ADVANCED LABORATORY TECHNIQUES

IMMUNOFLUORESCENCE AND RADIOIMMUNOASSAY (RIA)

Sawa University — جامعـــــة ســـــــاوة الاهلية

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. third Stage — المرحلـــــــــــــة الثالثة

محاضرة رقم 5

Lecture No. 5

الجانب النظري

Theoretical

تدريسي المادة : م.م طيبة شاكر

(INTRODUCTION)

Both **immunofluorescence (IF)** and **radioimmunoassay (RIA)** are highly sensitive immunological techniques used for **detection and quantification** of antigens or antibodies.

They are based on **antigen–antibody interactions**, but differ in the type of **label** used for detection:

- **Fluorescent dyes** in immunofluorescence
- **Radioactive isotopes** in RIA

Immunofluorescence (IF)

Principle

Immunofluorescence uses **fluorescently labeled antibodies** (such as FITC – *fluorescein isothiocyanate*, or TRITC – *tetramethylrhodamine isothiocyanate*) to detect specific antigens in cells or tissues.

When exposed to **ultraviolet (UV) light**, the dye emits visible fluorescence that marks the location of the antigen–antibody complex

It was described in 1942 and refined by Coons in 1950, which used a fluorescence microscope able to read the specific immunological reaction and cellular slide preparations.

It is an effective method for visualizing intracellular processes, structures, and conditions as well.

- **In Vitro type of Ag-Ab reaction.**
- **Detects surface antigens or antibodies.**
- **Fluorescent dyes are used for the visualization of Ag-Ab reactions.**

Types of Immunofluorescence

◈ 1. Direct Immunofluorescence (DIF)

•A *single* fluorescent-labeled antibody directly binds to the target antigen in the sample.

•Used mainly to detect **antigens** in tissue or cell preparations.

•The slide is washed and observed under a fluorescence microscope.

•It provides **rapid results** but **less sensitivity** because only one fluorophore binds per antigen site.

◈ 2. Indirect Immunofluorescence (IIF)

•Used to detect **antibodies** in a patient's serum.

•The test antigen (e.g., a tissue section) is first incubated with the patient's serum.

•If antibodies are present, they bind to the antigen.

