

# Serological Tests

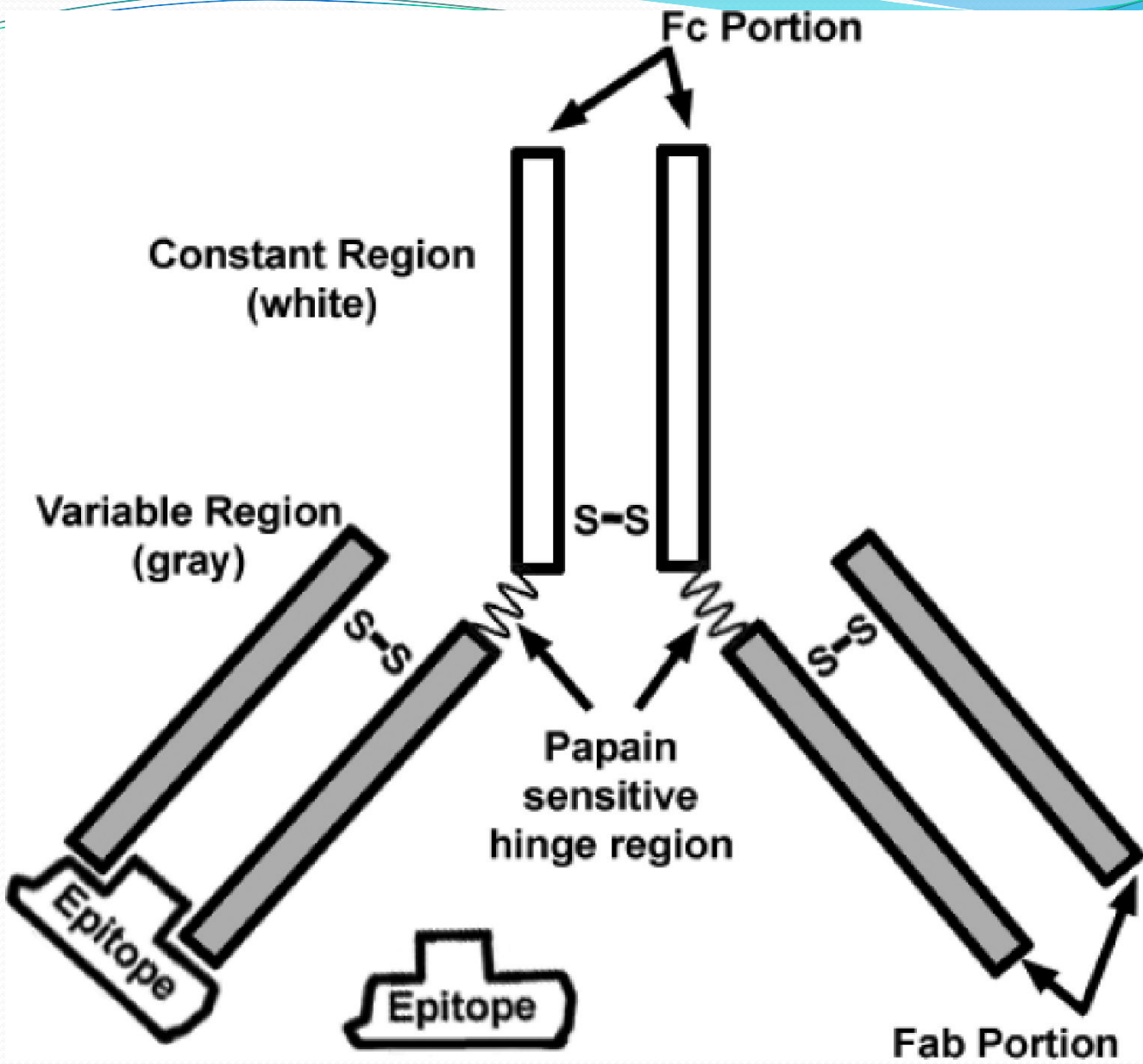
- **Serology** :is the science of measuring antibody or antigen in body fluids. The immune reaction is the production of antibody (substances) that protect the body against the antigen.
- **Serologic reactions**: that are in vitro Antigen-antibody reactions provide methods for the diagnosis of disease and for the identification and quantitation of antigens and antibodies. Simple serological techniques are called simple, because, these procedures involving direct demonstration and observation of reactions, they do not require the participation of accessory factors such as; indicator system, or specialized equipment. Some examples of these tests are the agglutination reactions, and precipitation reactions

