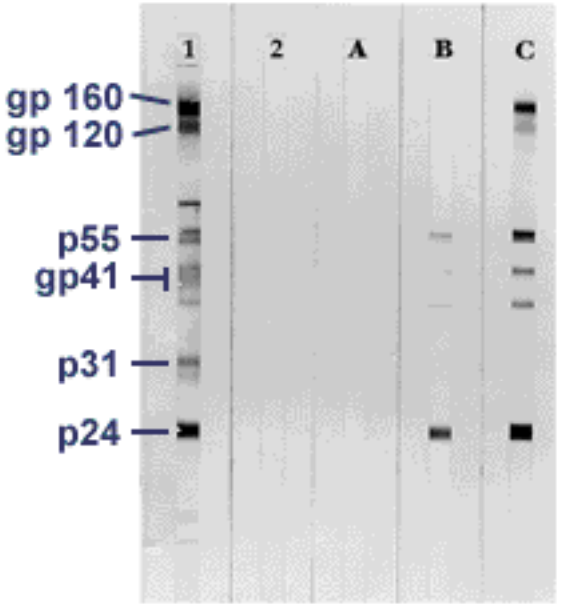
Kuby Figure 6-13

HIV Western Blot

No bands present……..Negative

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

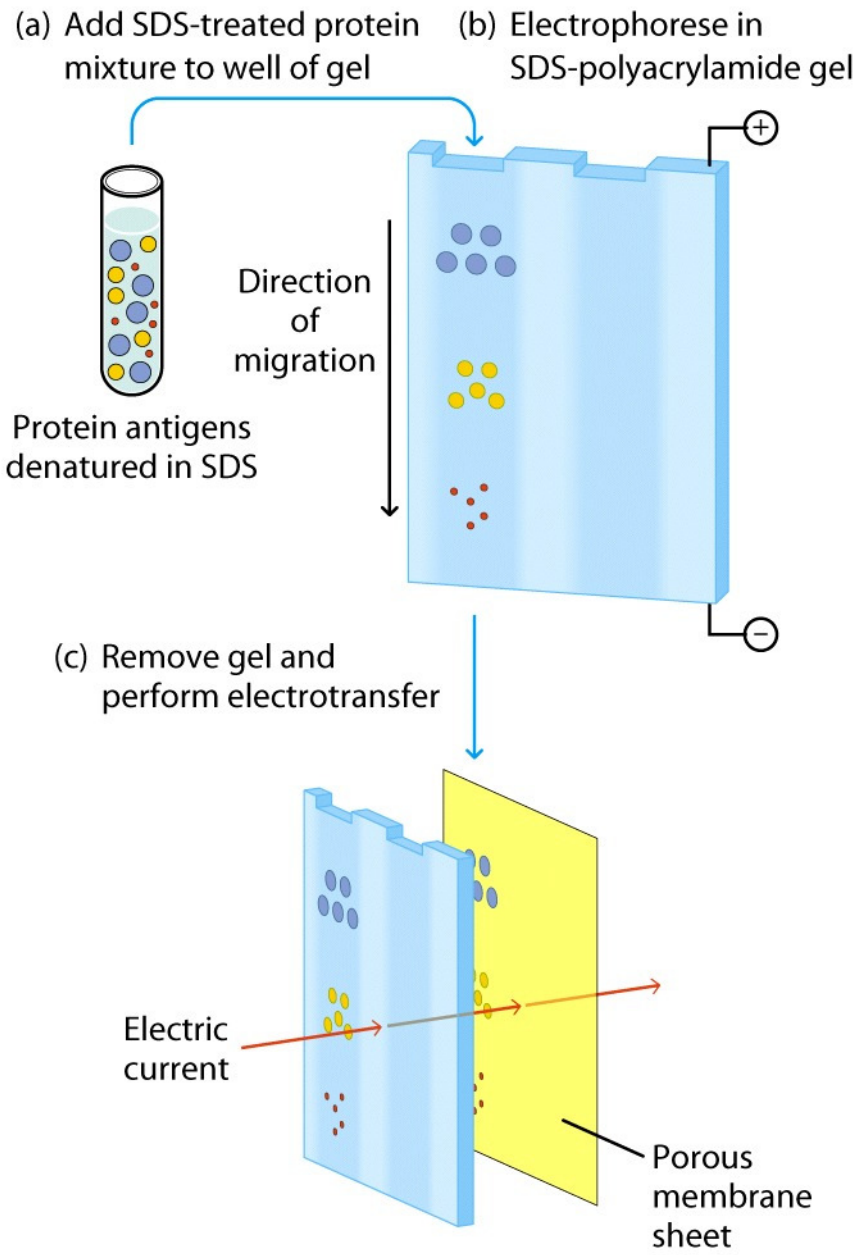
Bands present, but pattern does not
Meet criteria for positivity……..Indeterminate



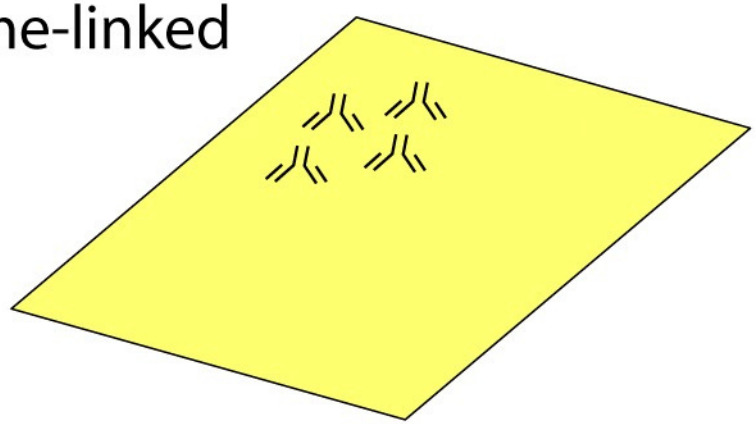
1.Lane 1, HIV+ serum (positive control)
2.Lane 2, HIV- serum (negative control)

