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# Antibody-Based Clinical Tests

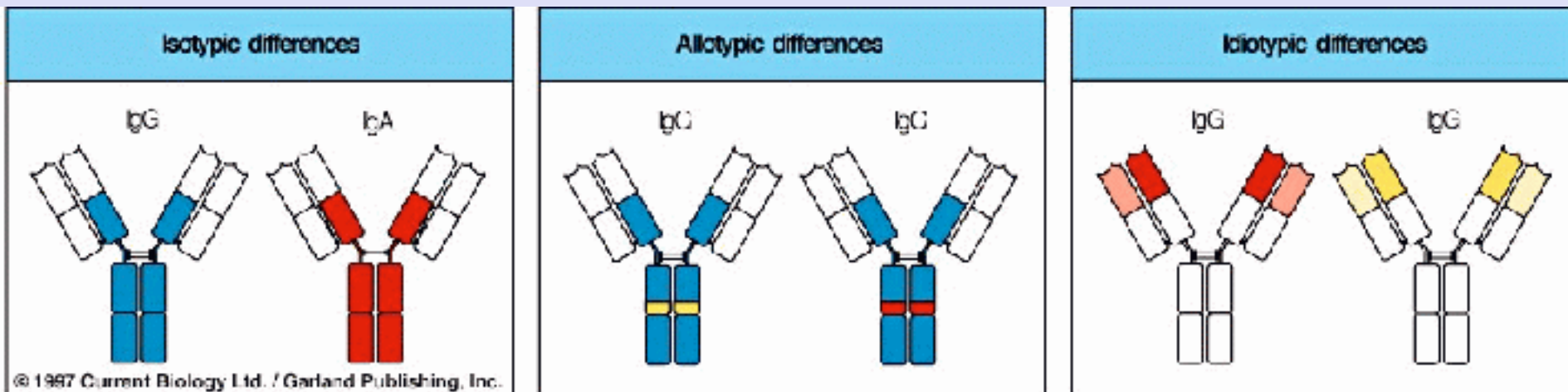
## M1 – Immunology Sequence

Winter 2009



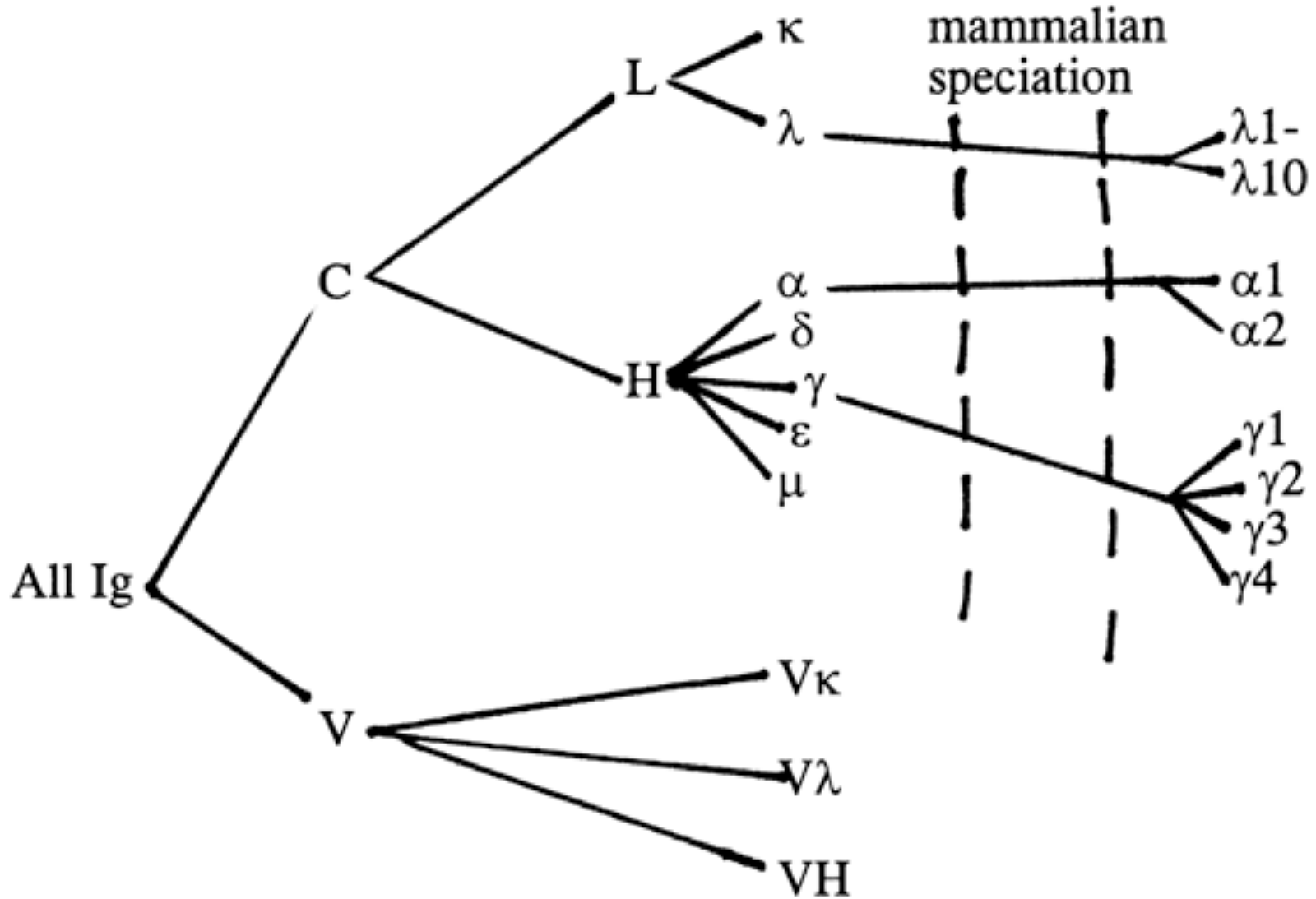
1. Antigenic determinants used to describe parts of immunoglobulins: isotypes and idiotypes.
2. Immunological tests:  
immunoelectrophoresis,  
agglutination,  
radioimmunoassay (RIA),  
enzyme-linked immunosorbent assay (ELISA)
3. Why is production of monoclonal antibodies by hybridomas so useful?

Isotype: An antigenic determinant on an immunoglobulin that is expressed by all members of a species.



If one injects a rabbit with a human immunoglobulin with a  $\gamma 1$  heavy chain and a  $\kappa$  light chain, the determinants on the constant region of the human antibody will be recognized as foreign, and the rabbit will make antibodies against them. Those rabbit antibodies recognize isotypic determinants, and will react with immunoglobulins in the serum of virtually all humans, since all humans express  $\gamma 1$  heavy chains and  $\kappa$  light chains.

Each isotype is encoded by one gene: mu, kappa, lambda, gamma-1, etc.



Idiotypes. Serological term--antigenic determinant unique to a given immunoglobulin. Used to describe the complementarity determining regions unique to a given antibody.

