LAB-4-
IMMUNOFLUORESCENCE

Submitted by

assist.Lec.Hiba Hadi Rashid

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- Principle
- Requirements
- Methods
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- Advantage
- Disadvantage

➢ HISTORY

- Immunofluorescence, Studies are considered the **“gold standard”** for diagnosis of autoimmune blistering disease.
- **Sir George G.Stockes(1852),** first observation that the mineral “fluorspar” exhibits fluorescence when illuminated with ultraviolet light , and he coined word “fluorescence”.
- **August kohler(1904): devised the fluorescence microscope .**
- **M.Haitinger(1933):**first stain of histological specimens with fluorescent dyes.
- **Coons et al .(1941)** developed the immunofluorescence technique for the first time, a discovery which made possible to observe microscopically antigens, antibodies and their related substances on tissue sections or on cell smears.
- **Beutner and Jorden(1960):** IF was introduced into Dermatology when revealed through this technique tissue and circulating antibodies in autoimmune vesicobullous dermatoses.
- **Weller& Coons(1954):** Diagnosed Herpes- Zoster Ag by IF.

DEFINITION

IMMUNOFLUORESCENCE:

Is a technique allowing the visualization of a specific antigen by binding a specific antibody chemically conjugated with a fluorescence dye.

FLUOROCHROME(FLUOROCEIN): are dyes which have the ability to absorb the short wavelength UV radiation and emit light of longer wave length fluorescence (visible green light).

