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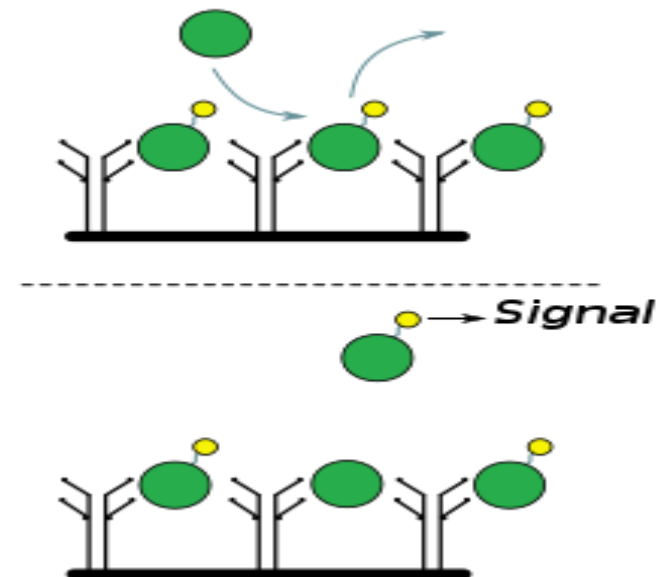
INTRODUCTION

- An immunoassay is a biochemical test that measures the concentration of substance in liquid (a portion of a biological specimen) using the reaction of an antibody or antibodies to its antigen. OR
- An immunoassay is an analytical method which uses antibodies as reagents to quantitate specific analytes.
- They are used in a lot of laboratories including hospital labs and have been widely used in the special area of forensic toxicology to screen for drug and other chemicals in body.

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- An antibody: antigen complex is also known as an immuno-complex.
 - The assay operates the immune system to identify a substance (usually a protein) based on it's capacity to act like an antigen.
 - Highly specific "lock and key" system: antibody – antigen reaction.

PRINCIPLE

- The immunoassay is a technique which incorporates the binding reaction of a target substance (antigen) with an antibody.
- Antibodies are basically immunoglobulins that bind to different natural and synthetic antigens in the body such as carbohydrates, lipids, proteins and nucleic acids.



ANTIBODIES

- An antibody is a protein that is produced by the body in response to an invading (foreign) substance.
- Antibodies are produced as part of the body's immune response to protect itself.
- Antibodies (Ab) are a type of protein called immunoglobulin.
- The most common one is immunoglobulin G(IgG).
- IgG is a protein composed of two main structural and functional regions.