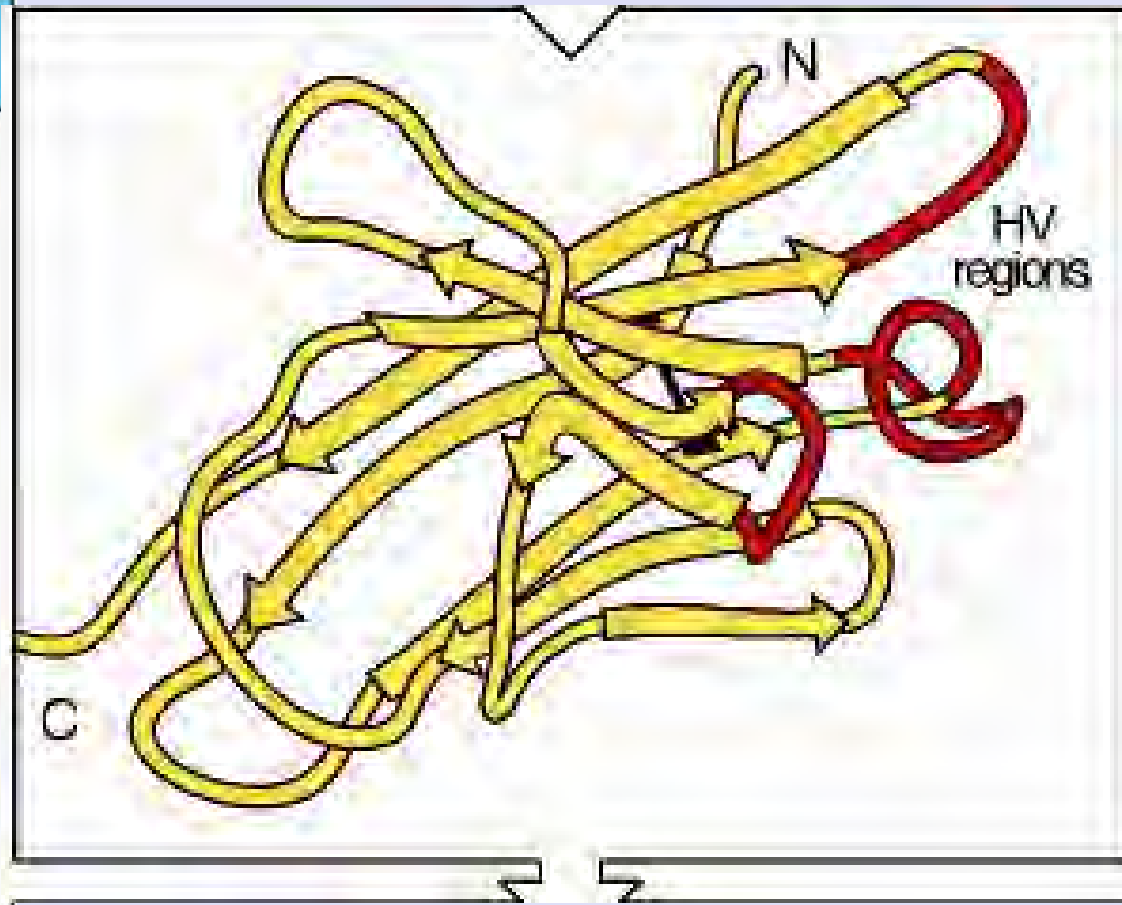
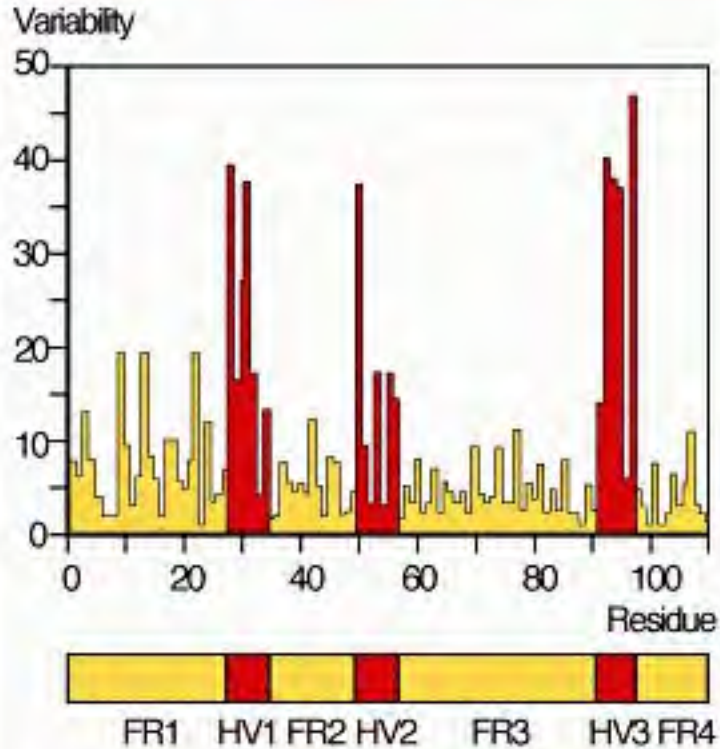
Not a constant region determinant

Not a variable region framework determinant

The antibodies used to detect idiotypes almost always bind to hypervariable regions.



## Light-chain V region



© PD-INEL Janeway. Immunobiology: The Immune System in Health and Disease. Current Biology Ltd./Garland Publishing, Inc. 1997

Idiotype is a useful term for: the unique shape of the epitope combining site made by the combination of the VH and VL hypervariable regions.

Many clinical laboratory tests use antibodies to detect various proteins (for example, insulin in serum). In tests for presence of an infection, or tests of immune status, antigens are often used to detect antibody expression in serum.

# Terms for antibody:antigen interactions:

Affinity is the dissociation constant for the interaction of a single epitope with a single Fab.

$$\text{Dissociation constant} = \frac{[\text{antigen}] [\text{antibody}]}{[\text{antigen:antibody complex}]} = 10^{-6} \text{ to } 10^{-10}$$

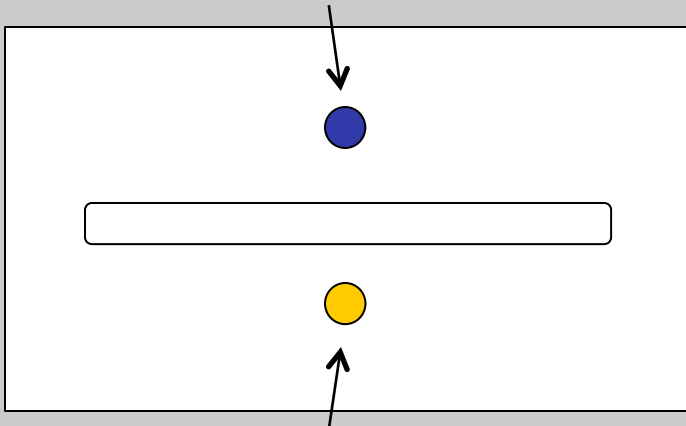
Avidity is used to describe the interaction of multivalent antigens with multivalent antibodies.

# Immuno-electrophoresis

Serum proteins are fractionated in an electrical field. Separation is by net charge.

Serum samples are added to immunoelectrophoresis plate

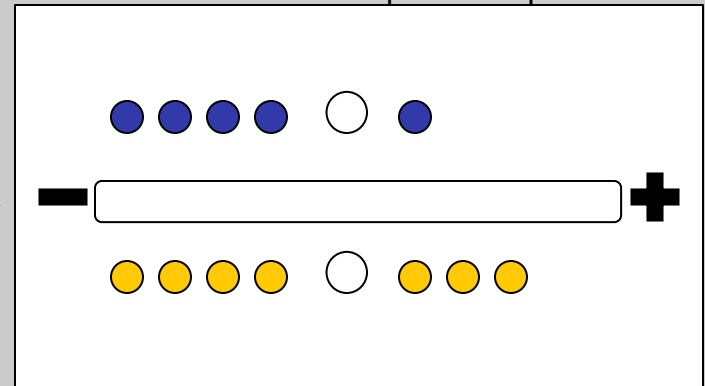
Serum from patient with recurrent infection



Serum from normal individual

Serum components are separated by electrophoresis

Albumin      Globulins  
                  α                    β                    γ