EXAMPLE: fluorescein isothiocyanate (FITC) emits green light

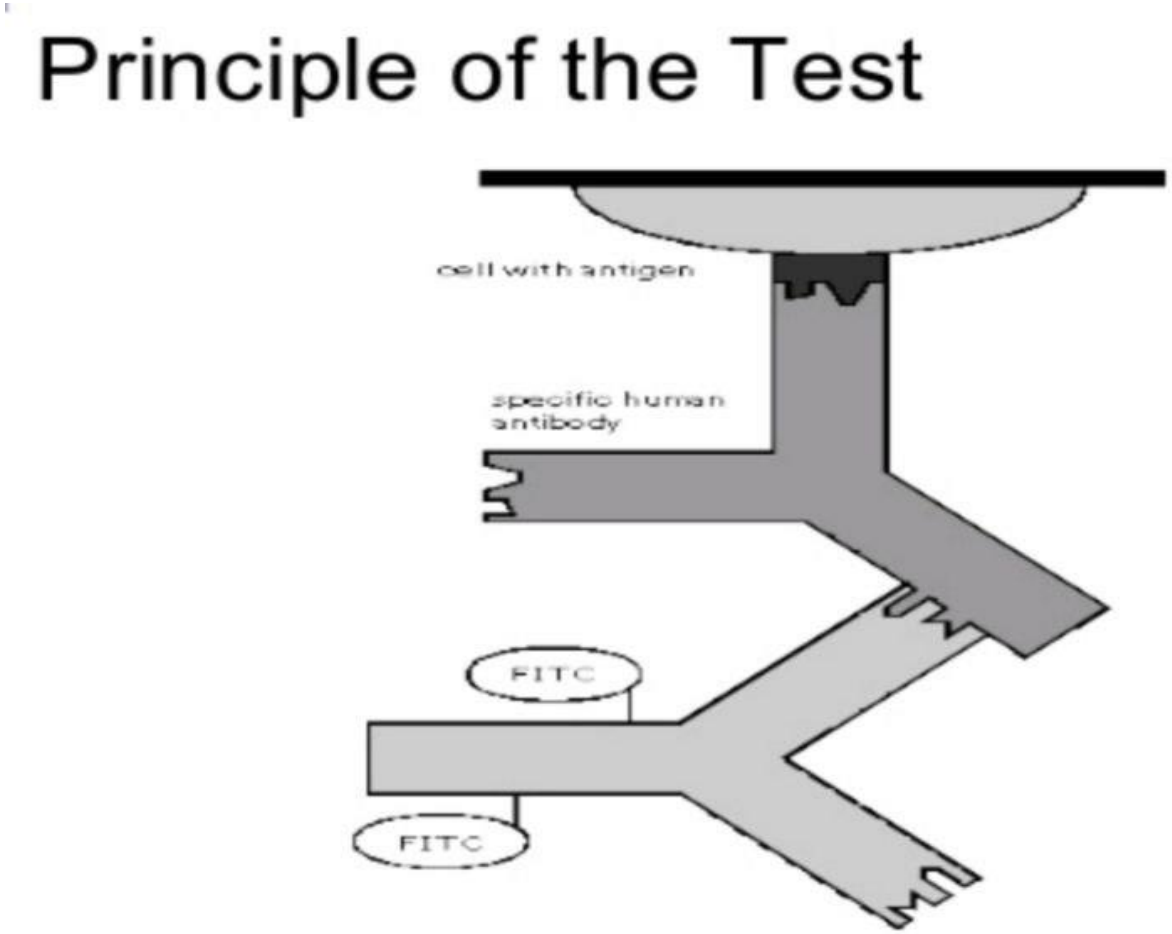
Tetramethylrhodamine-emits red light

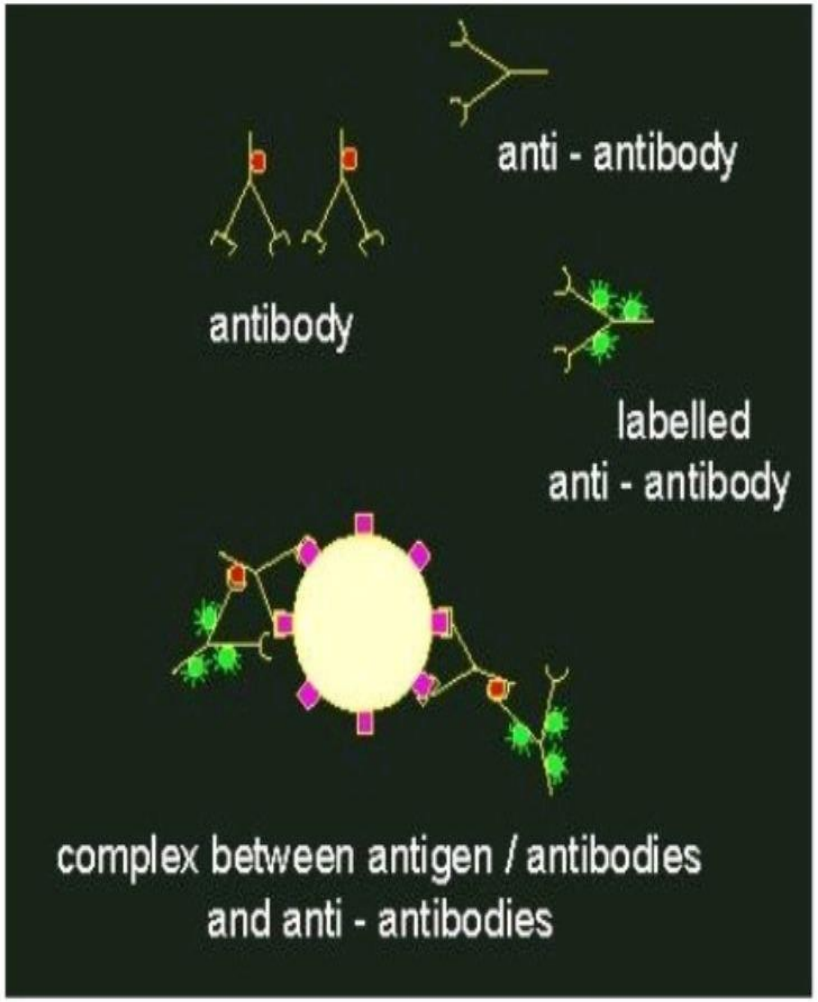
*This technique is sometimes used to make Viral Plaques more readily visible to the human eye.

PRINCIPLE OF IF

- Employs Ag-Ab reaction
- Ab tagged with a fluorophore is introduced to specimen.
- Ab binds to Specific Ag.
- Specimen is viewed under a UV light with dark background in fluorescent microscope
- Fluorophore absorbs radiant energy and is excited.
- Fluorophore goes back to aground state , emitting light energy of longer wavelength and lower energy.
- Phosphorescence emission continues for milliseconds to minutes after the energy source has been removed.

