Immunofluorescence Assay

Immunofluorescence is an assay which is used primarily on biological samples and is classically defined as a procedure to detect antigens in cellular contexts using antibodies.

Immunofluorescence may be used for,

- Antigen detection in cells inoculated with field samples
- Antigen detection in naturally infected cells (nasal epithelium, semen)
- Antigen detection in pathological material
- Antibody detection
- Other fields (virus titration, histology, etc.)

Conjugate: Antibody labeled with fluorescent compounds.

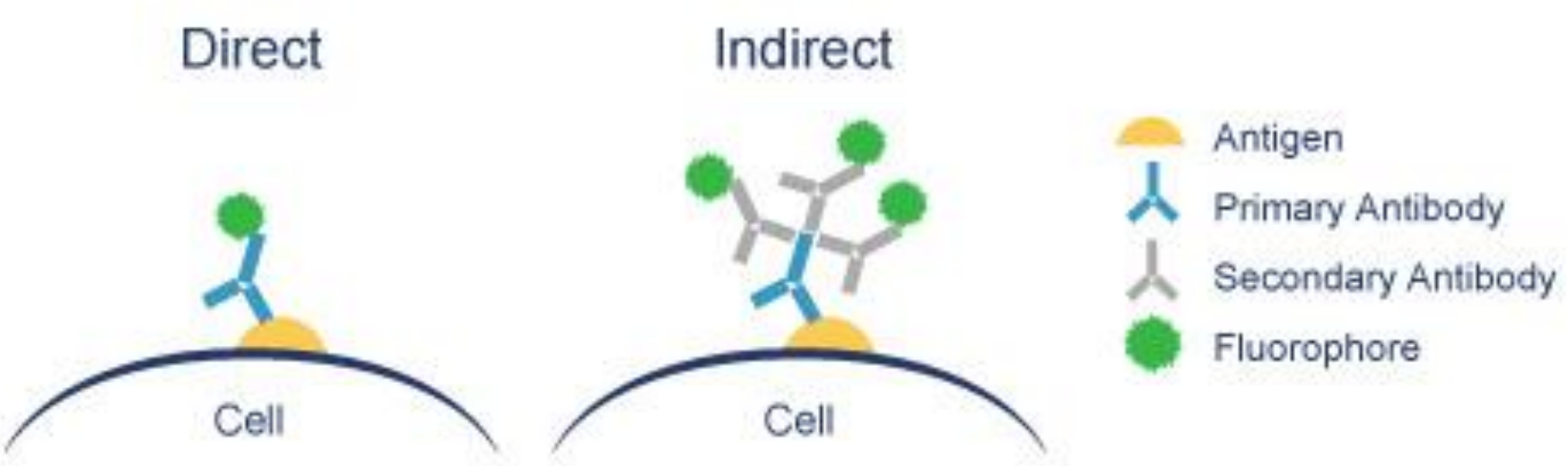
- Marker substance
- Fluorescent compounds; Are fluorescent brighteners under fluorescent light.
- The most important markers are rodamin and derivatives of fluorescein.
- Rhodamine compounds: Red violet TMRTC: tetramethyl rhodamine thiocyanate)
- Fluorescein compounds: yellow-green luminescence (FITC: fluorescein isothiocyanate)

Antibody in conjugate

- The assay is specific for the agent (eg if the IF test is performed to investigate the presence of the "A" antigen, the antibody is directed against the "A" antigen)
- If there is an antibody present in bovine serum, the anti-bovine IgGs are found in the conjugate structure
- Direct IF and NIF tests were performed against conjugate antigens and indirect IF tests were performed against primer antigens.

Methods

- Direct IF test (for Ag detection)
- Indirect IF test (for Ag or Ab determination)
- Neutralization IF test (for Ab detection)


Direct
Cell
Indirect
Cell
Antigen
Primary Antibody
Secondary Antibody
Fluorophore

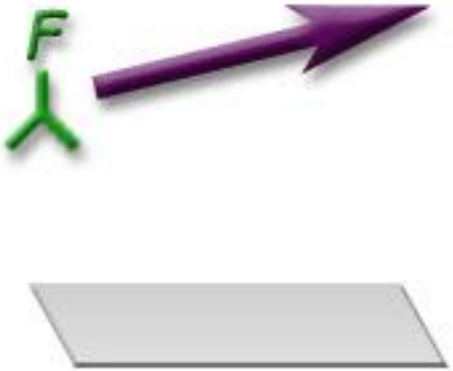
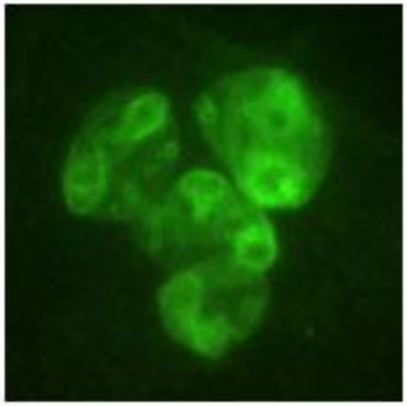
Materials

- Cell culture
- conjugates
- Virus (known or suspected)
- Serum (known or suspected - excluding Direct IF)
- Fluorescent microscope

Direct IF assay

- Inoculation is made from material suspected to cell cultures produced as monolayer in tubes.
- Following the appropriate incubation period (24-72 hours) cell surfaces are washed.
- Add the medium conjugate. After incubation (usually 1 hour) the cell surfaces are washed again and the cells are checked under a fluorescent microscope.