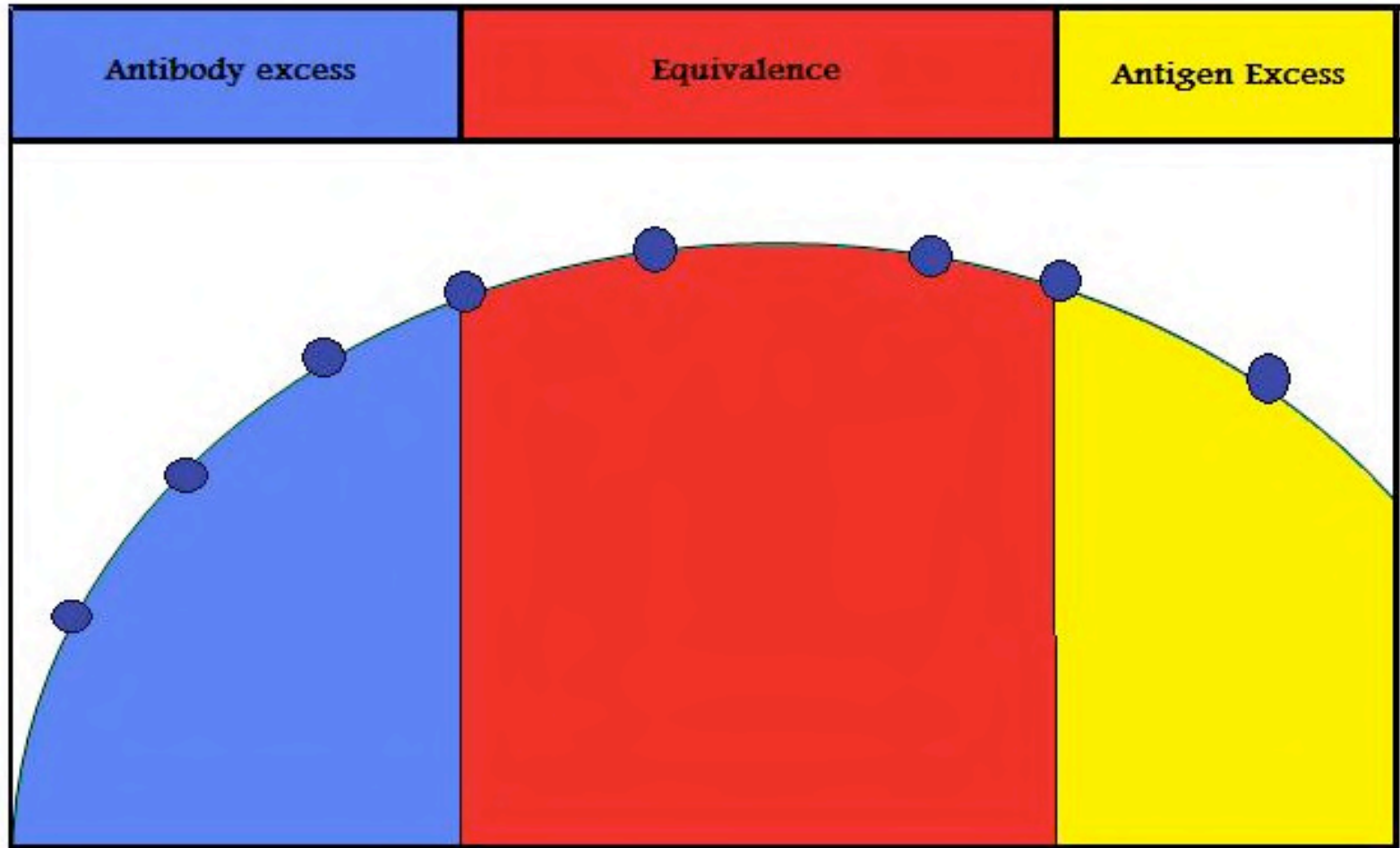
# Immuno-electrophoresis

Next, specific isotypes are detected by diffusion of appropriate rabbit anti-human antisera from a trough toward the lane of the fractionated serum proteins.

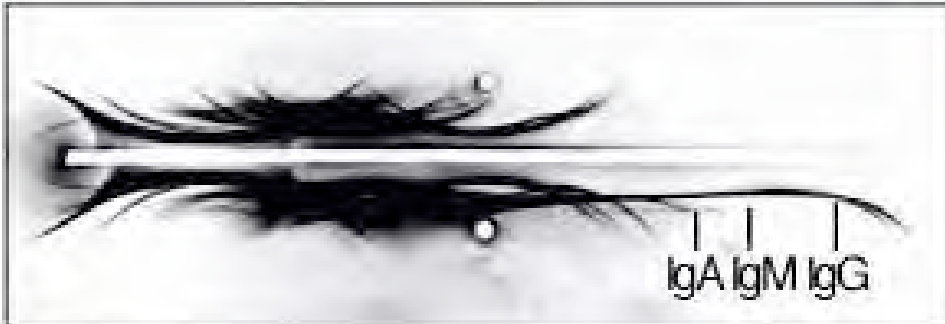
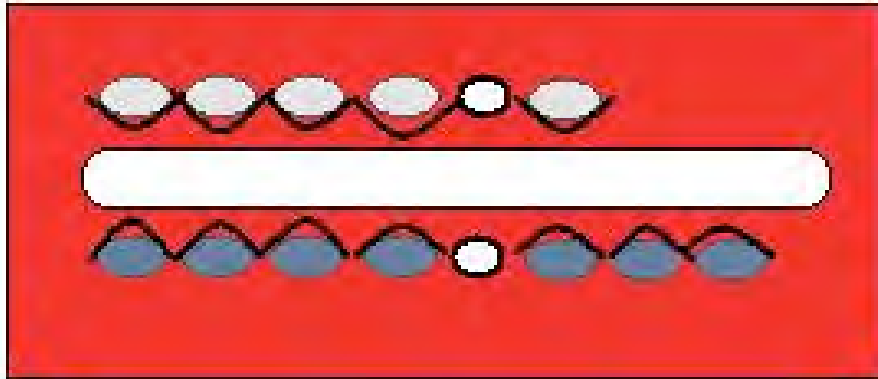
Antibodies in the human serum are detected as arcs of antibody-antigen precipitation, which occurs at the position where antibody and antigen concentration are about equal.

Since immunoglobulins have many VH and VL regions, they have a large variety of net charges, thus immunoglobulins migrate as a broad peak of reactivity.

Precipitation reactions require an optimal concentration of both antibody and antigen.



Rabbit anti-human serum is added to the central trough and diffuses into the plate, forming precipitin lines