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

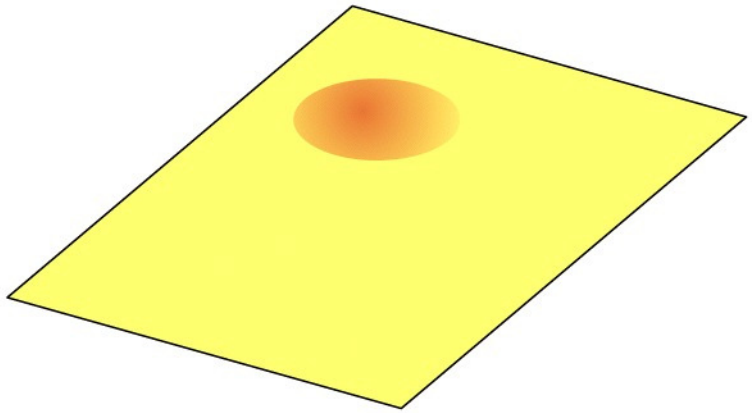
http://www.biology.arizona.edu/immunology/activities/western_blot/west2.html


(a) Add SDS-treated protein mixture to well of gel
(b) Electrophorese in SDS-polyacrylamide gel
Direction of migration
Protein antigens denatured in SDS
(c) Remove gel and perform electrotransfer
Electric current
Porous membrane sheet

(d) Bind antigen of interest with enzyme-linked antibodies

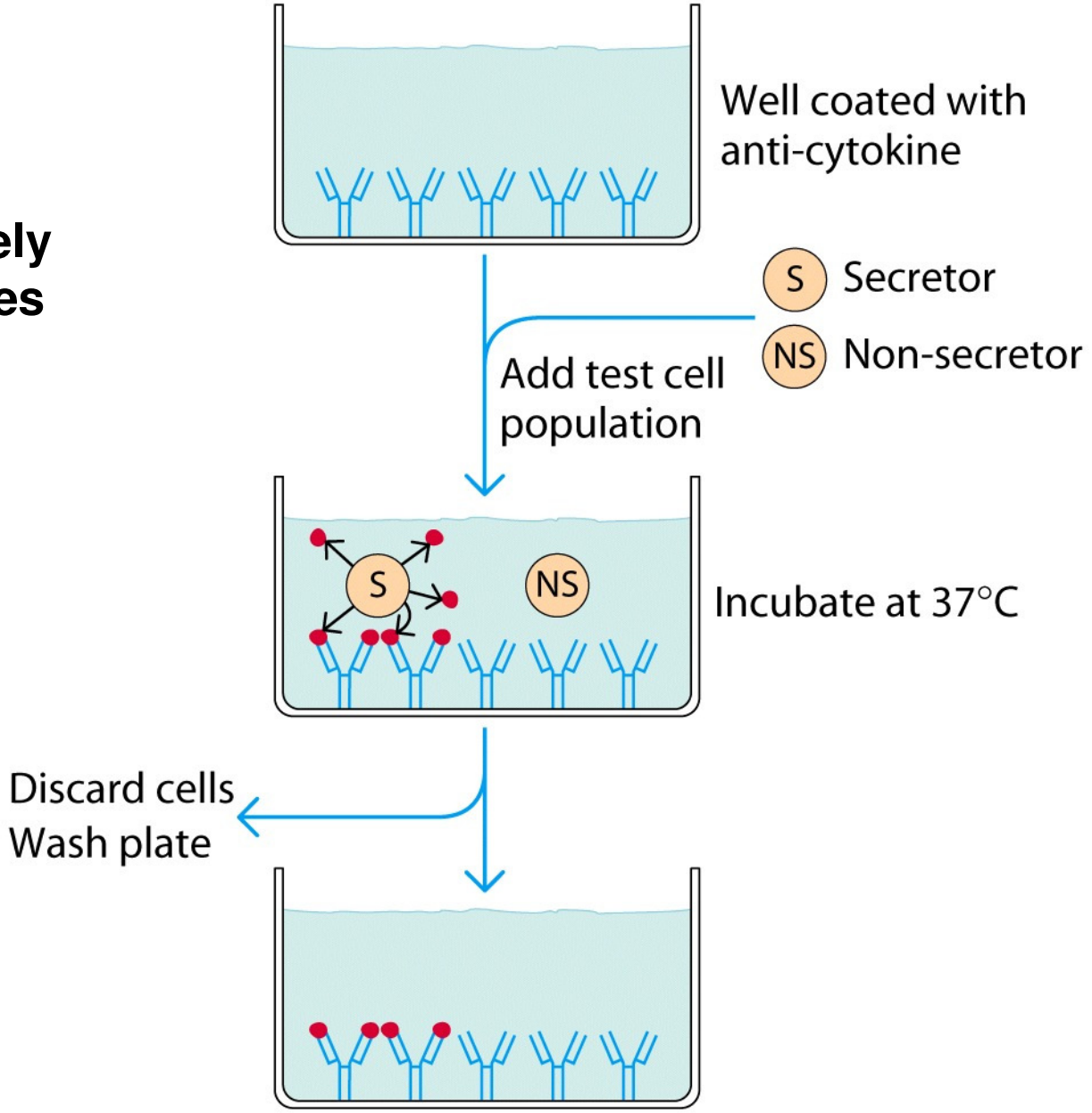


(e) Add substrate to activate color reaction



ELISPOT

1) **Detects cell actively secreting cytokines**
2) **Capture antibody**
3) **Activated cells**