PRINCIPLE OF IF

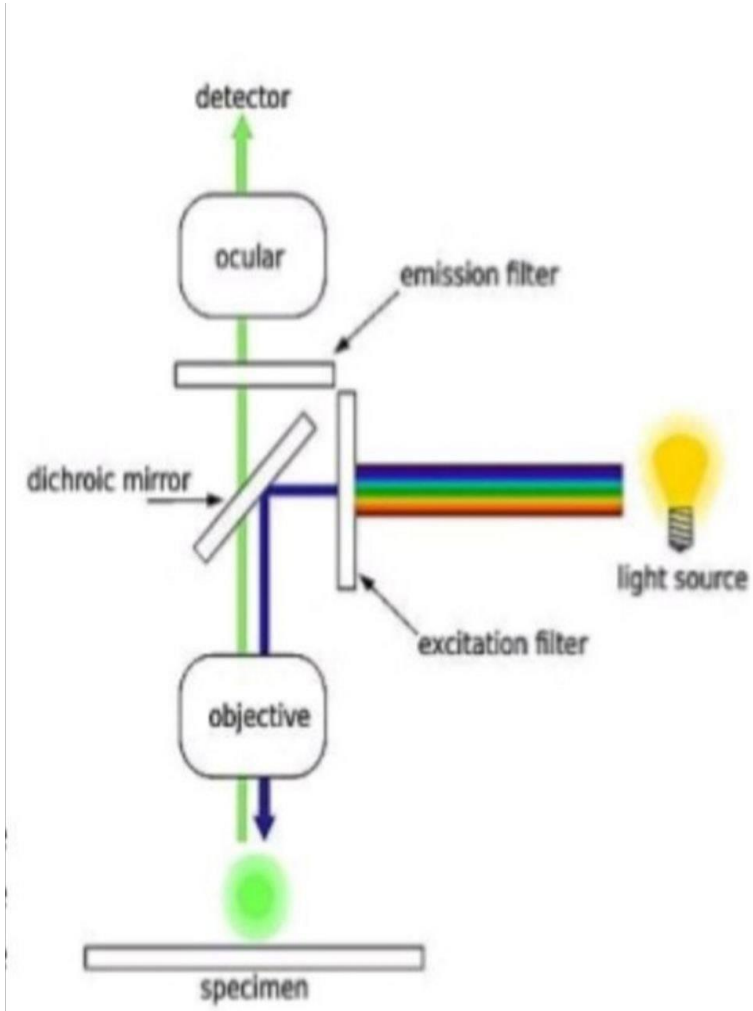


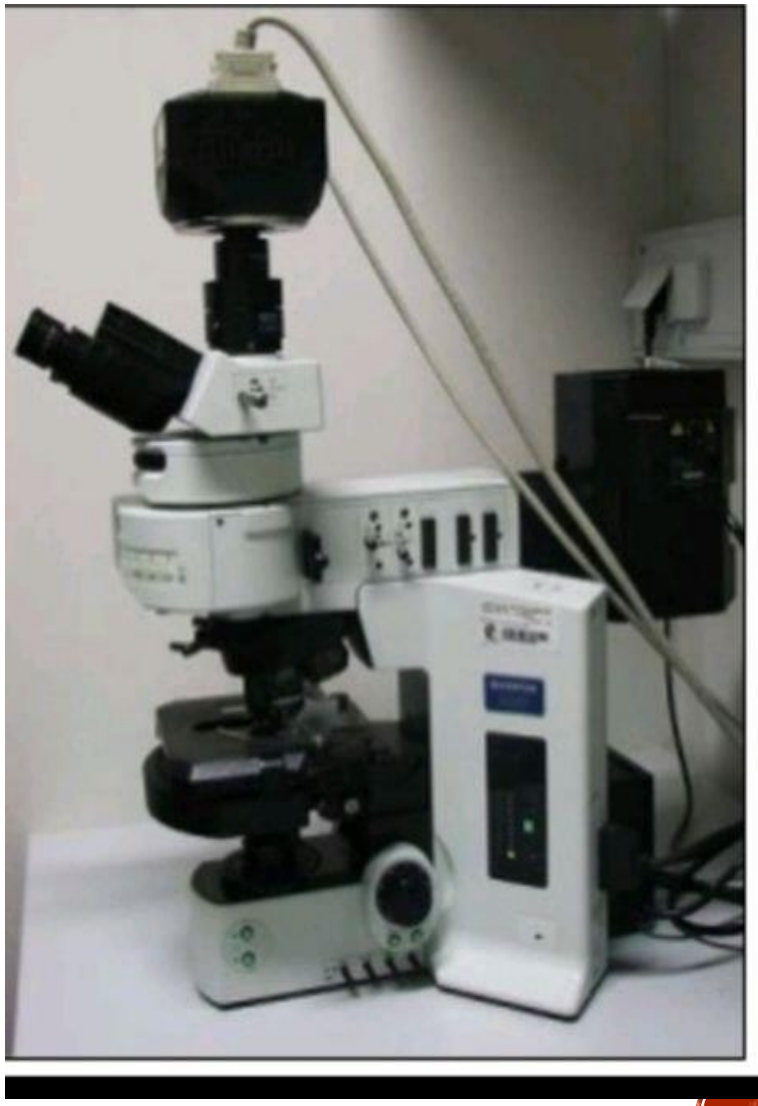


REQUIREMENTS

- Slide
- Tagged Antibody