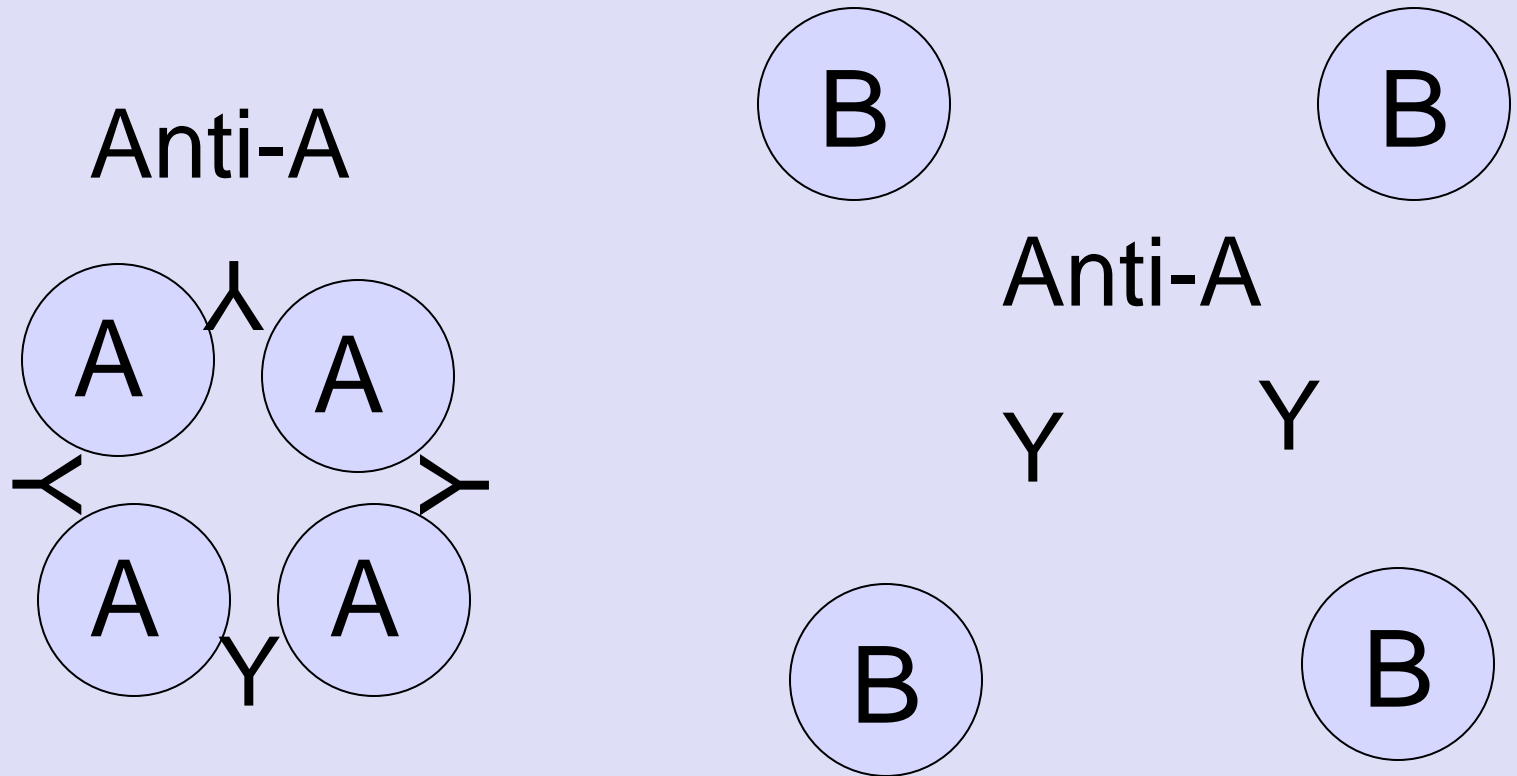


Agammaglobulinemia

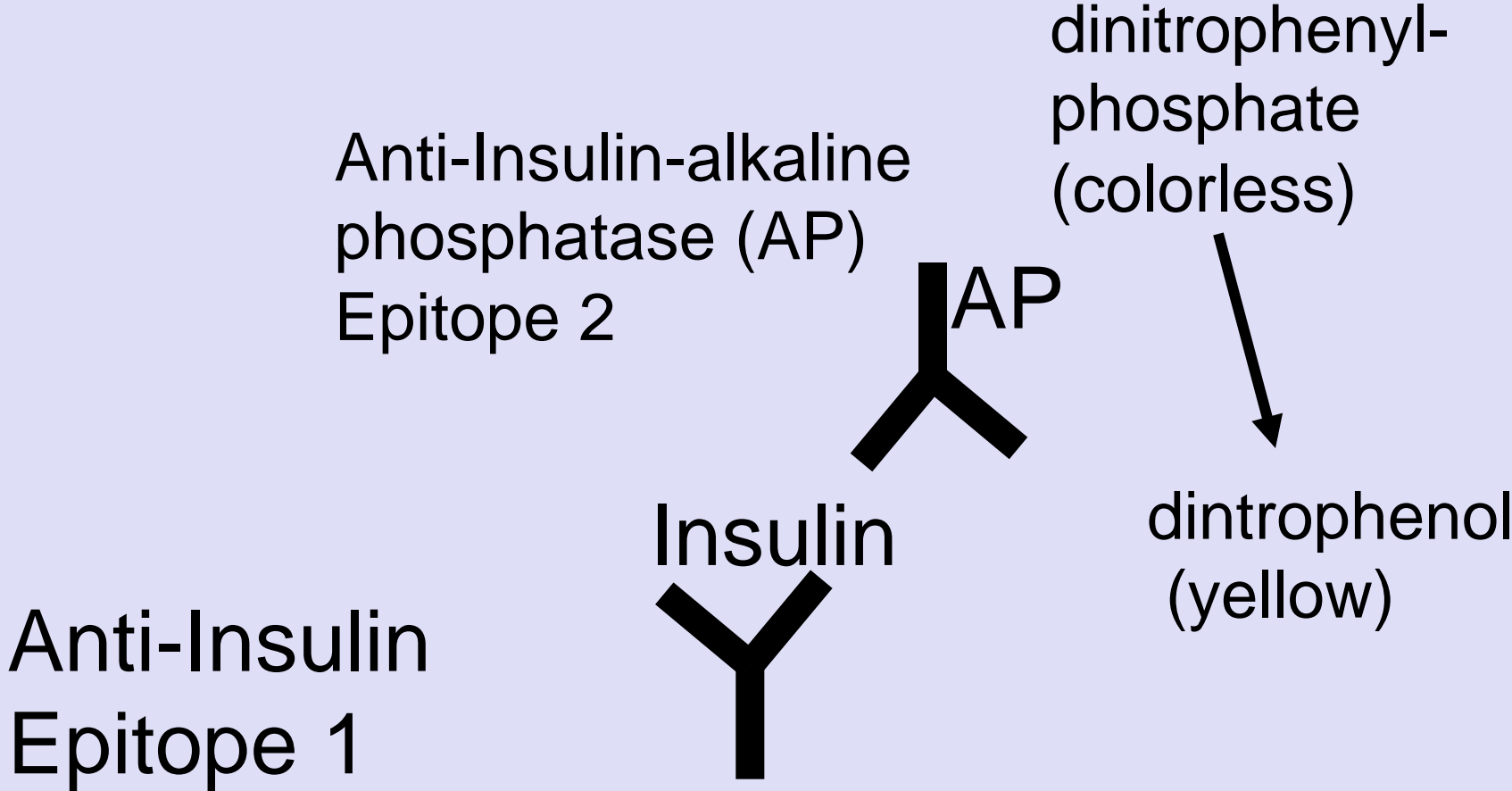
Normal

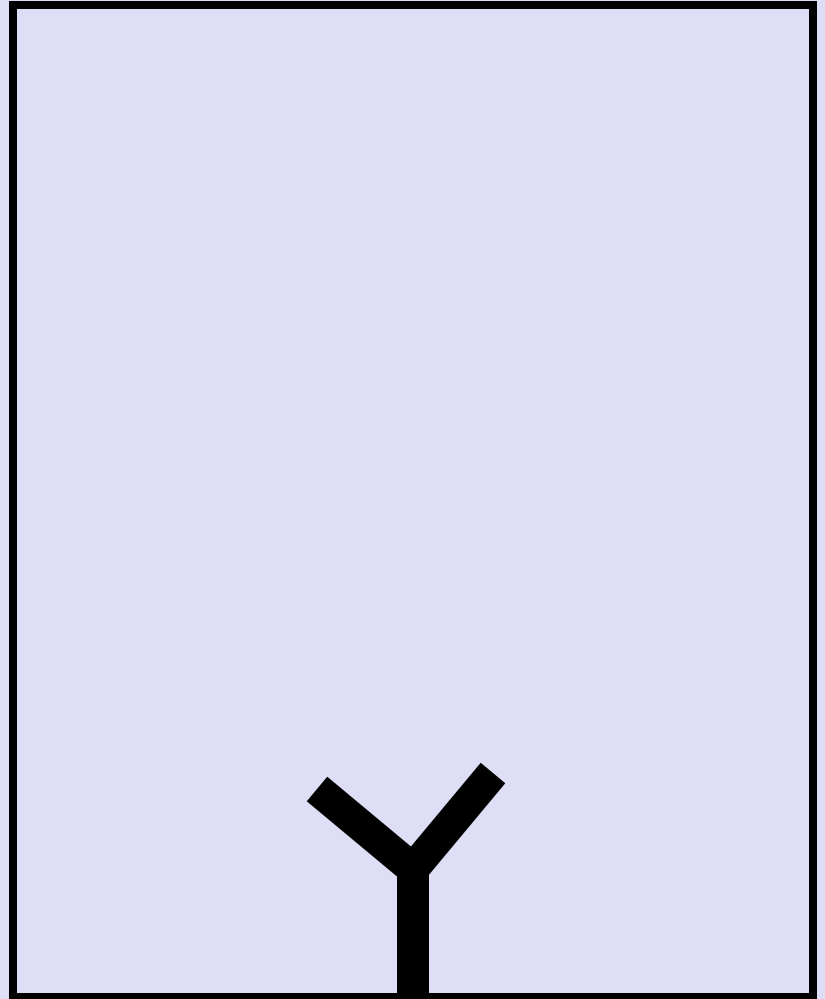
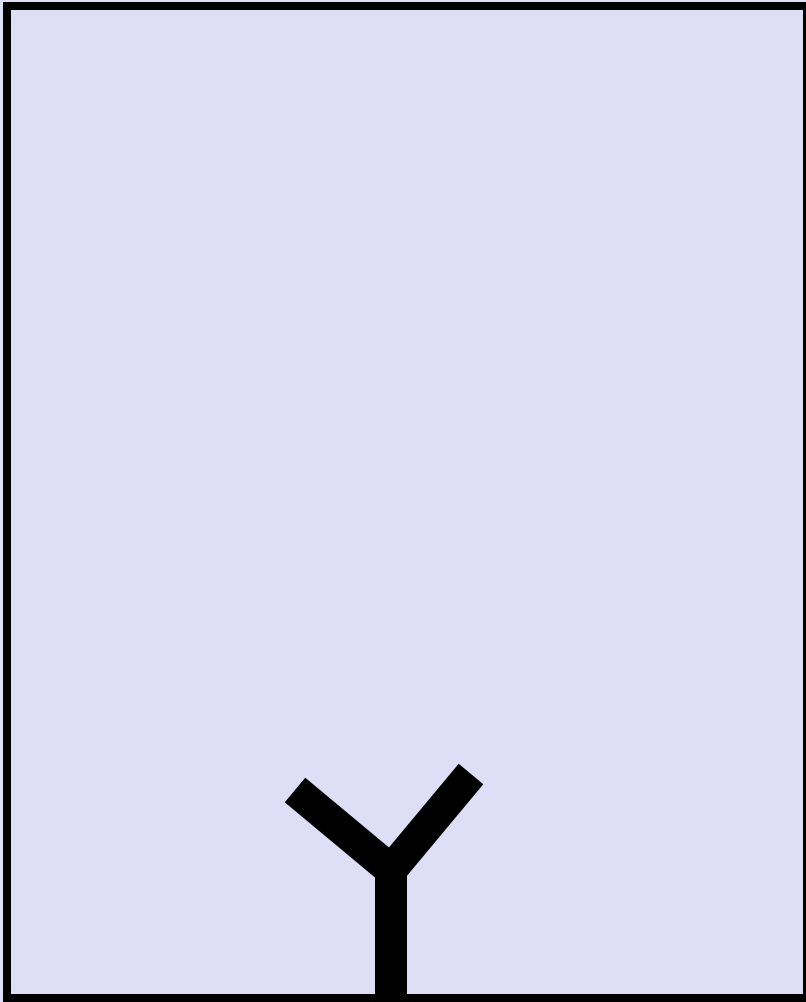