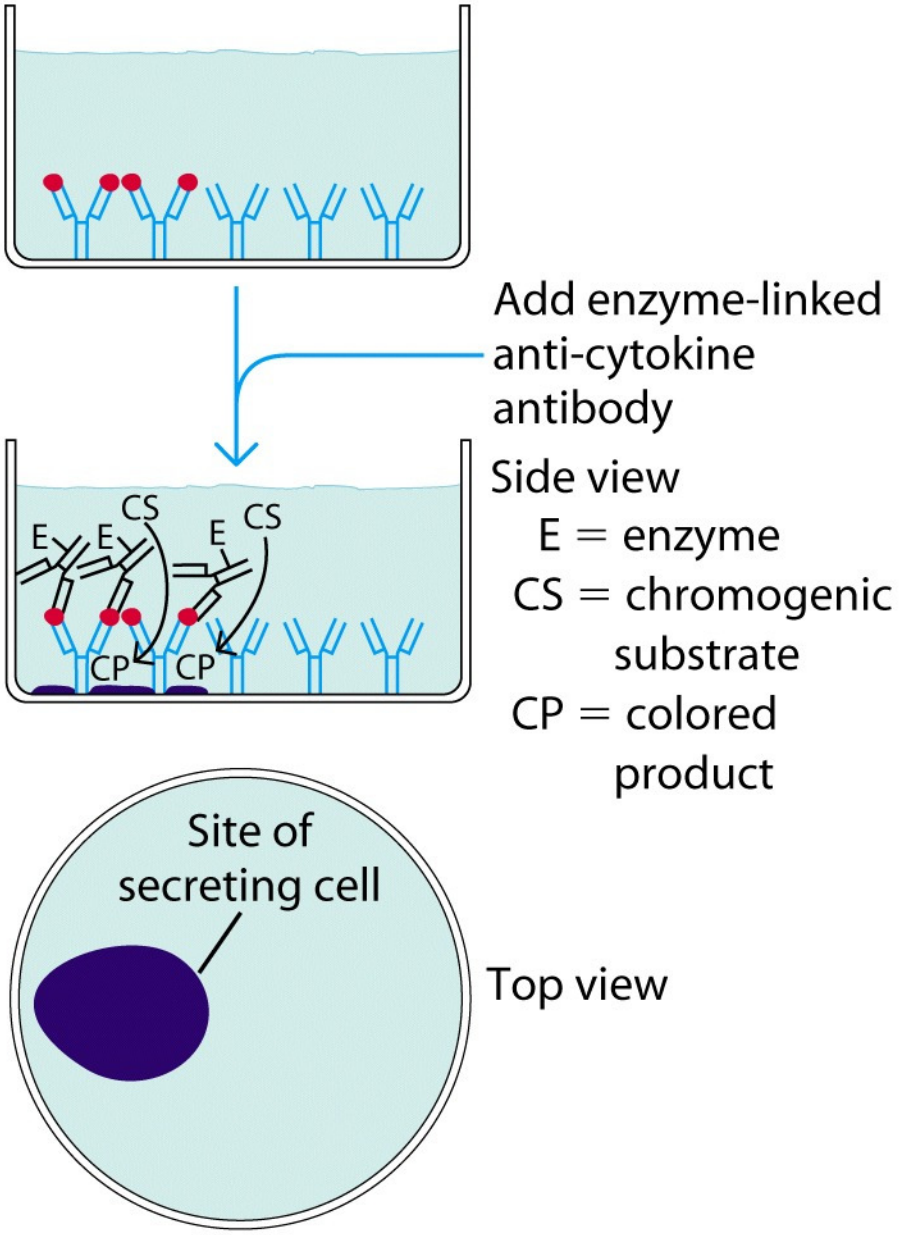


ELISPOT

4) Anti-cytokine antibody linked to reporter enzyme

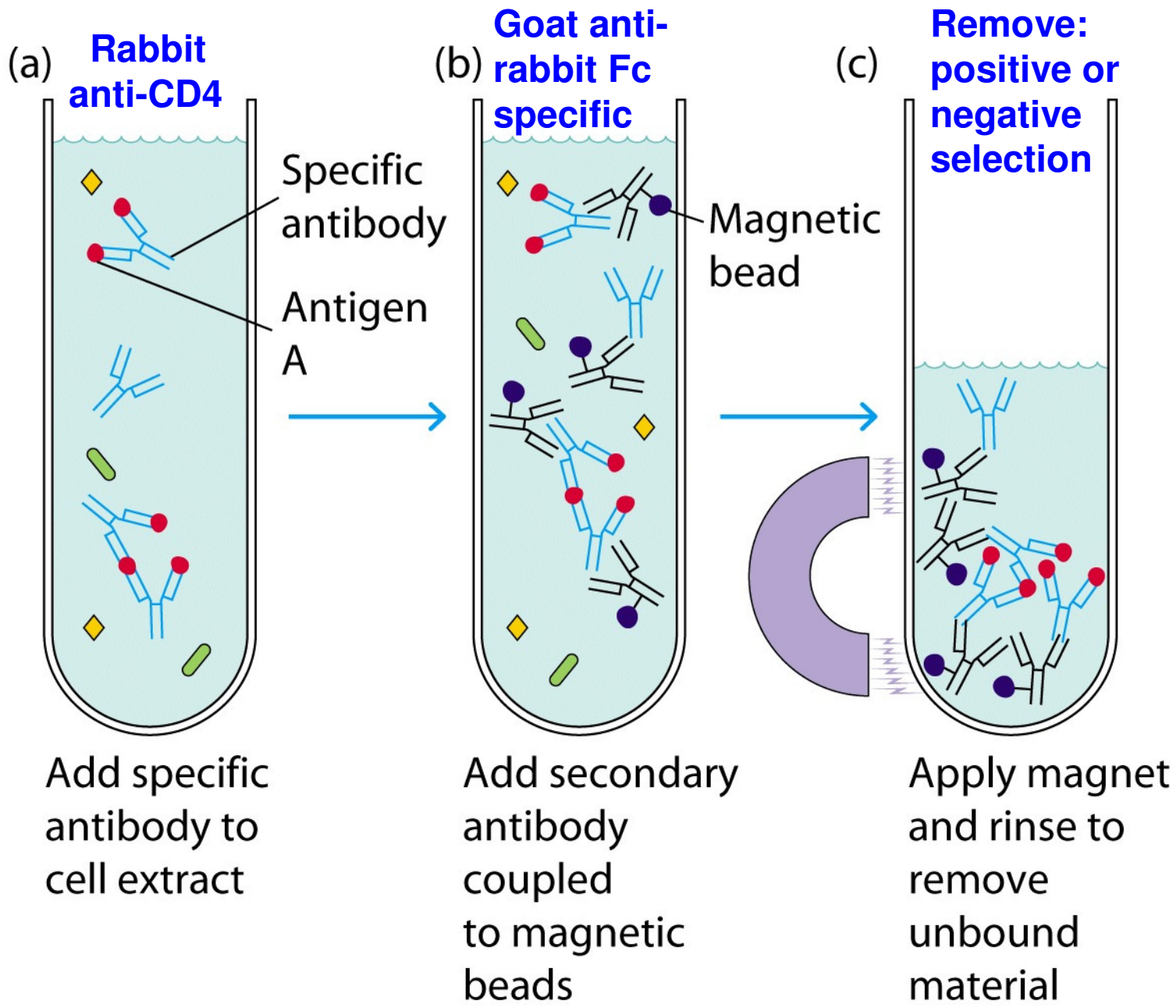
5) Substrate

