IMMUNOFLUORESCENCE ASSAY

- Definition:

It is an immunocytochemical method in which the presence of the agent (or Ag) or antibodies against them in the suspicious material is investigated by using antibodies labeled with fluorescent compounds.

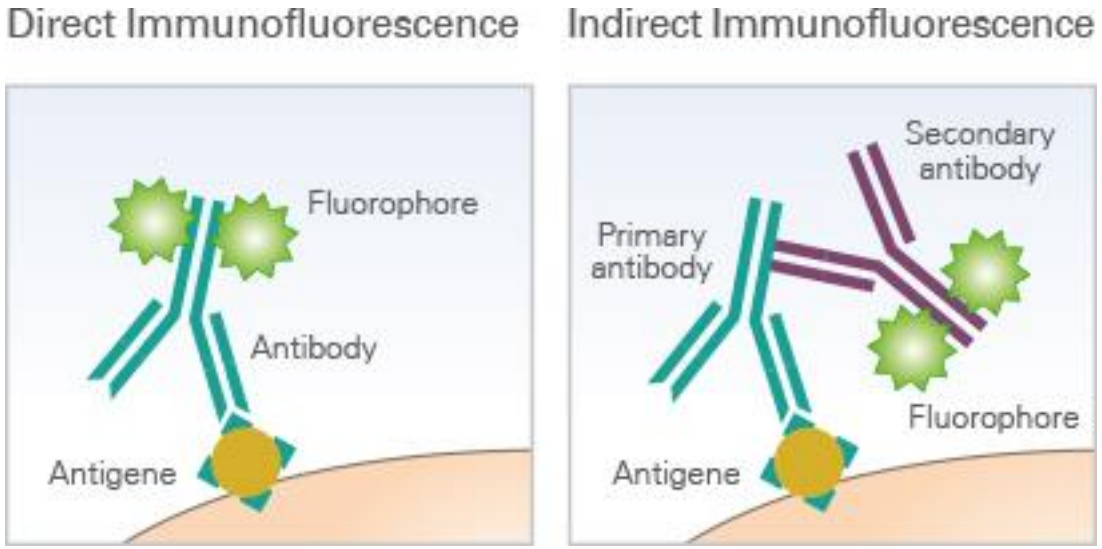
Purposes of Use

1. Antigen detection in cells inoculated with a morbid material
2. Antigen detection in naturally infected cells (nasal epithelium, sperm)
3. Antigen detection in pathological material
4. Antibody detection
5. Other (virus titration, histology, etc.)

What we need to performed the test?