Immunofluorescence Assay (IFA) or Fluorescent Antibody (FA) Test

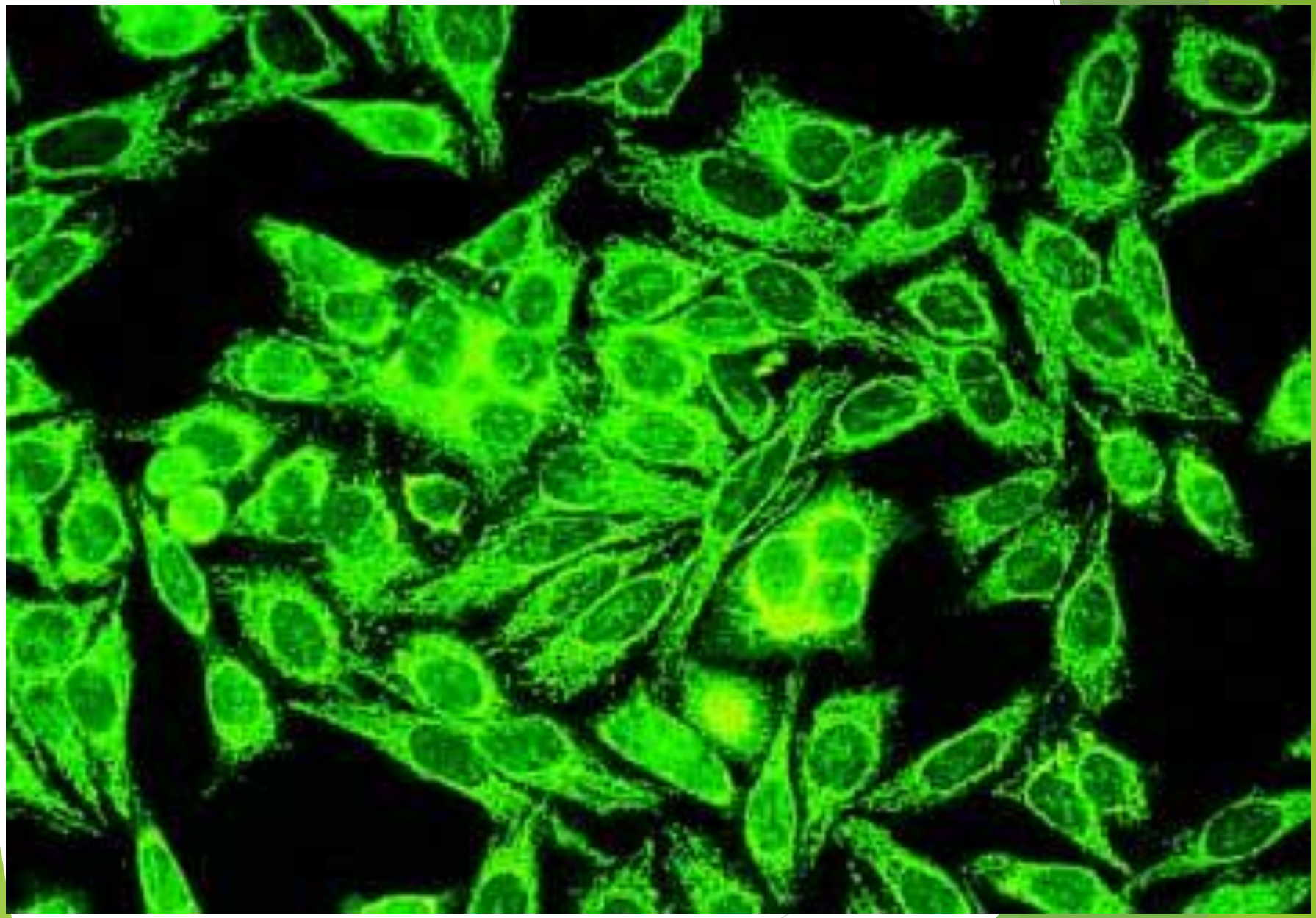
REAGENTS:	POSITIVE SAMPLE	NEGATIVE SAMPLE
unknown sample fixed to slide (may contain Ag of interest) 	Ag is present on slide 	Ag is not present on slide 
REAGENTS: fluorescein-labeled Ab to Ag of interest  Allow time to react Wash away unbound Abs		
PROCEDURE: observe under a fluorescence microscope  POSITIVE: green fluorescence NEGATIVE: no fluorescence		

Result

- Fluorescent (+) direct IF (+).
- Fluorescent (-) direct IF (-).

Epithelium Cells

Immunofluorescence staining of epithelium cell (Hep-2) mitochondria.



Indirect Immunflorescence assay

- The test is used to detect antibody or Ag presence. It is often applied to investigate the presence of Ab.
- Antibodies in the conjugate used in the test are specific to the primer antibody.

Method

- Known viruses are inoculated into monolayer-layered cell cultures in tubes.
- After the appropriate incubation period, the medium is placed in suspected serum and incubated for 1 hour at 37 ° C.
- Following incubation, the cell surface is washed and the conjugate is added and incubated for 1 hour at 37 ° C.
- Following incubation and washing, cells are examined under a fluorescent microscope.

Result

- If the fluorescent (+) Indirect IFT (+). (There is known virus-specific antibody in the suspected serum).
- If the fluorescent (-) Indirect IFT (-). (There is no known virus-specific antibody in the suspect serum)