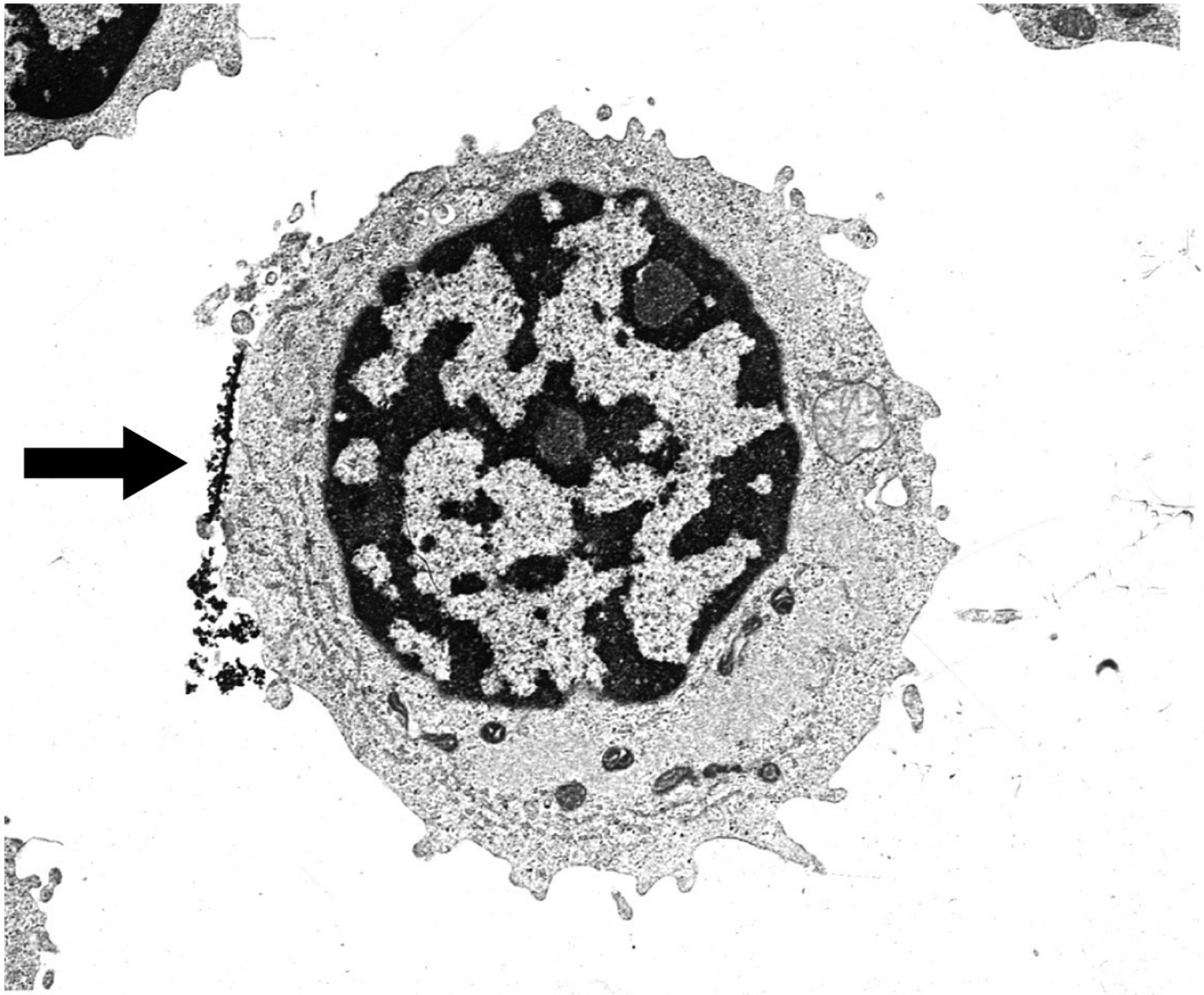
6) Quantify spots. Each spots represent the an actively secreting cell



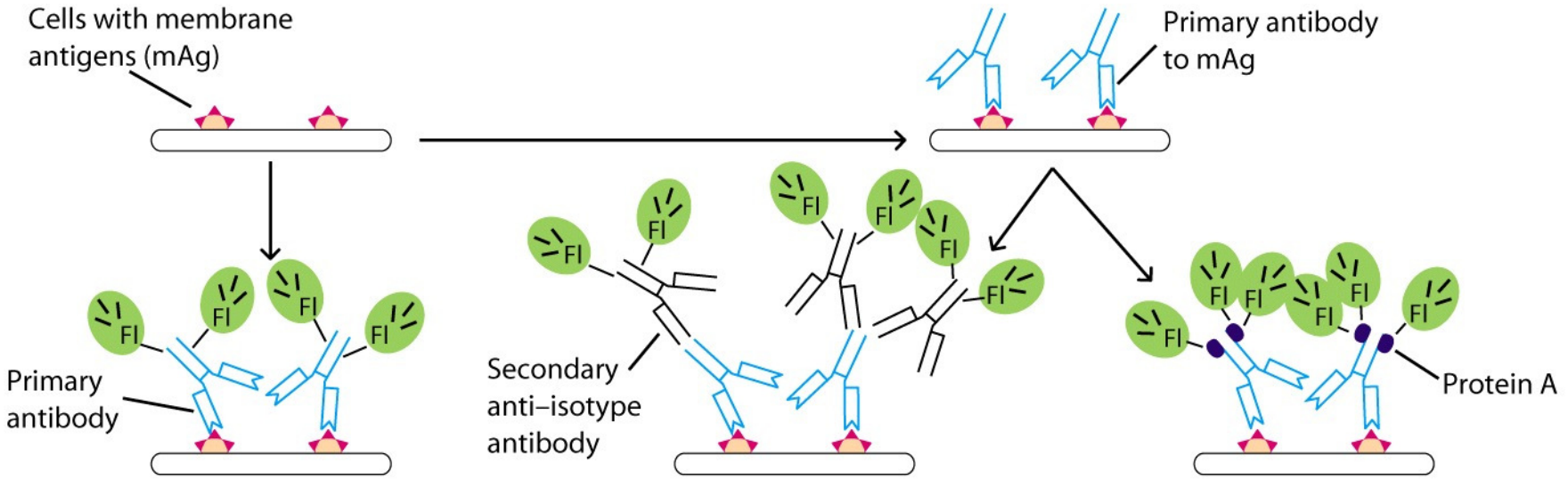
Immuno-magnetic separation (IMAS)