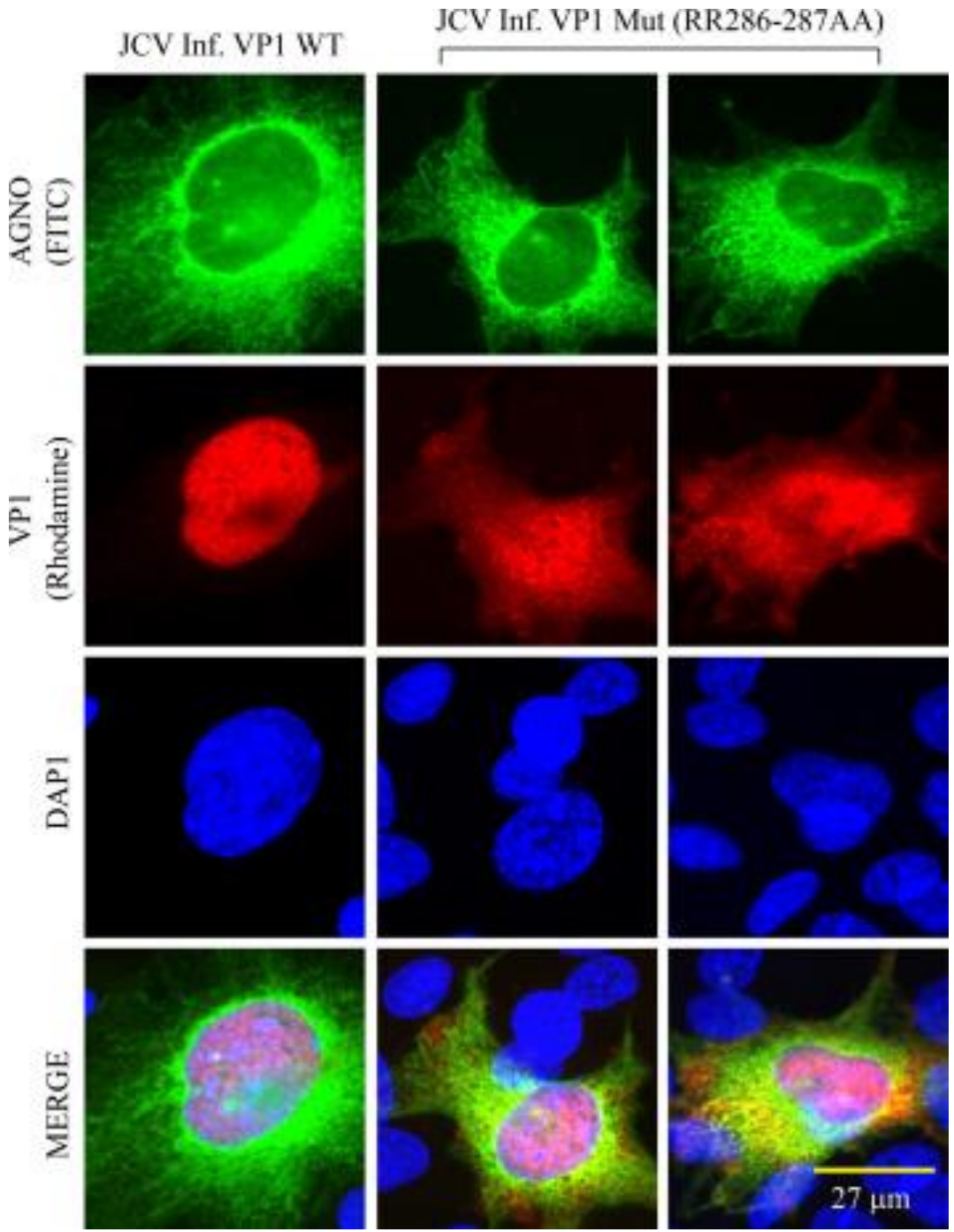
- Cell culture
- Conjugate
- Virus (Known or suspected)
- Serum (known or suspected – not for Direct IF)
- Fluorescence microscope

- Conjugate: Antibody labeled with fluorescent compounds.



https://ibidi.com/content/364-the-principle-of-immunofluorescence-assays

- Fluorescent compounds; They are substances that produce a colored glow under fluorescent light.
- The most important markers are rhodamine and fluorescein derivatives.
  - Rhodamine compounds: Red rhodamine TMRTC:Tetramethylrhodamine isothiocyanate)
  - Fluorescein compounds: Yellow-green luminescence (FITC: Fluorescein isothiocyanate)



A. Sami Saribas, Anna Bellizzi, Hassen S. Wollebo, Thomas Beer, Hsin-Yao Tang, Mahmut Safak (2023) Human neurotropic polyomavirus, JC virus, late coding region encodes a novel nuclear protein, ORF4, which targets the promyelocytic leukemia nuclear bodies (PML-NBs) and modulates their reorganization, Virology,587, 109866, https://doi.org/10.1016/j.virol.2023.109866.

https://bitesizebio.com/33529/fluorescence-microscopy-the-magic-of-fluorophores-and-filters/

Antibody in the Conjugate

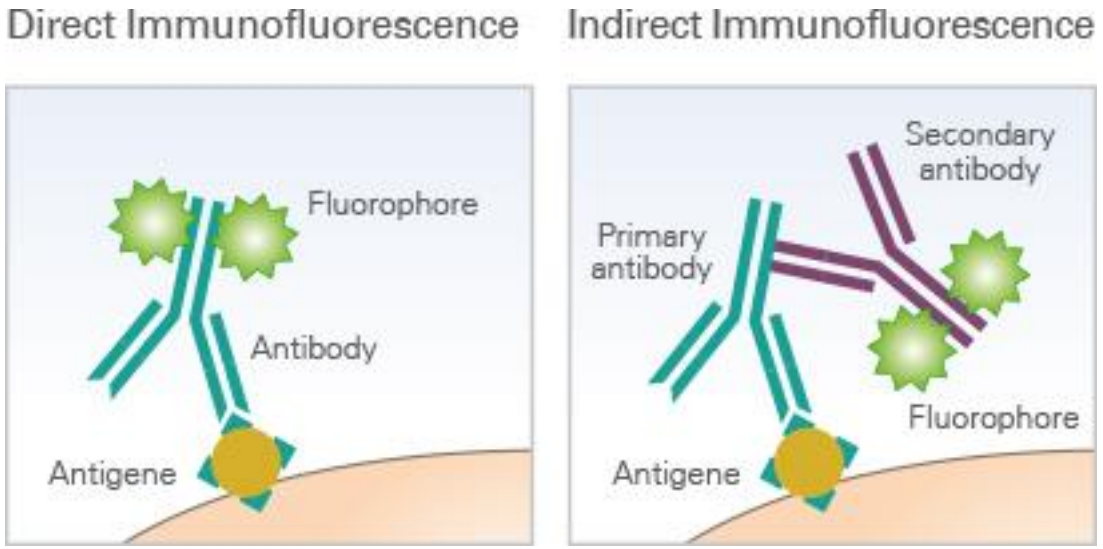
- It is specific to the virus to be detected