# Antigens

- A molecule which can be specifically bound by an antibody (typically a protein or carbohydrate recognized as "foreign").
- **Antigen** : is substance which when introduced parentally into the body stimulates the production of an antibody with which it reacts specifically and in an observable (fever, rash).
- The antigens may classified according to their immunogenicity & immunoreactivity into:
- **I-Complete antigen** : substances with both immunogenecity and immunoreactivity By convention , we call complete antigen as antigen.
- **II- Incomplete antigen (hapten)**: substances only with immunoreactivity Hapten +carrier complete antigen (immunogens) Hapten: Only possess immunoreactivity Carrier: Make hapten obtain the immunogenicity B

# ANTIBODY

- **ANTIBODY**
- The molecule present in serum and other body fluids which mediates humoral immunity, and which can bind specifically to an antigen.
- Serum which contains antibodies (directed against one or more antigens) is termed an antiserum.
- **Antibody Molecules** :Also known as an **immunoglobulin (Ig)**, is a **protein produced by plasma cells that is used by the immune system to identify and neutralize pathogens such as bacteria and viruses.** large, Y-shape



# Factors affecting serological test (antigen antibody reaction)

- Many factors affect the interaction between antigen and antibody; these include:
- **1-Specificity:** The ability of a particular antibody to combine with one antigen instead of another is referred to as specificity. This property depends on the antigen – binding fragment of an immunoglobulin molecule. Antigen – antibody reactions can
- show a high level of specificity.
- **2-Cross reactivity:** Unrelated molecules can have antigens with similar antigenic determinants. This means a proportion of the antibodies directed against one kind of antigen will also react with the other kind of antigen. This is called cross reactivity. An example of cross reactivity is when; antibodies directed against a protein in one species may also react in a detectable manner with the homologous protein in another
- species.

- **3-Temperature:** The optimum temperature needed to reach equilibrium in an antibody – antigen reaction differs for different antibodies.
- IgM antibodies are cold reacting, with a thermal range of 4-220C,
- IgG antibodies are warm reacting with an optimum temperature of reaction of 370C.
- **4-pH:** Although the optimum pH for all reactions has not been determined, a pH of 7.0 is used for routine laboratory testing.
- **5-Ionic strength:** The concentration of salt in the reaction medium has an effect on antibody uptake by the membrane bound erythrocyte antigens. Sodium and chloride ions in solution have an inhibitory effect. These ions cluster around the opposite charges on antigen and antibody molecules which partially neutralizes them. This hinders the association of antibody with antigen. Reducing or lowering the ionic strength of a reaction medium, such as low-ionic strength salt, can enhance antibody uptake.

- **6-Concentration:** Under normal conditions, the concentration of antigen and antibody should be optimal, but sometime this is not the case. Excess antibody or antigen concentration will result in a false reaction, sometimes known as zonal reaction when the concentration of antigen is excess it is known as a
- post zone reaction; excess antibody is referred to as a prozone reaction. This phenomenon can be overcome by serial dilutions until the optimum amount of antigen and antibody is present.

## **7-Bond strength and intermolecular attractive force.**

Bonding of an antigen to an antibody takes place because of the formation of multiple, reversible, intermolecular attraction between an antigen and amino acids of the binding site. ●

- **8-Previous vaccination**
- **9-History of previous infection by the same organism**

# ❑ Collection, preparation and preservation of serological specimens

❑ Specimens that are commonly used for Serological tests include:

❑ Serum,

❑ plasma

❑ CSF.



# Classification of antigen- antibody interactions

# Types of binding Tests

## □ I-primary serological binding tests

- ❖ Primary binding tests are tests that directly measure the binding of antigen and antibody (i.e.; directly measure or visualize the immune complex). They are the most sensitive techniques in terms of the amount of detectable antigen or antibody.
  - 1. Immunofluorescence tests
  - 2. Enzyme linked immunosorbent assay (ELISA) .
  - 3. Radioimmunoassay (RIA)
  - 4. Radioimmunodiffusion (RID).



# II-Secondary binding tests

- Secondary binding tests are tests that detect and measure the consequences (secondary effect) of antigen-antibody interaction.
- These consequences include
  1. Precipitation of soluble antigens
  2. Agglutination of particulate antigens
  3. Complement fixation test
  4. Serum neutralization tests (SNT)
  5. Toxin-antitoxin test
- They are usually less sensitive than primary binding tests, but may be easier to perform



THANK YOU