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


(a)
Rabbit anti-CD4
Specific antibody
Antigen A
Add specific antibody to cell extract
(b)
Goat anti-rabbit Fc specific
Magnetic bead
Add secondary antibody coupled to magnetic beads
(c)
Remove: positive or negative selection
Apply magnet and rinse to remove unbound material



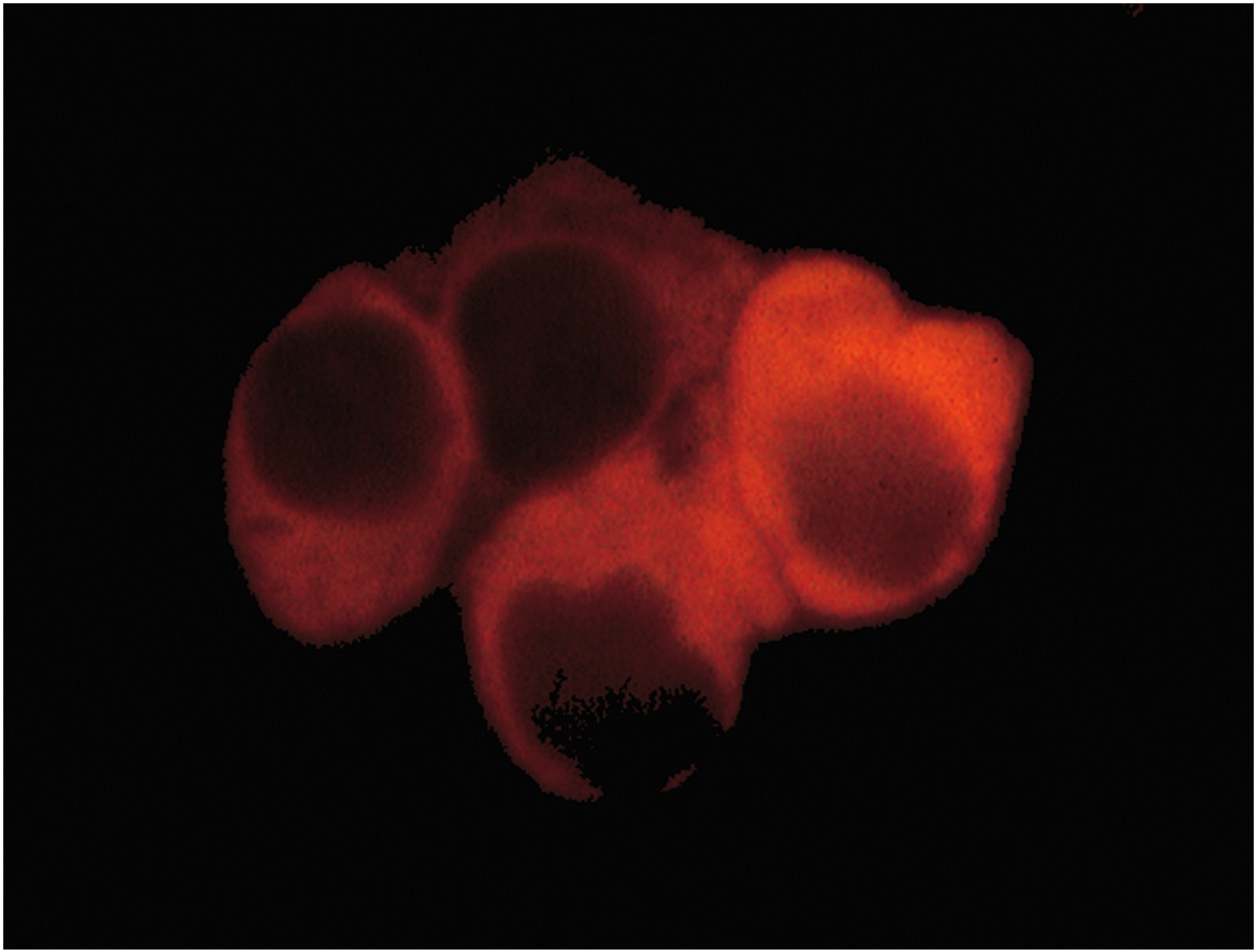
Immunofluorescence



1

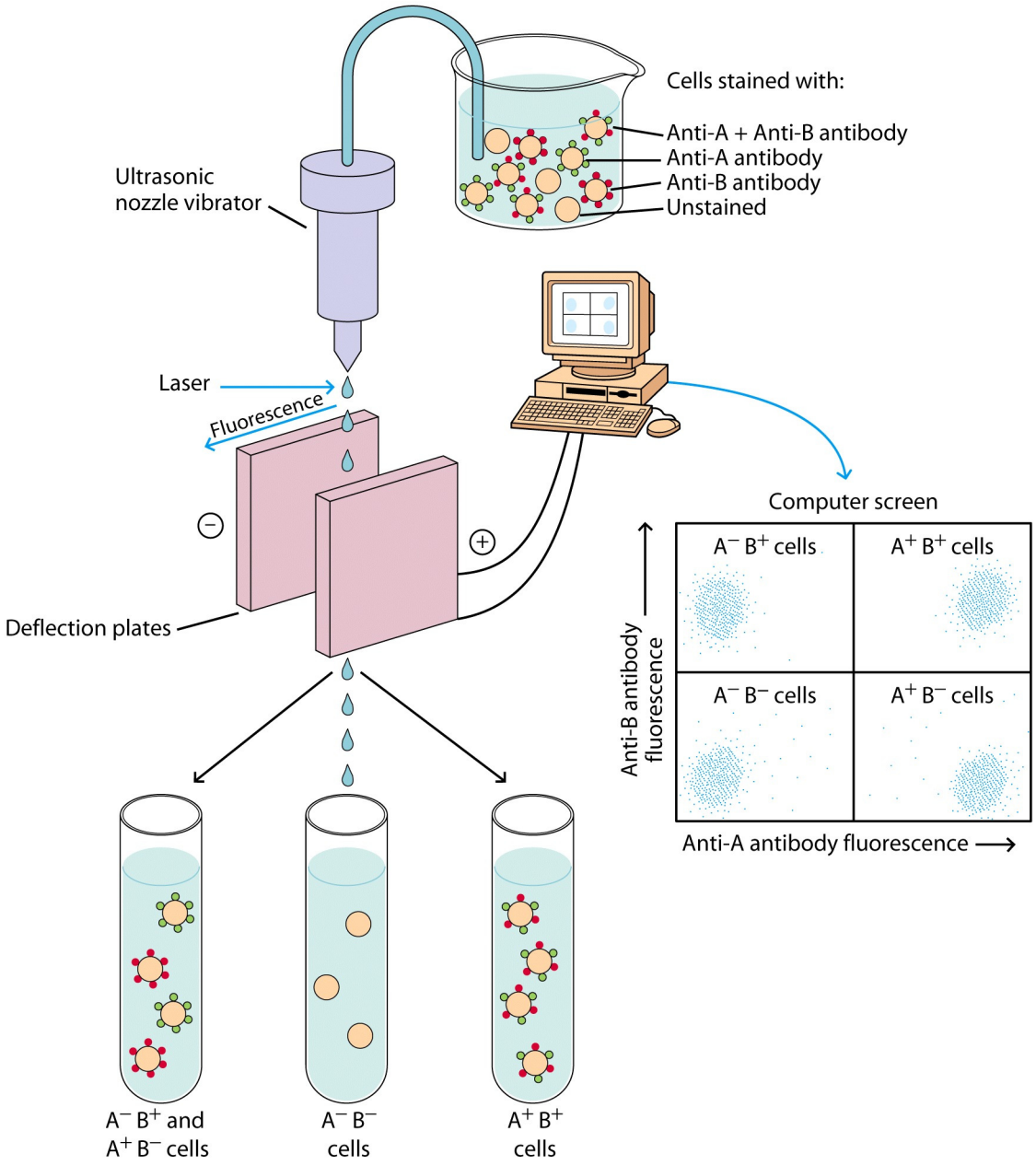
2

3

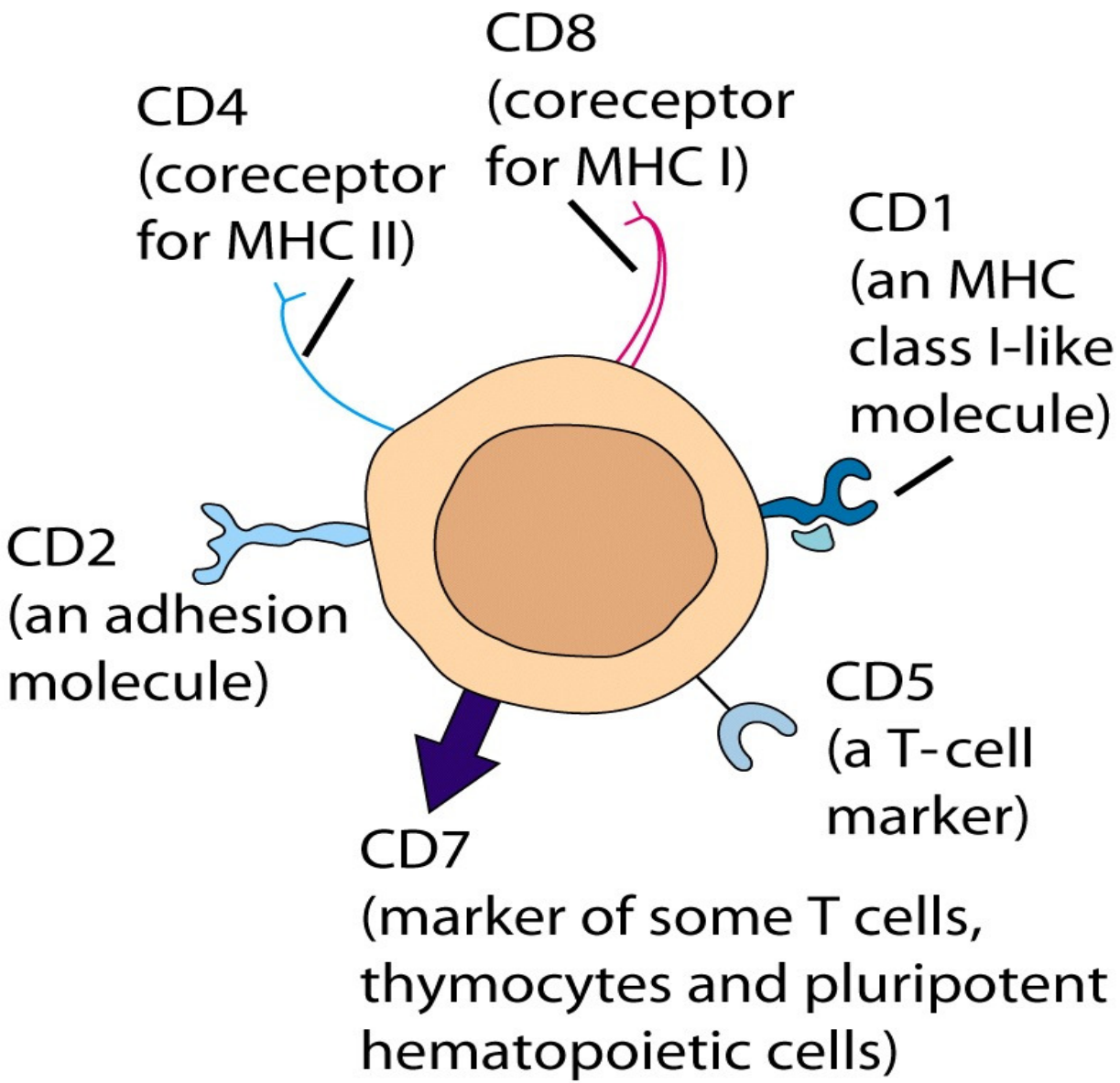


Fluorescence Acitvated Cell Sorter (FACS)