  For example, if the IF test is performed to investigate the presence of "A" antigen, the antibody has formed against the "A" antigen.)

- It is specific to the primary antibody.

  For example, the presence of an antibody in bovine serum is sought anti-bovine IgGs are present in the structure of the conjugate.

  If the presence of an antigen in a sample is sought anti-primer antibody antibody is in the structure of the conjugate.



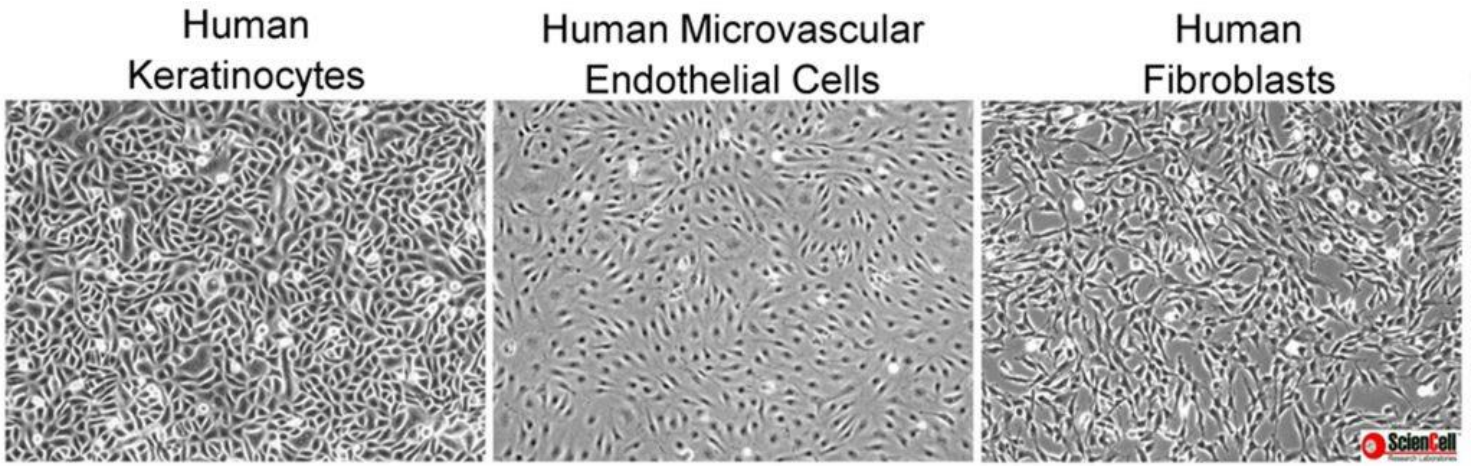
https://ibidi.com/content/364-the-principle-of-immunofluorescence-assays

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

Direct IF test:

1. Suspicious (virus ?) material is inoculated into monolayer cell cultures.
2. Following the appropriate incubation period (24-72 hours), cell surfaces are washed (with PBS).

https://kayfreesm.pics/product_details/18941246.html



https://sciencellonline.com/blog/13-technical-tips-for-successful-primary-cell-culture/

3. Add fixative material.
   - Fixation is an essential preliminary step in IF staining in order to prevent autolysis, mitigate putrefaction, and preserve morphology while maintaining antigenicity.
   - The ideal fixation method serves to immobilize target antigens without disturbing cellular architecture to allow antibodies maximum access to any targeted cellular components.

Neutral buffered Formalin, 10%.

Methanol, 100%. Bring temperature down to −20°C before use.

Acetone, 100%. Bring temperature down to −20°C before use.

4% Formaldehyde in PBS.