# Agglutination



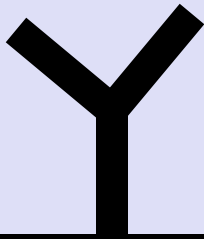
# Enzyme-linked Immunosorbent Assay (ELISA)





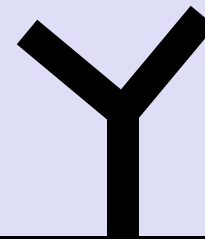
Anti-Insulin

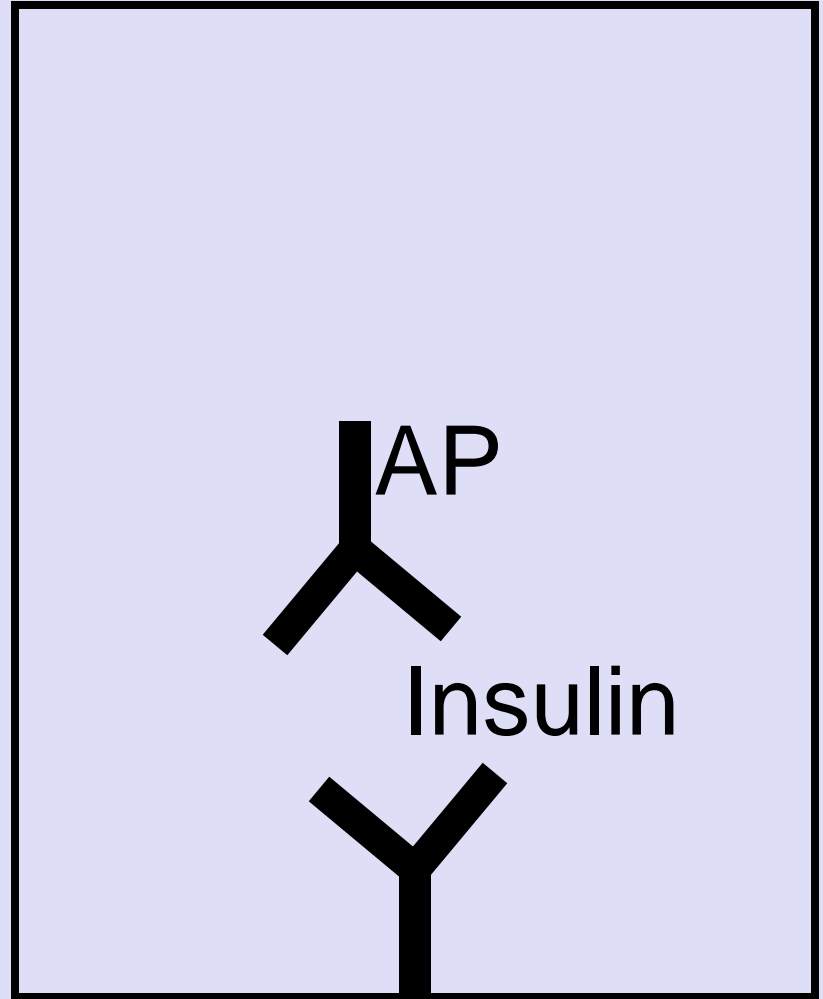
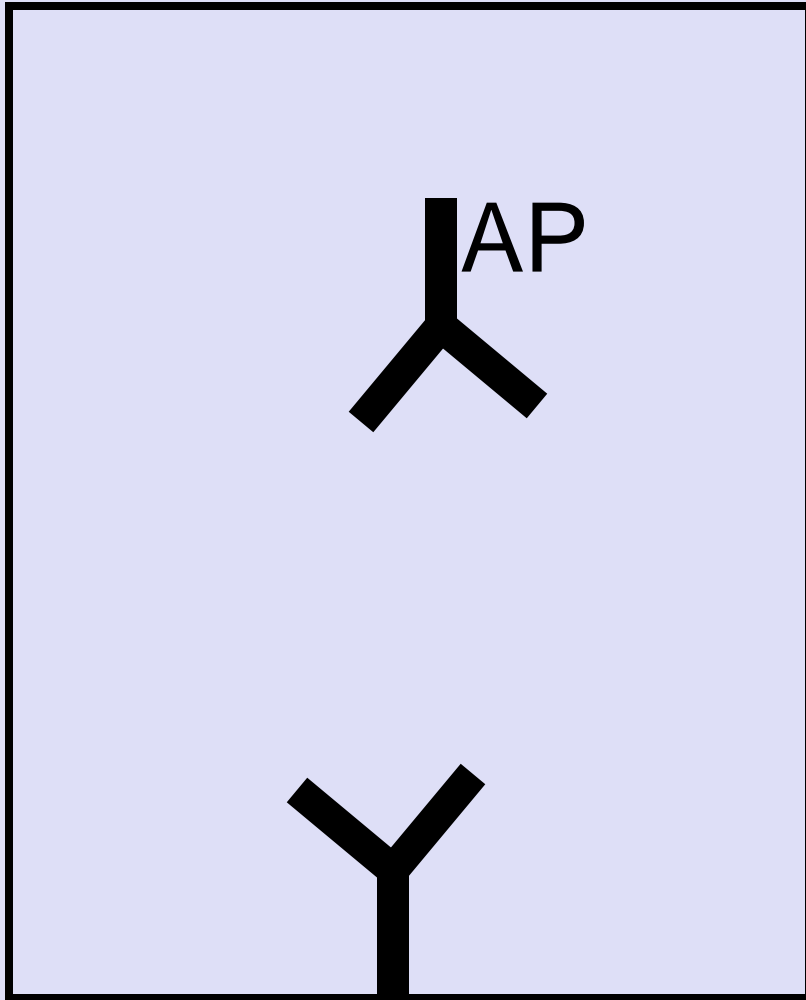
Serum lacking  
(or low in) insulin