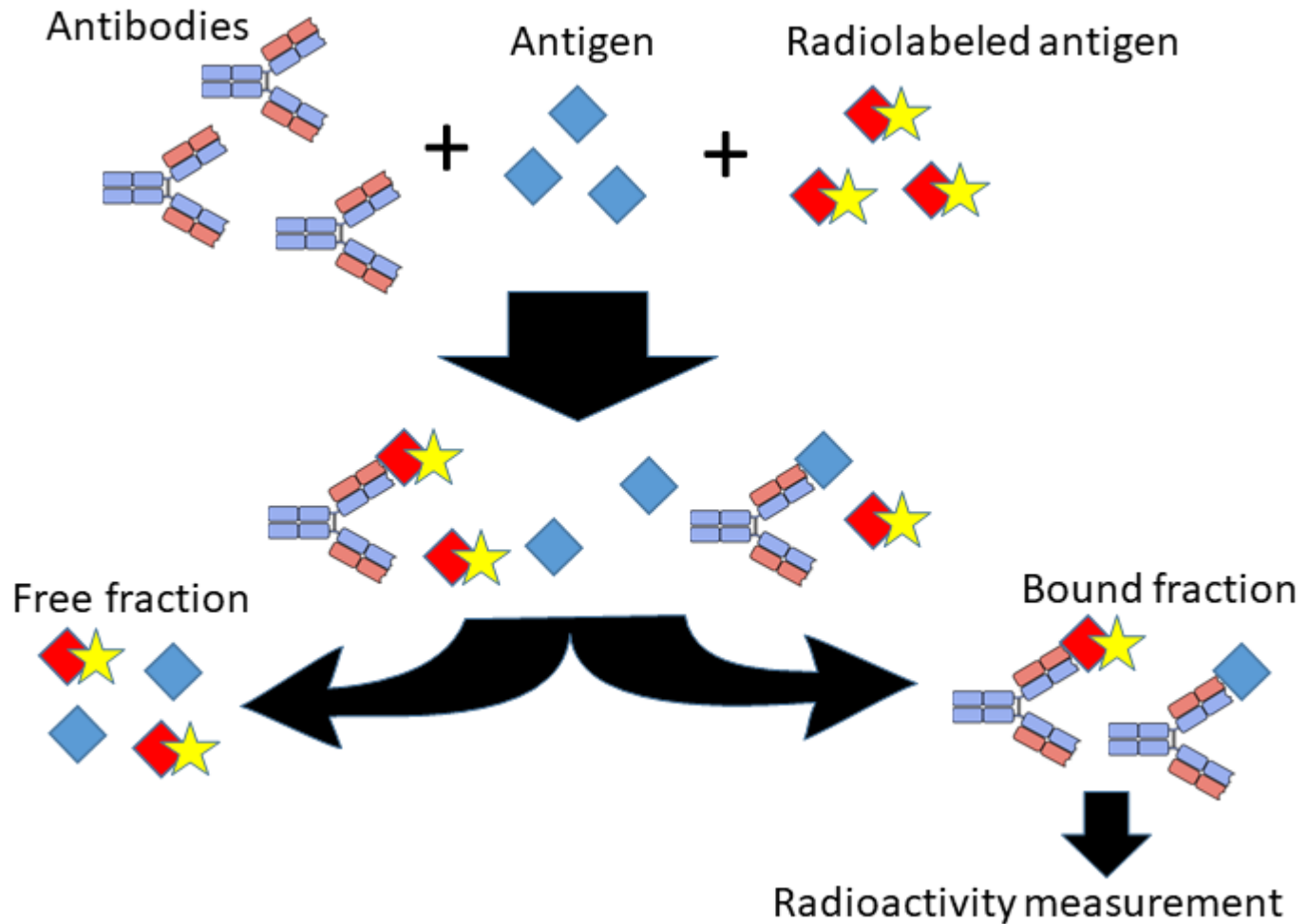
TYPES OF ANTIBODIES

- Polyclonal antibodies
- Monoclonal antibodies
- Polyclonal antibodies recognize multiple sites on antigens, or monoclonal antibodies recognize single sites on antigens.
- **ANTIGEN**
- An antigen is the substance that the body is trying to fight off (eliminate or reduce) by mounting an immune response.
- In a test to measure the concentration of a drug, e.g., the drug is the antigen that binds to the antibody.

Methodology For Immunoassay

- **Isotopic immunoassay:**
- Based on competition for antibody between radioactive indicator antigen and unlabelled antigen in test sample.
- Increase in count of unlabeled antigen in test sample decrease the labeled antigen in bound.
- The concentration of the test antigen can be determined by comparison with a standard calibrated curve with known concentration of purified antigen.
- Examples; Radioimmunoassay

PRINCIPLE OF RADIO-IMMUNOASSAY



USES OF RIA

- ❖ Narcotics drug detection
- ❖ Early cancer detection
- ❖ Blood bank screening for the hepatitis
- ❖ Diagnosis and treatment of peptic ulcer
- ❖ Tracking of leukemia virus
- ❖ Measurement of growth hormone levels

Nonisotopic Immunoassay

- Non-isotopic immunoassay is a powerful and innovative technique used in the field of diagnostics, clinical research, and molecular biology to detect and quantify specific molecules, such as proteins, hormones, or antibodies, without the use of radioactive isotopes.
- This technology has gained prominence as a safer, more convenient, and environmentally friendly alternative to traditional isotopic immunoassays.

METHOD USED IN NIA

Enzyme-Linked Immunosorbent Assay (ELISA):

- Enzymes, such as horseradish peroxidase or alkaline phosphatase, are conjugated to antibodies or antigens.
- The enzyme reacts with a substrate to produce a detectable signal, such as a color change (enzyme-linked colorimetric assay) or light emission (enzyme-linked chemiluminescent assay).

Fluorescence-Based Assays:

- Fluorescent markers or dyes are conjugated to antibodies or antigens.
- When excited by a specific wavelength of light, these markers emit fluorescence, which is detected and quantified using a fluorometer or a microplate reader.

USES OF NIA

- Forensic Analysis:
- Pregnancy Testing:
- Monitoring of Therapeutic Biologics:
- Research and Development in Biotechnology:
- Drug Screening:
- Environmental Monitoring:
- Food and Beverage Industry:
- Diagnosis of disease

Application of immunoassay in food Industry

- Many of the macromolecule that can found in food are good antigen and antibodies are capable of recognizing them and small molecule.
- Composition of raw material and final product.
- Harmful and useful minor substances with biological activity (toxins and allergens).
- Enzyme detection.
- Contaminants detection and determination (hormones, drug, pesticides residue).