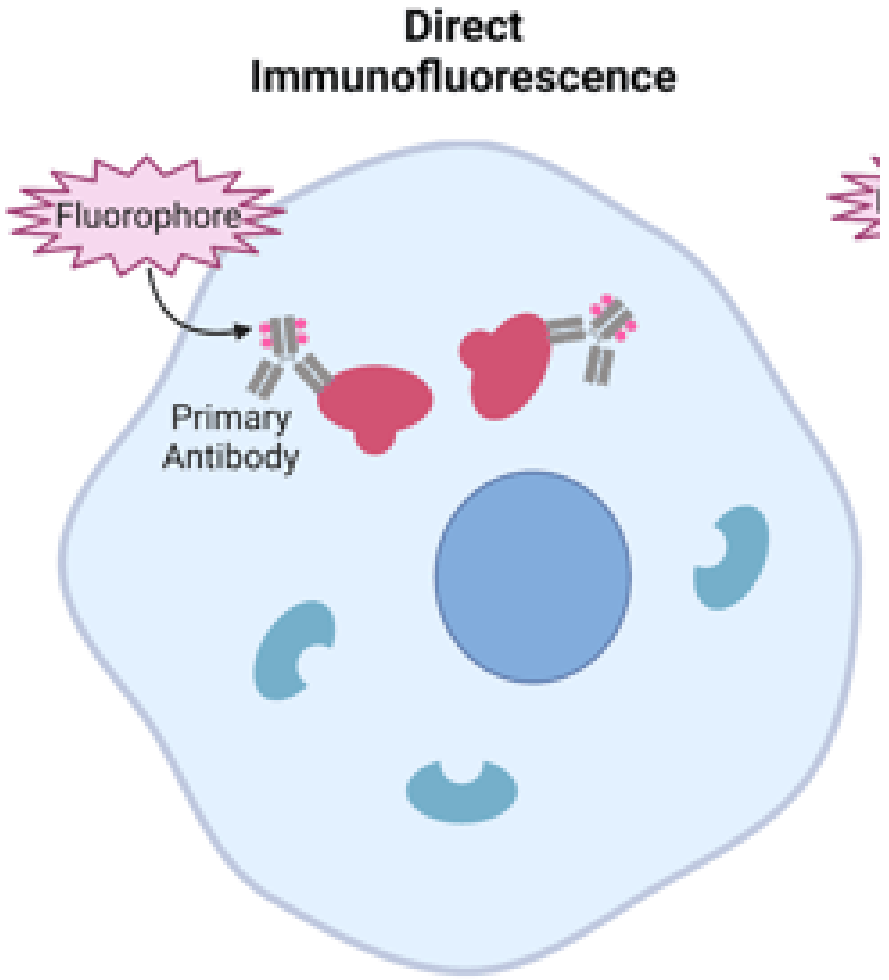
Etc.

Im K, Mareninov S, Diaz MFP, Yong WH. An Introduction to Performing Immunofluorescence Staining. Methods Mol Biol. 2019; 1897:299-311. doi: 10.1007/978-1-4939-8935-5_26. PMID: 30539454; PMCID: PMC6918834.