Normal serum

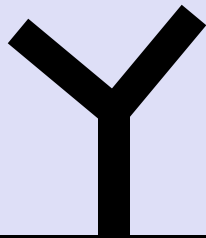
Insulin



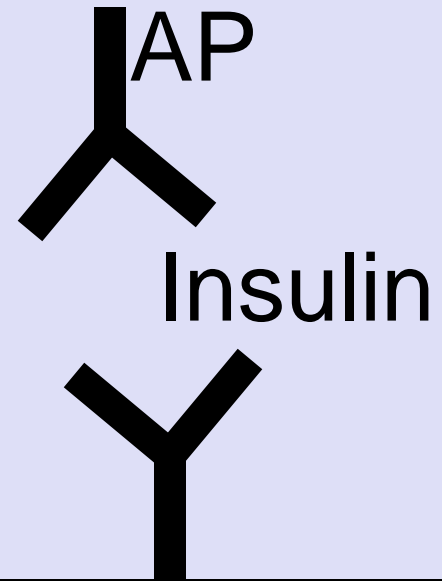


Anti-Insulin conjugated to alkaline phosphatase (AP)

dinitrophenyl-  
phosphate

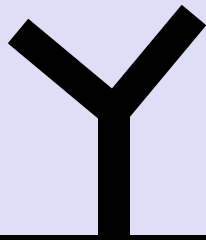


dinitrophenyl-  
phosphate

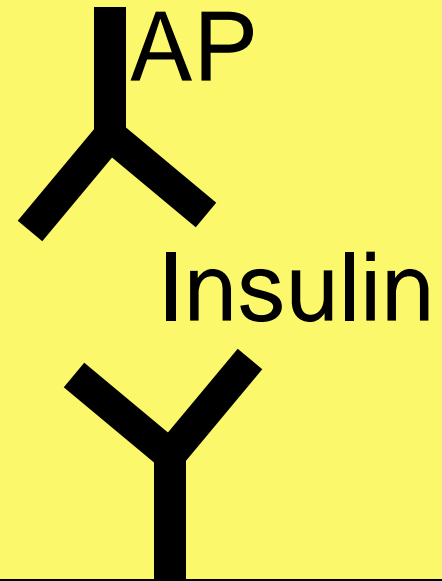


Substrate that turns yellow when cleaved  
by alkaline phosphatase (AP)

dinitrophenyl-  
phosphate



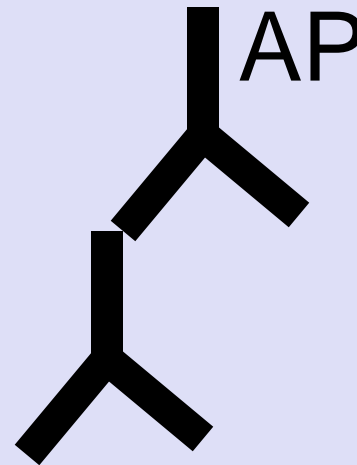
dinitrophenol



Anti-Insulin conjugated to alkaline phosphatase (AP)

# Rabbit anti-human IgG--alkaline phosphatase

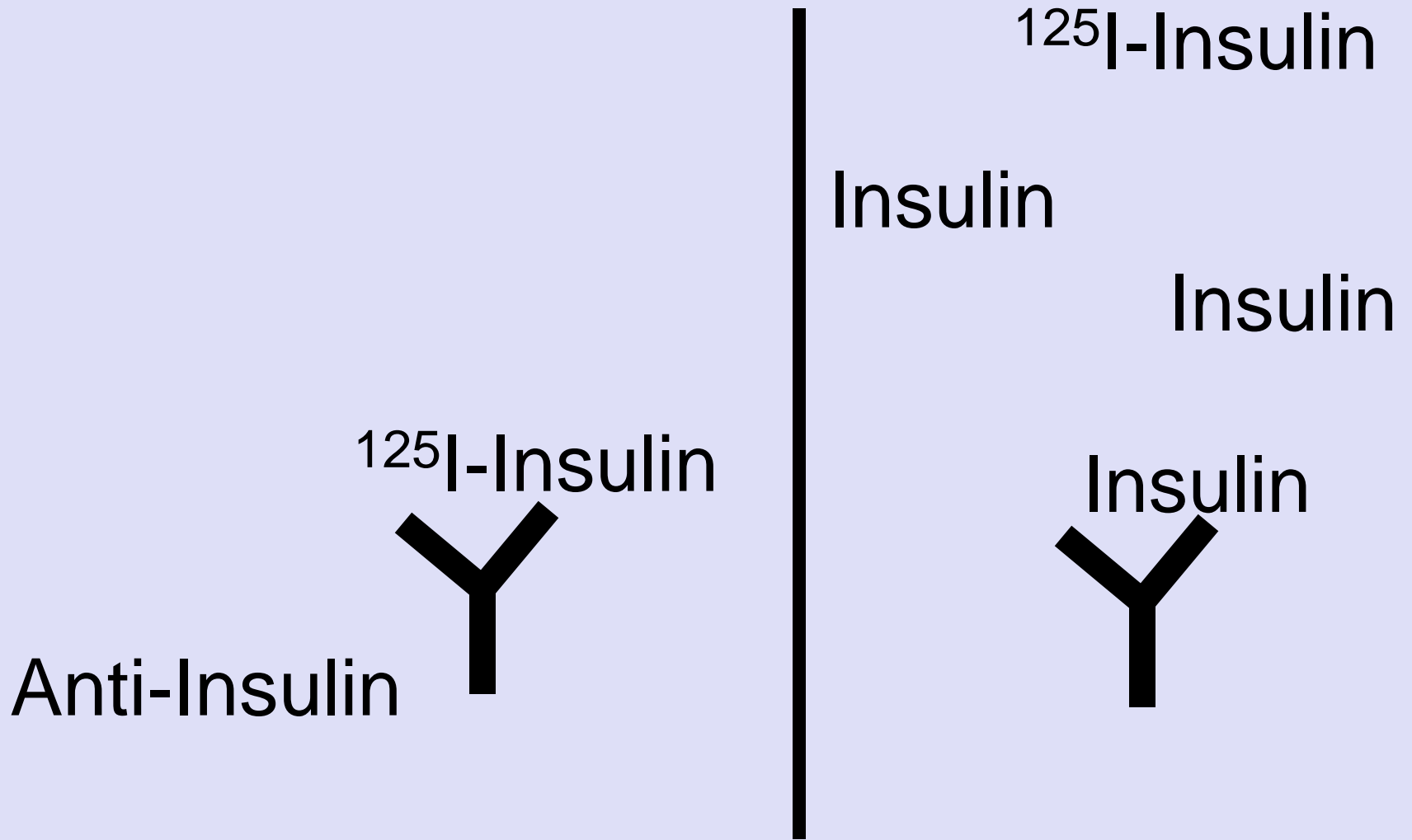
Patient Serum  
(with anti-hepatitis B)