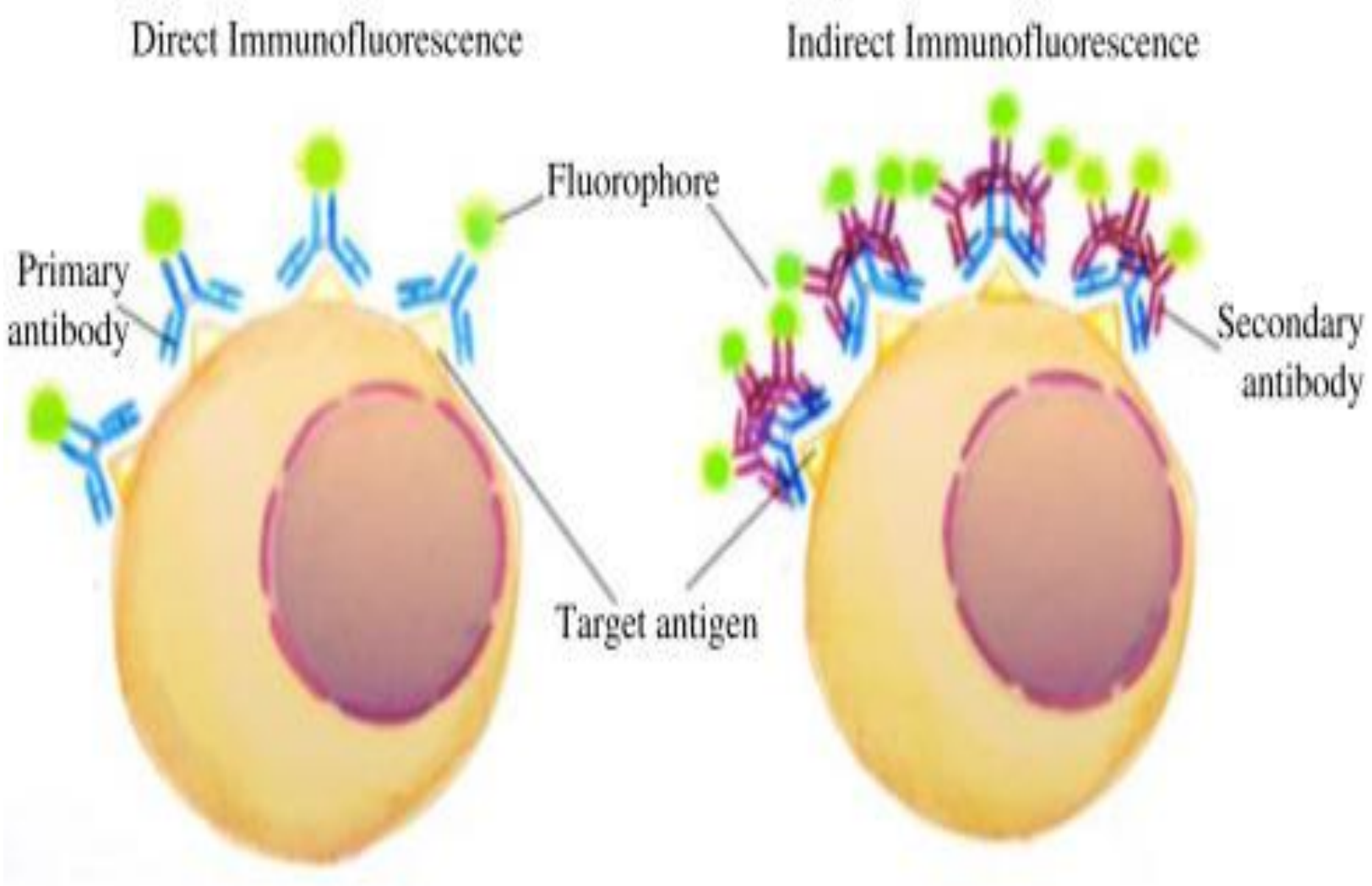
•A **second antibody**, labeled with a fluorescent dye, is then added; it binds to the patient's antibody (anti-IgG).

•This method is **more sensitive** due to amplification of the fluorescent signal

Direct immunofluorescence:

uses a **single**, **primary** antibody, **chemically linked** to a fluorophore or fluorochrome. **The primary antibody recognizes the target molecule (antigen) and binds to a specific region called the epitope**. The attached fluorophore can be detected via fluorescent microscopy.

direct immunofluorescence, although somewhat less common, has notable advantages over the indirect procedure. The direct detection of the antigen by a primary antibody directly labeled with a fluorochrome reduces the number of steps in the procedure, saving time and reducing non-specific background signal.


Direct Immunofluorescence
Indirect Immunofluorescence
Fluorophore
Primary antibody
Secondary antibody
Target antigen

Indirect immunofluorescence:

Highly sensitive immunofluorescence detection systems for the detection of IVD antibodies related to autoimmune conditions. Our innovative IF detection systems and high affinity antibodies, have opened the doors for a faster and accurate immunofluorescence and immunohistochemistry applicable to autoimmune disease like nephropathies and lupus. These detection systems are intended for use in immunofluorescence (IF) applications of formalin-fixed paraffin-embedded tissues (FFPE), frozen tissue sections and cell preparations.

Types of immunofluorescence

1. Direct immunofluorescence (DIF)

- A *single* fluorescent-labeled antibody directly binds to the target antigen in the sample.
- Used mainly to detect **antigens** in tissue or cell preparations.
- The slide is washed and observed under a fluorescence microscope.
- It provides **rapid results** but **less sensitivity** because only one fluorophore binds per antigen site.

2. Indirect immunofluorescence (IIF)

- Used to detect **antibodies** in a patient's serum.
- The test antigen (e.G., A tissue section) is first incubated with the patient's serum.
- If antibodies are present, they bind to the antigen.
- A **second antibody**, labeled with a fluorescent dye, is then added; it binds to the patient's antibody (anti-igg).
- This method is **more sensitive** due to amplification of the fluorescent signal.

Applications

- Detection of autoantibodies (e.G., In autoimmune diseases).
- Identification of infectious agents within cells.
- Localization of proteins in cellular or tissue structures.
- Cancer diagnostics and immunopathology.