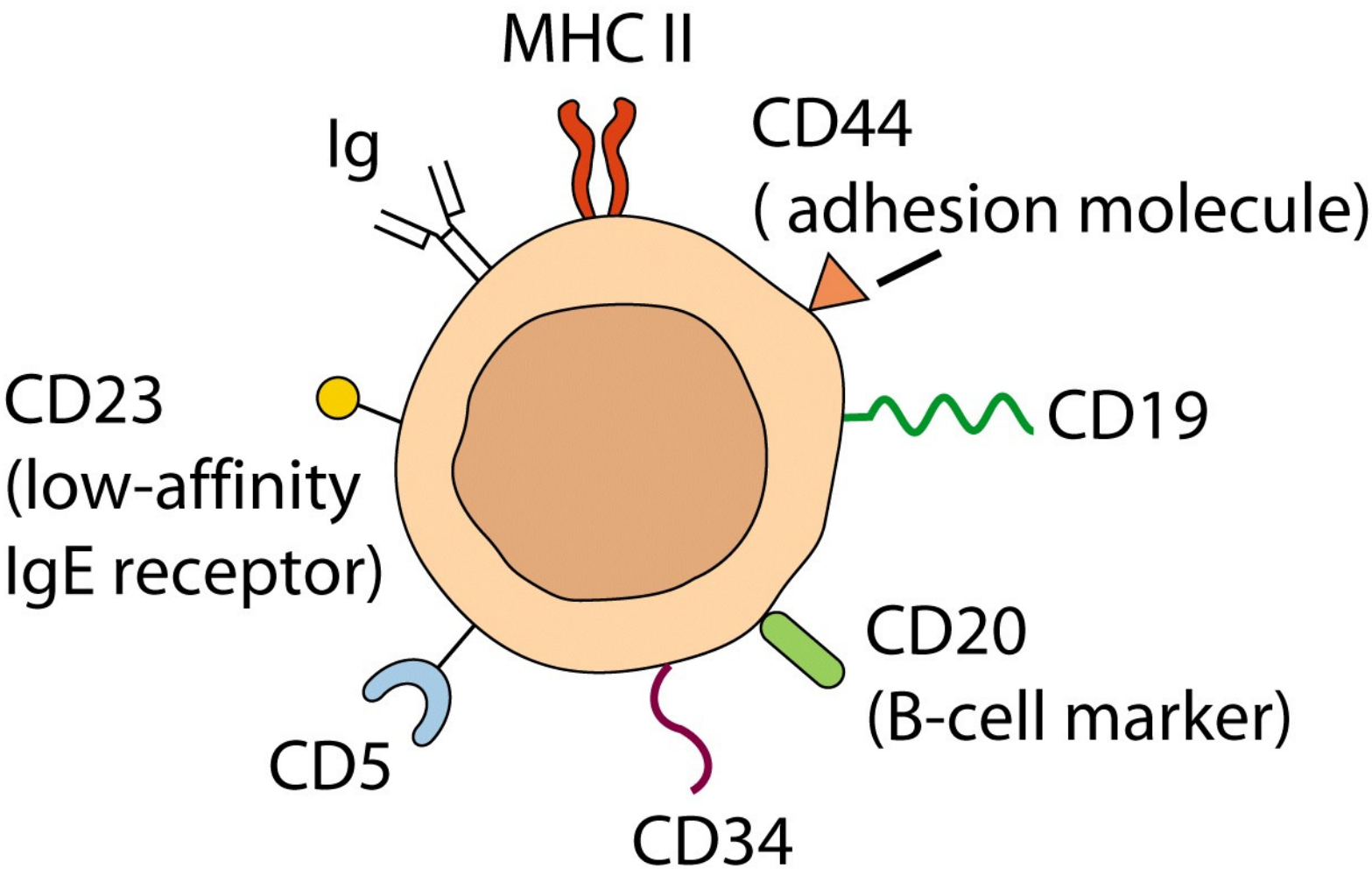
- Mixed cell population
- Requires two different fluorochromes
- Commonly used: FITC, PE, Texas Red, etc


Cells stained with:
Anti-A + Anti-B antibody
Anti-A antibody
Anti-B antibody
Unstained
Ultrasonic nozzle vibrator
Laser
Fluorescence
Deflection plates
Computer screen
A⁻ B⁺ cells
A⁺ B⁺ cells
A⁻ B⁻ cells
A⁺ B⁻ cells
Anti-B antibody fluorescence
Anti-A antibody fluorescence
A⁻ B⁺ and A⁺ B⁻ cells
A⁻ B⁻ cells
A⁺ B⁺ cells