- Fluorescent Microscope

Specimens

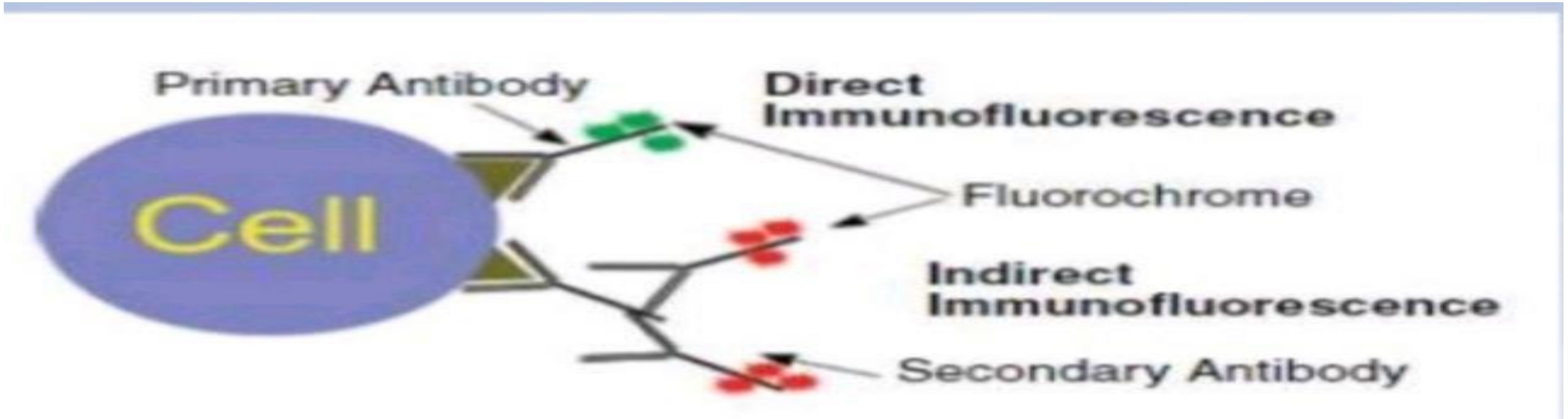




METHODS OF IF ASSAY

- There are two Types of IF :