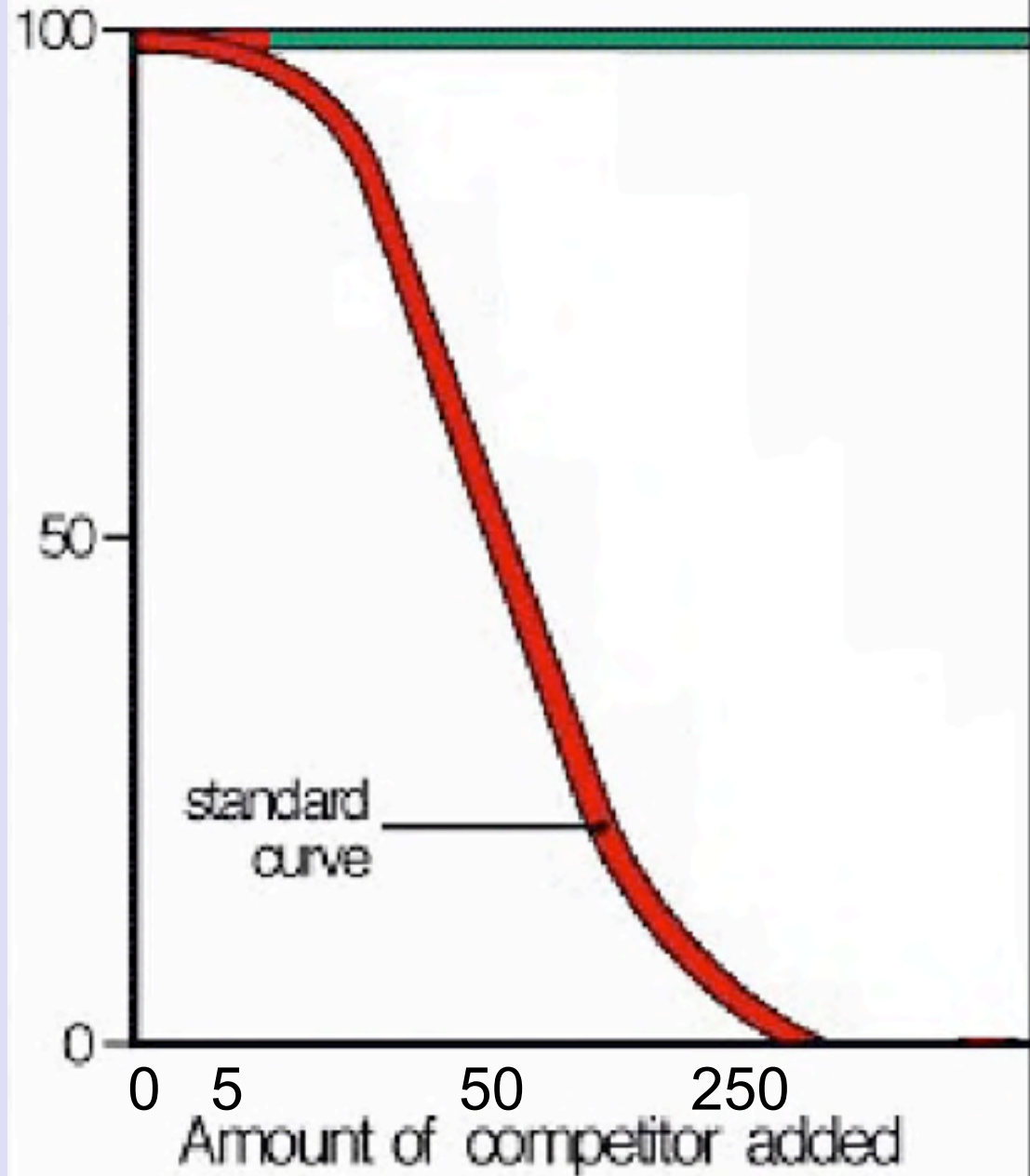
Hepatitis B Antigen



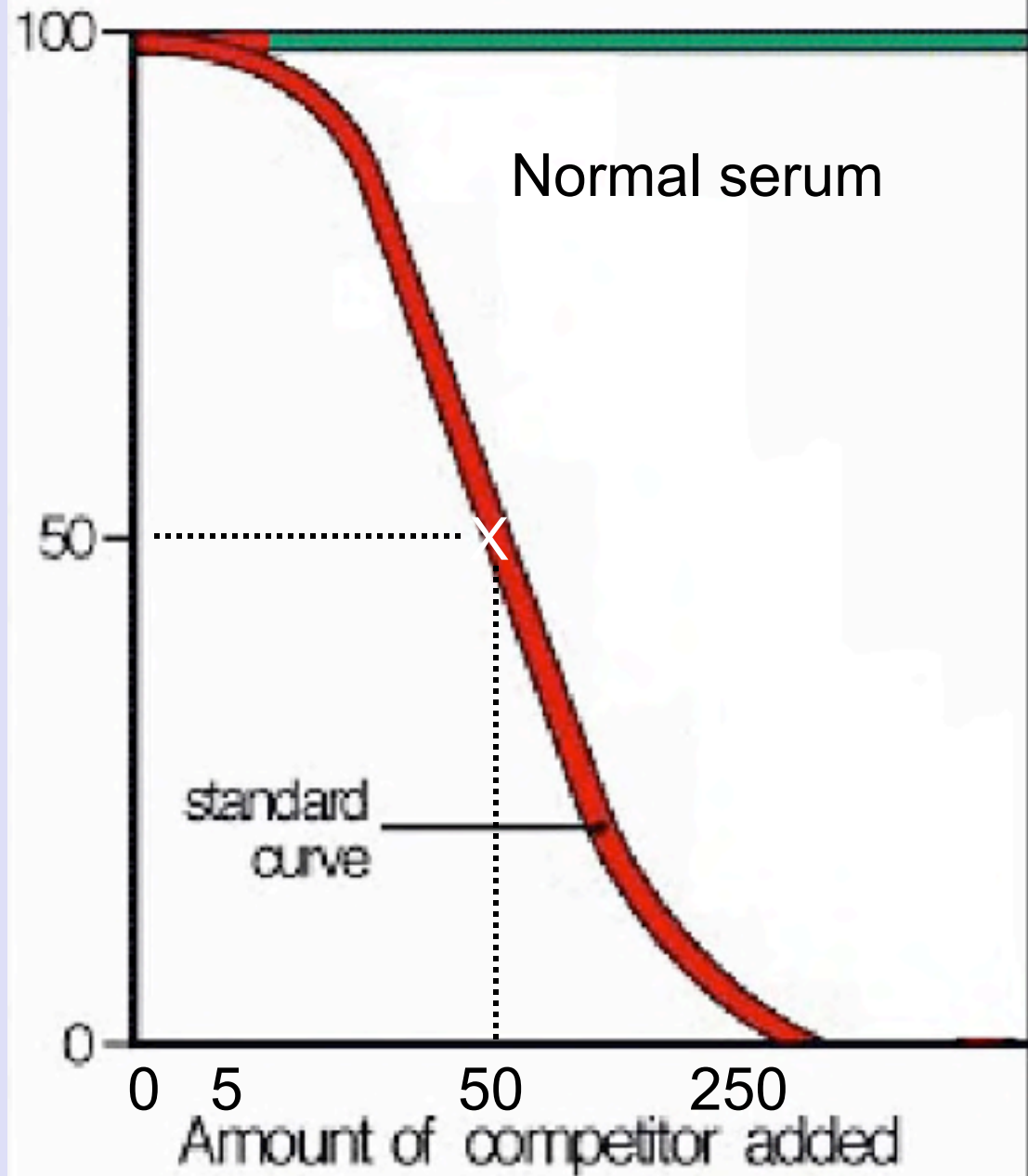
Radioimmunoassays (RIA) use radiolabeled antigens or antibodies. They are most used as a competitive assay. Clinical samples (not radioactive) are used to compete for binding to antibodies with a constant amount of radiolabeled standard.