ALL of the T lineage



A B-lineage CLL



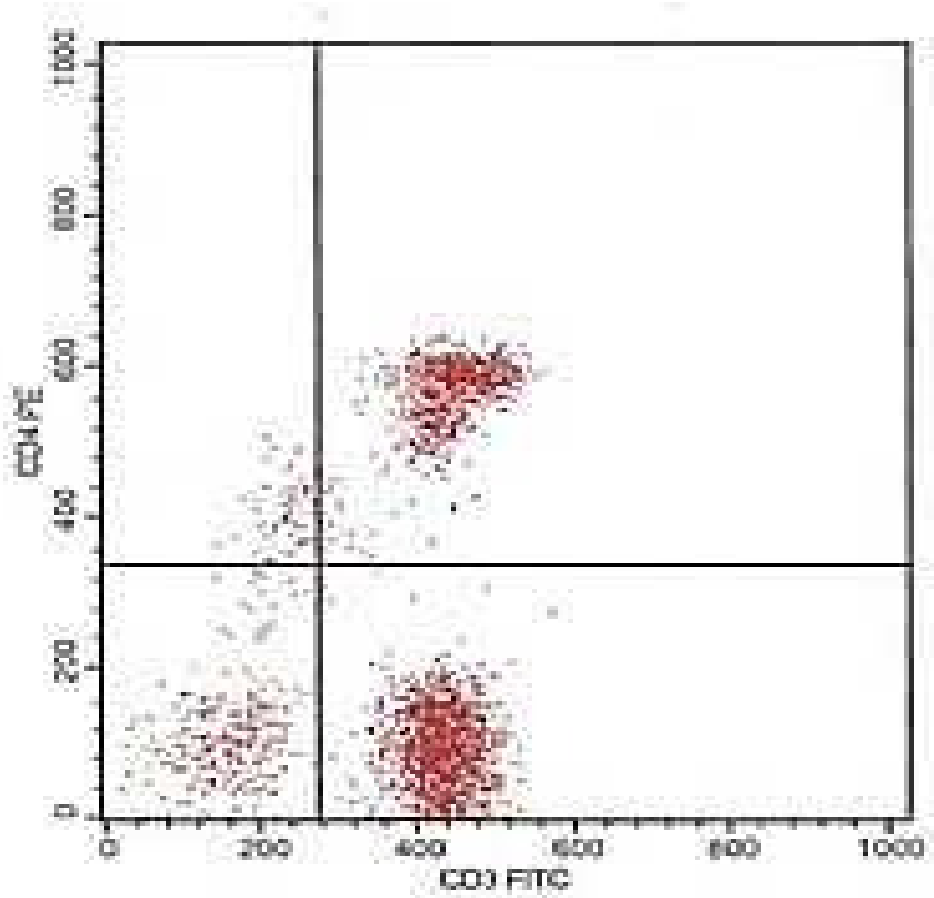
FACS Analysis

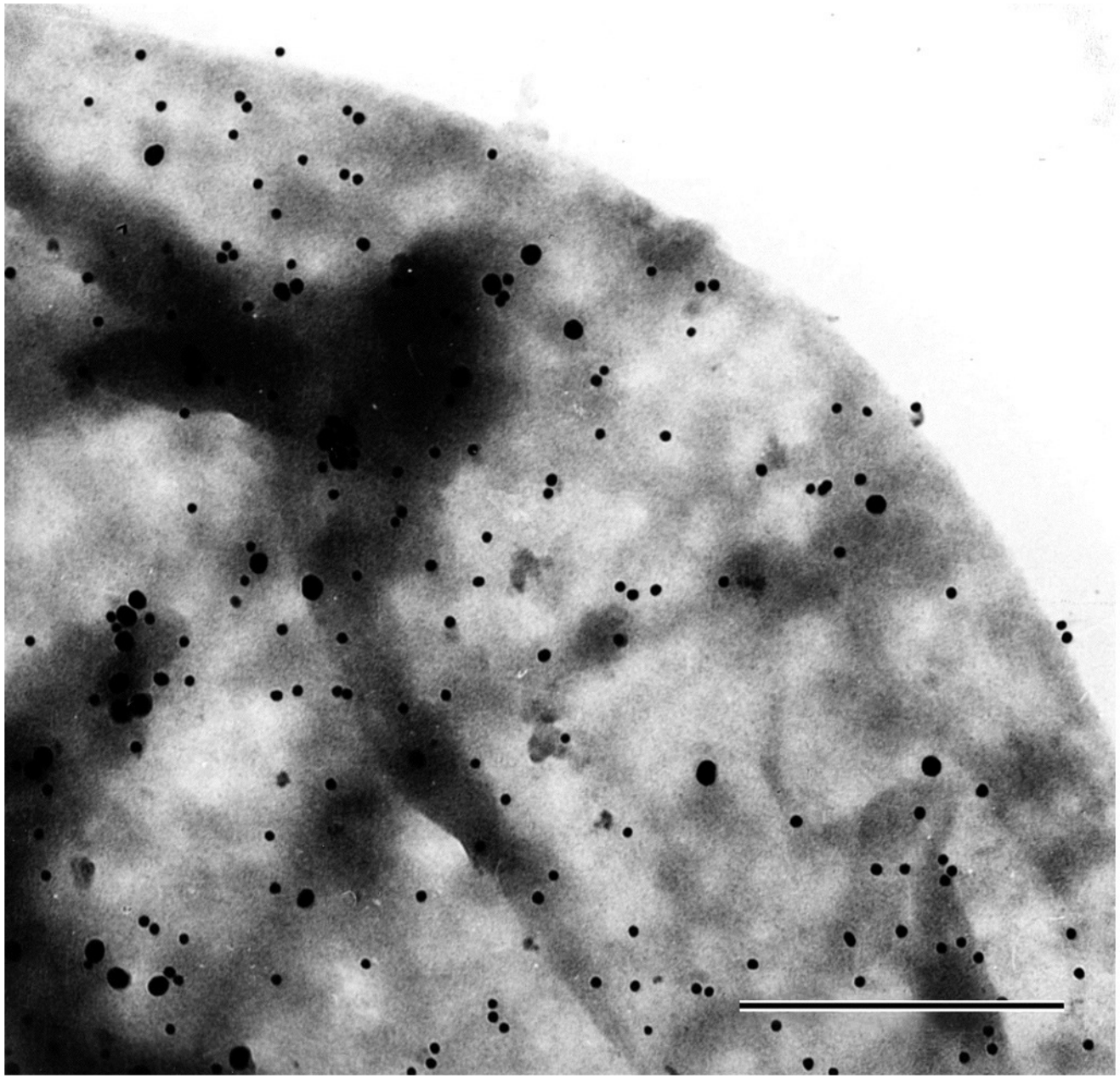
Reagents:

CD4-PE Phycoerythrin (PE)

VS

CD3 Fuorecein (FITC)





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		