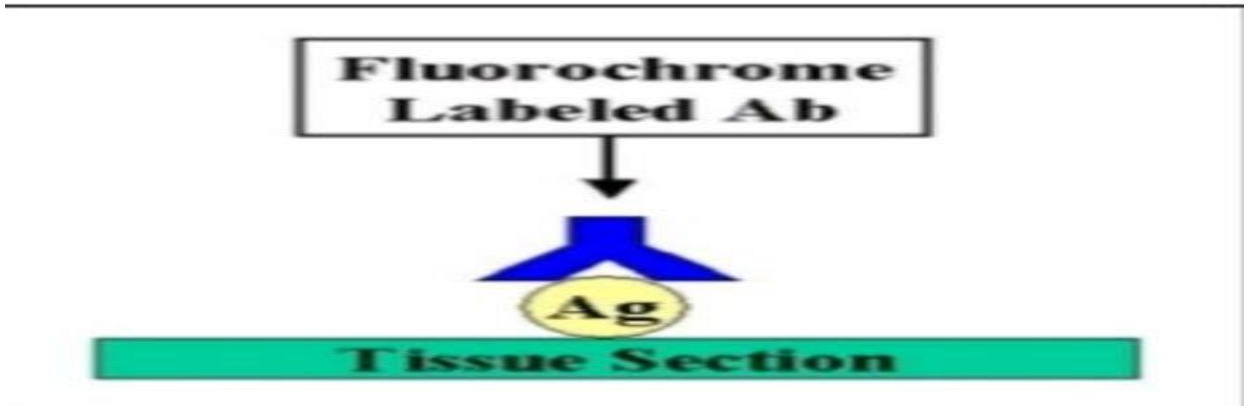
1. Direct IF assay (DIFA)
2. Indirect IF Assay (IDIFA)