4. Conjugate is added.

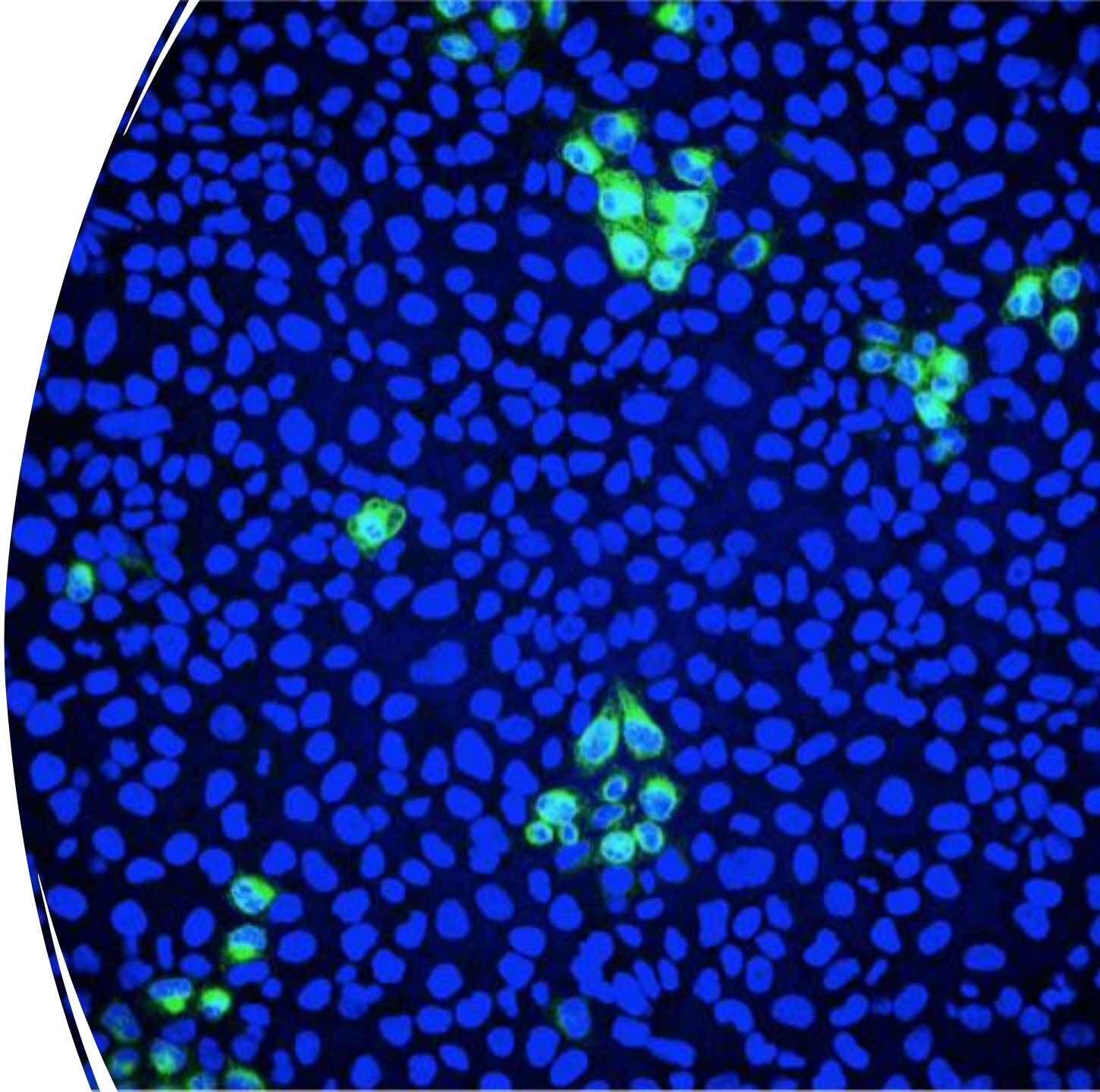
5. After incubation (usually 1 hour) the cell surfaces are washed with PBS again and the cells are checked under a fluorescence microscope.