Method	Advantages	Disadvantages
Direct immunofluorescence	• Simplified workflow and shorter sample staining time • No cross-reactivity between secondary antibodies • Best solution for specific staining of multiple targets	• Lower sensitivity for low-abundance targets with limited number of fluorescent dyes that can be attached to primary antibody • Limited number of commercially available fluorescently labelled primary antibodies
Indirect immunofluorescence	• Greater sensitivity for low-abundance targets due to signal amplification by secondary antibodies • Numerous inexpensive secondary antibodies commercially available with various dyes and tags attached	• Cross-reactivity between secondary antibodies, in particular when performing multilabel experiments • Reaction of secondary antibodies with endogenous immunoglobulins leading to high background fluorescence

Radioimmunoassay (ria):radioimmunoassay (RIA) is a sensitive and quantitative method used to measure the concentration of antigens *in vitro*. It is first development in 1950s. Nanomolar and picomolar concentrations of hormones in biological fluids can be detected by RIA. It is the first immunoassay technique to analyze small amounts of analyte.

Principle

RIA is a **competitive binding assay** that uses **radioactively labeled antigens or antibodies** to measure very low concentrations of biological substances such as hormones, drugs, or viral antigens.

The principle is based on competition between:

- **Radiolabeled antigen (ag*)**

and

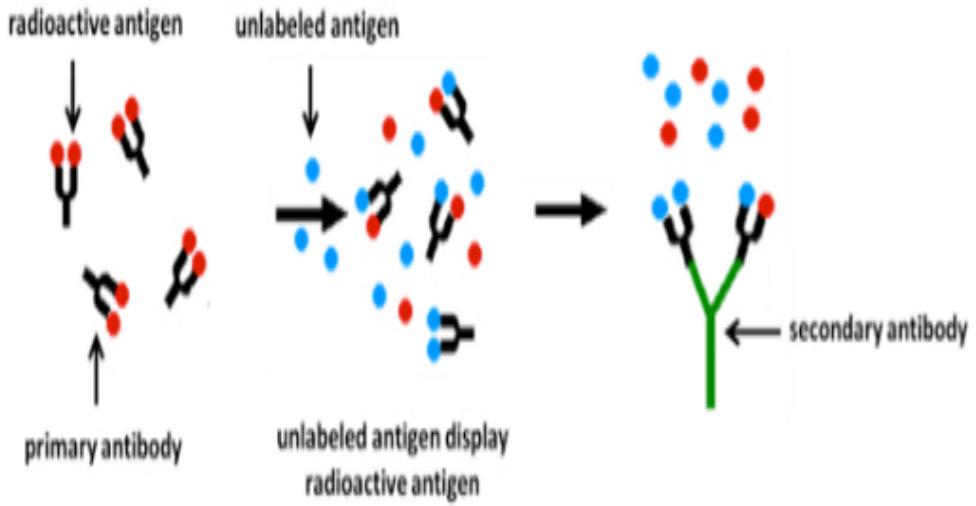
- **Unlabeled antigen (ag)** from the sample

for limited **antibody binding sites**.

The amount of radioactivity bound to the antibody is **inversely proportional** to the concentration of the antigen in the sample.

What is the principle of RIA?

RIA is performed by using antibody-antigen binding and radioactive antigen. The basic principle of RIA is competitive binding reaction, where the analyte (for example, antigen) competes with radio-labeled antigen for binding to the fixed antibody or the binding sites of receptor. Binding of the unlabeled antigen to the fixed and limiting amount of antibody causes displacement of radio-labeled antigen and results in decreasing the radioactivity of antigen-antibody complex.



Advantages

- **Extremely high sensitivity** (can detect picogram levels).
- Highly specific due to antigen–antibody recognition.
- Suitable for hormones, drugs, and viral antigen quantification.

Disadvantages

- Requires handling of **radioactive materials** (safety hazards).
- High equipment cost and strict disposal regulations.
- Replaced in many labs by safer **elisa** and **chemiluminescent immunoassays**

QUIZ:

Both immunofluorescence and radioimmunoassay rely on antigen–antibody reactions.

Why do you think RIA is more sensitive even though IF provides direct visualization?

ANY QUESTION?

Thank You