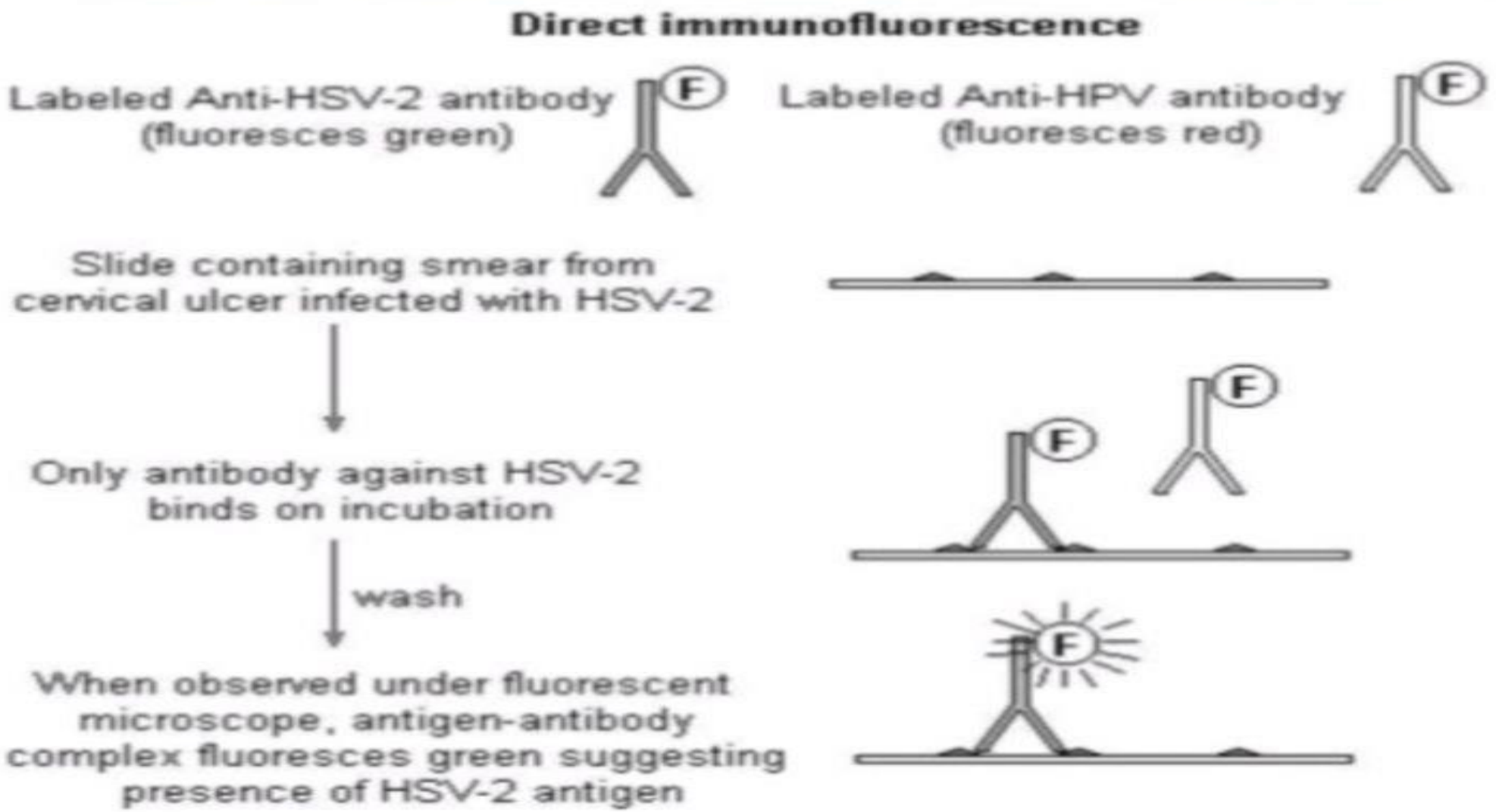
METHODS OF IF ASSAY

1. **Direct IF assay (DIFA)**

- Tagged Ab is directly added to unknown Ag fixed to a slide
- Requires incubation and wash step
- Ag are seen as bright apple green or Orange – yellow against a dark backgrounds under fluorescent microscope.
- Suitable for detection of specific Ag in tissue or body fluids and viruses (Respiratory Syncytial–virus kit in blood sample)



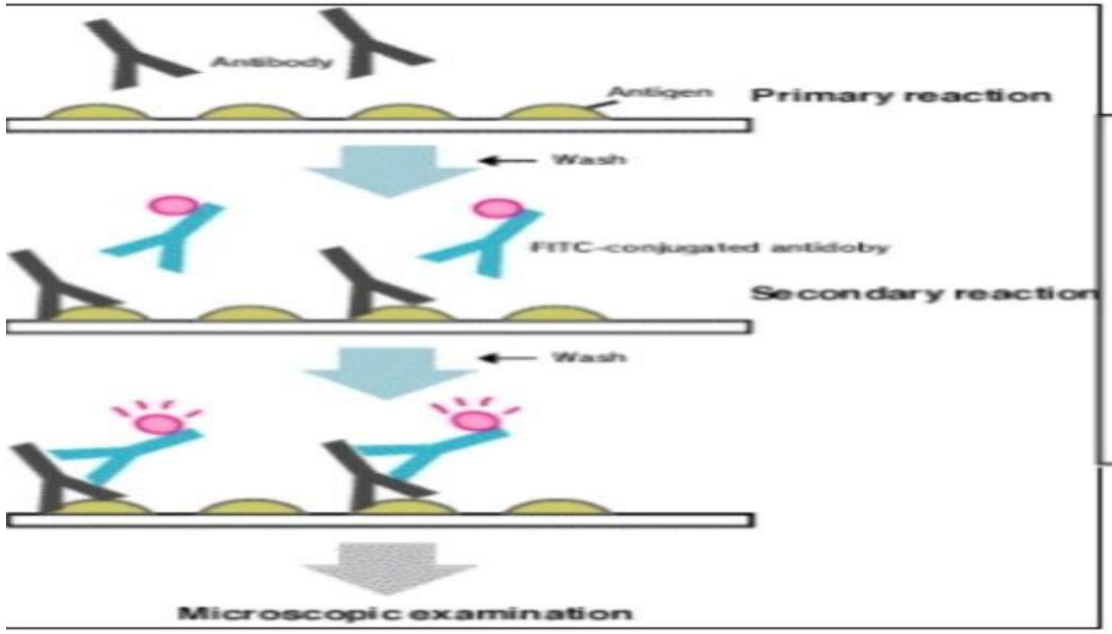
Direct IF assay (DIFA)