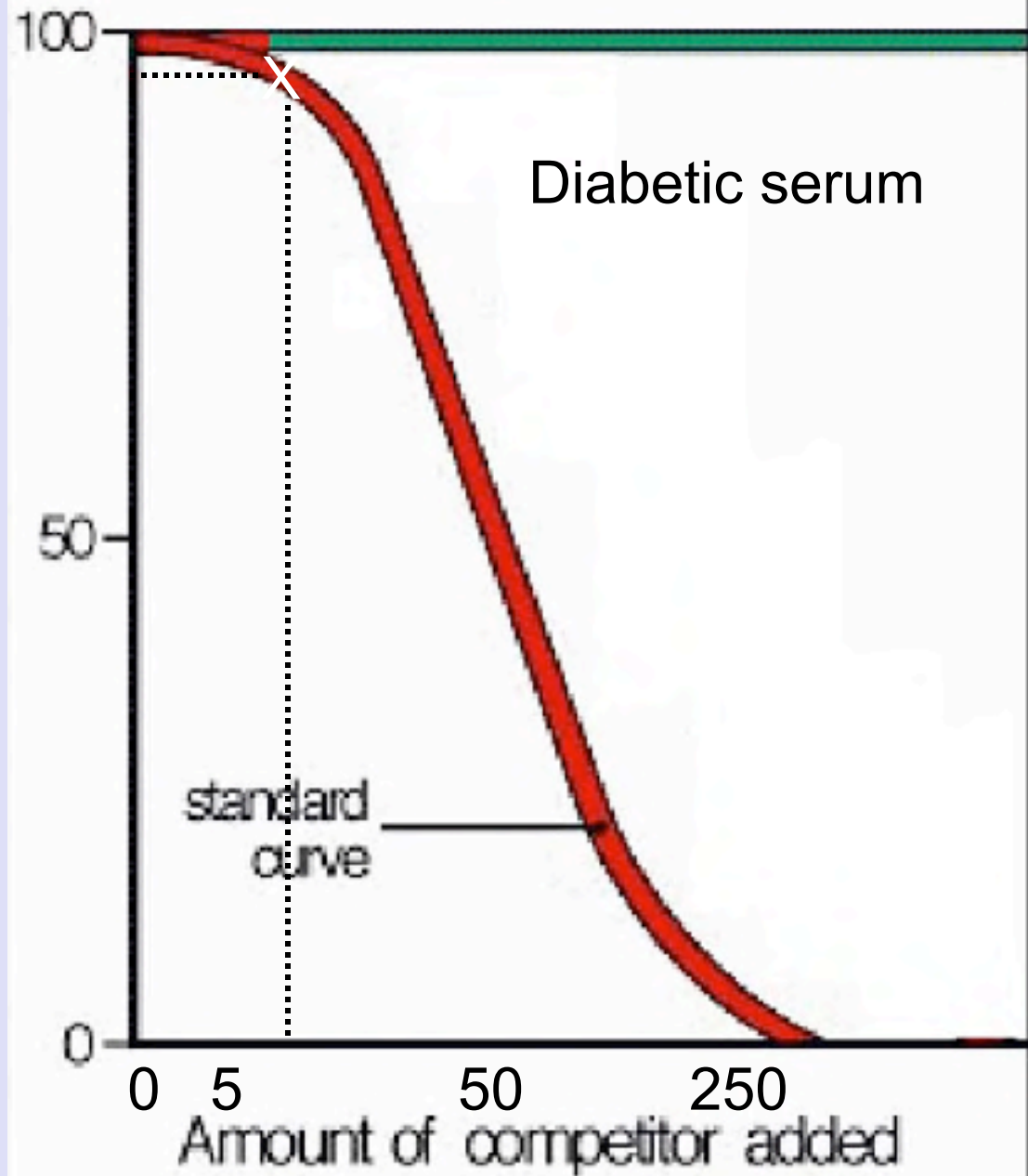
# Percent Radioactivity Bound



Percent  
Radioactivity  
Bound



Percent  
Radioactivity  
Bound



Results are reported as:

Negative or Positive (for example, more than 50% inhibition of the cpm for a standard at a dilution of 1:20 or greater)

Quantities ( $\mu\text{g/ml}$  or units)—compared to a standard curve with purified standard of known quantity.

Titers (Positive at a dilution of 1:250, 1:500, etc.)

The amount of antibody against a particular pathogen, whether expressed as micrograms, units, or titer, should be zero or low in an individual who has never experienced that pathogen. For example, in many healthy individuals, the antibody titer to hepatitis B virus is less than 1:5.

After recovery from an infection, the amount of antibody in serum (“convalescing serum”) should be significantly higher.

At the height of an acute infection, the immune response has not reached the effector phase yet, and so the amount of antibody in serum is low. Serum from a patient during active infection is often taken to compare to convalescing serum later on.

Pathogen-specific antibody titers can be elevated when a patient is first seen with a subacute or chronic infection or one that has a long incubation period.

The mixture of serum antibodies in a conventional antiserum is not perfect for immunological tests.

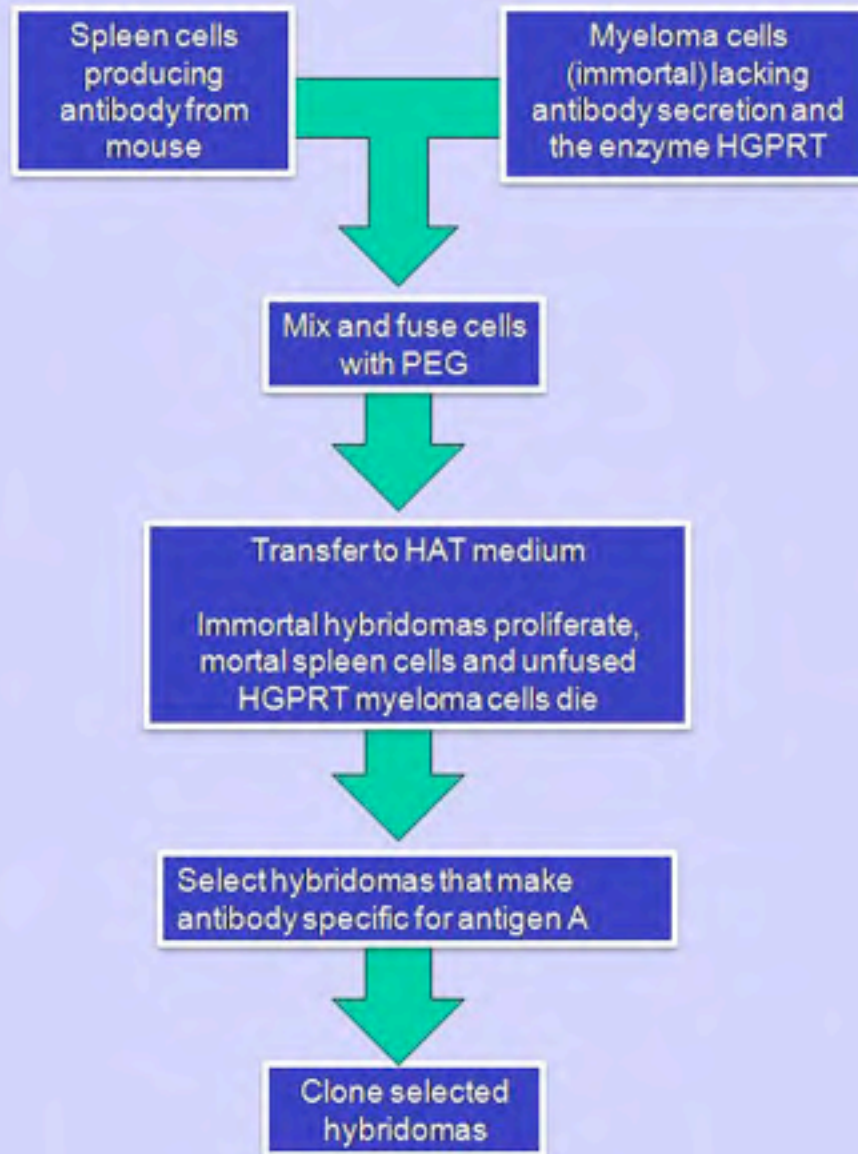
Serum is a mixture of antibodies that represents all of the antigens an individual has ever encountered.

Each antigen, with many epitopes, results in the production of many antibodies in serum.

Some of those antibodies, which bind to the antigen which elicited them with low affinity, may also bind a related antigen with low affinity.



Monoclonal antibodies from hybridomas solve most of the problems inherent in conventional antisera.



The *mouse* myeloma has been engineered to die in the presence of a drug (aminopterin in “HAT” media).

Plasma cells will not divide in tissue culture, and will die in a few days.

The supernatants of each hybridoma can be screened via ELISA for secretion of a particular antibody (to insulin, for example).

# Advantages of hybridomas

1. Immortal.
2. Monoclonal, and hence specific.
3. The hybridoma can be grown in large quantity.

# Uses of Monoclonal Antibodies

Reagents for sandwich ELISA for hormones, clotting factors, etc.

Reagents for detection of bacterial and viral antigens.

Reagents for tissue typing, or analysis of CD expression.

Detection of virtually any protein.

1. Antigenic determinants on immunoglobulins: isotypes and idiotypes
2. Immunoelectrophoresis, agglutination, radioimmunoassay, and enzyme-linked immunosorbent assay detect antigens or antibodies.
3. Hybridomas are useful because the antibody produced is monoclonal, and therefore highly specific. In addition, hybridomas live essentially forever and can be produced in large quantity.

# Additional Source Information

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- Slide 5: Janeway. Immunobiology: The Immune System in Health and Disease. Current Biology Ltd./Garland Publishing, Inc. 1997
- Slide 7 : Wesley Dunnick
- Slide 9: Janeway. Immunobiology: The Immune System in Health and Disease. Current Biology Ltd./Garland Publishing, Inc. 1997
- Slide 13: Regents of the University of Michigan
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