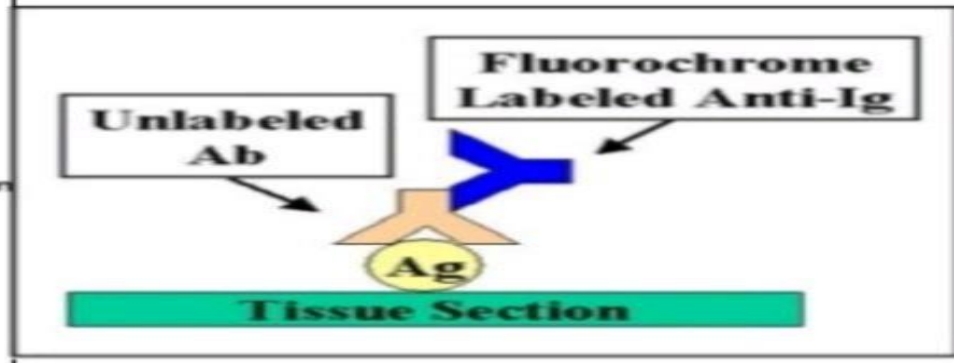


METHODS OF IF ASSAY

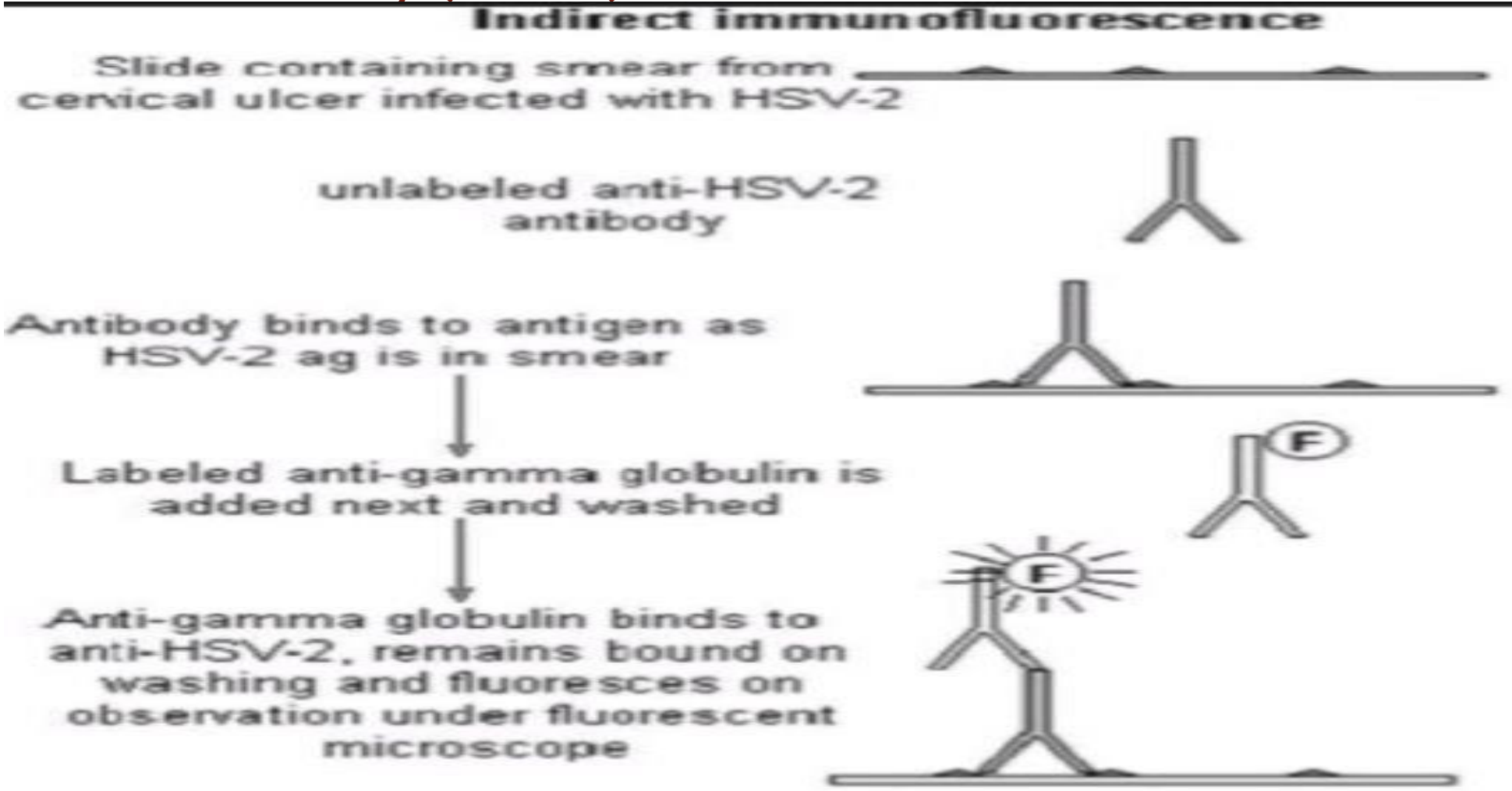
***Indirect IF Assay (IDIFA)**

- Indirect test uses two antibodies ;
- The unlabeled primary antibody specifically binds the target molecule.
- Secondary antibody Treated with a fluorochrome – conjucated anti- immunoglobulin serum and recognizes the primary antibody and binds to it .

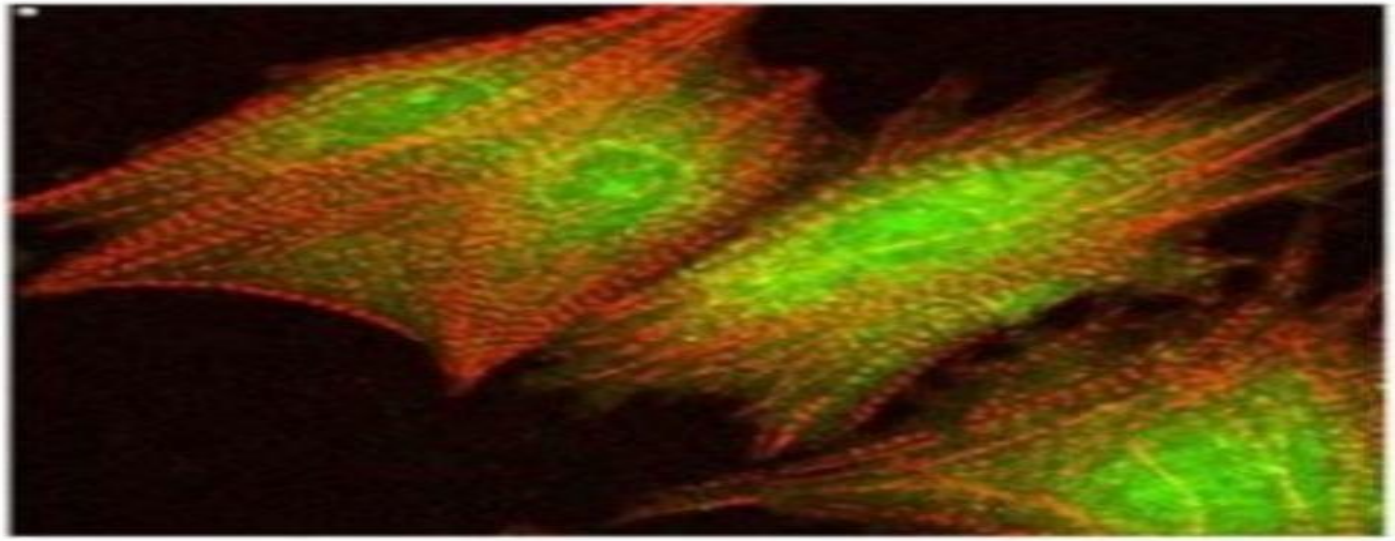




Indirect IF Assay (IDIFA)



RESULT



Confocal image to detect phosphorylated AKT (green) in cardiomyocytes infected with adenovirus

APPLICATIONS OF IFA

▪ Direct immunofluorescence	▪ Indirect immunofluorescence
1-used to detect , pathogens(virus) or their Ag in tissue or in pathological sample & tumour antigens from patient specimens. 2-another application is identified the infections in (skin , renal, lung and liver biopsy) to detect an antigen within a tissue or within compartments of cells.	1- It often used in the detection circulating pathogenic autoantibodies. 2-Commenly used in(Dermatology) to detect of anti-nuclear antibodies (ANA) found in the serum of patients with SLE(Systemic Lupus Erythematosus).

DIF ASSAY (VS) IDIF ASSAY

	Direct IF	Indirect IF
Time	Shorter procedure, single labelling step	Longer, double labelling step
Cost	Expensive	Relatively inexpensive
Complexity	Less steps	Additional steps involved
Flexibility	Limited flexibility	Greater flexibility
Sensitivity	Less	High, amplification of the signal
Species Cross-reactivity	Minimised	May cross-react with species other than the target

THANK YOU