*incubation: in incubator 5% CO2 at 37°C

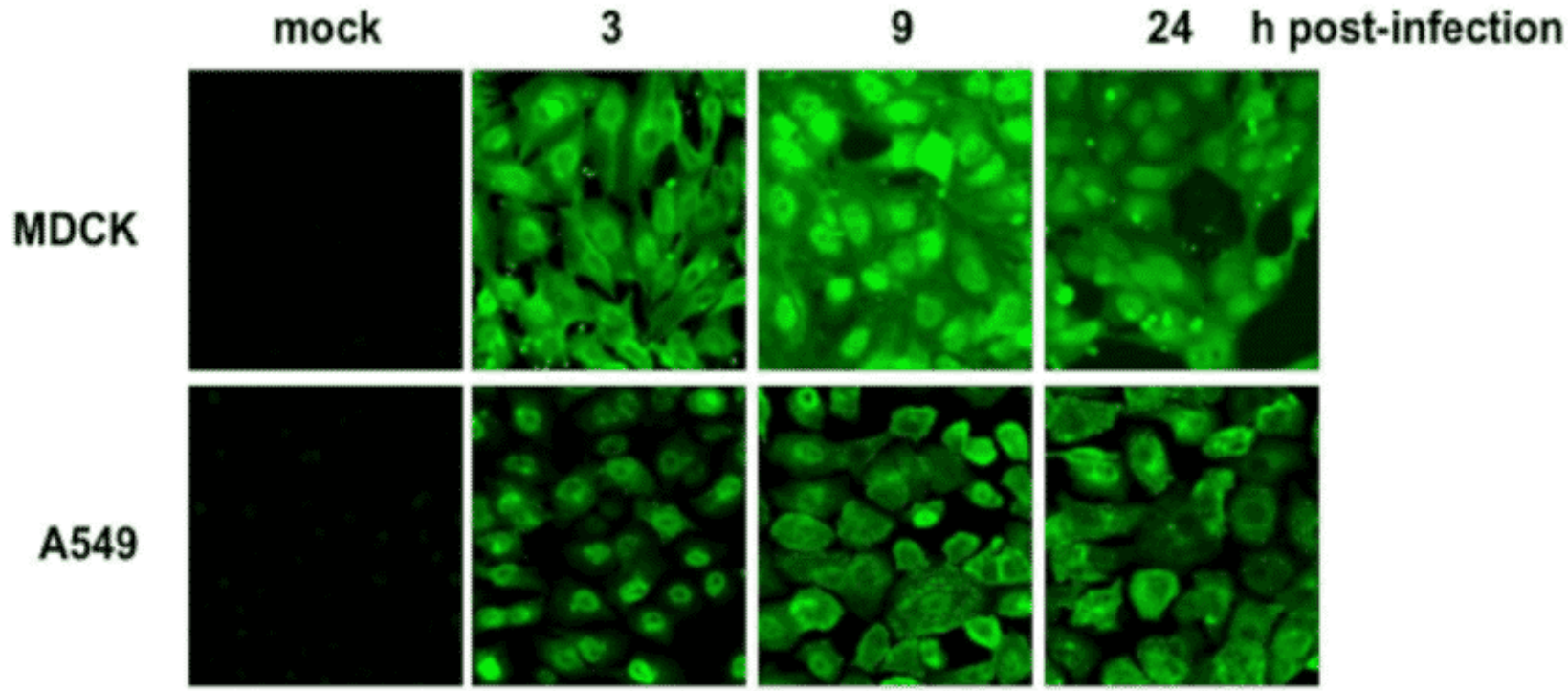


https://blog.addgene.org/introduction-to-immunofluorescence Image created with BioRender.com

- *Fluorescence microscopy can be used to quantify the percentage of infected cells. Green: Zika virus infected cells; Blue: cell nuclei.*



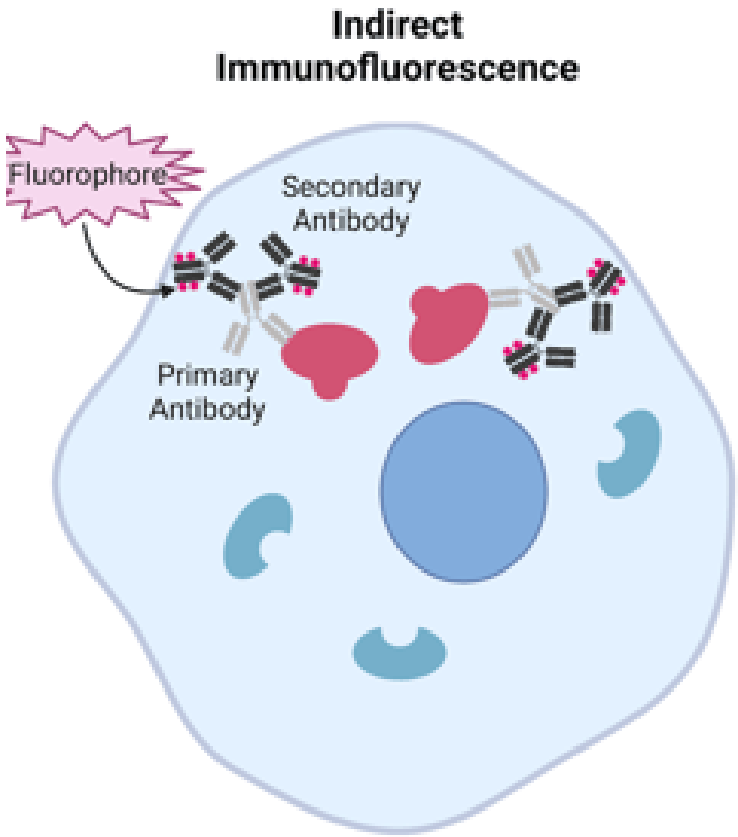
https://thenativeantigencompany.com/visualising-viruses-with-immunofluorescence-microscopy/



https://www.antibodies.com/influenza-a-virus-nucleoprotein-antibody-c43-a335

Indirect Immunflorescence assay

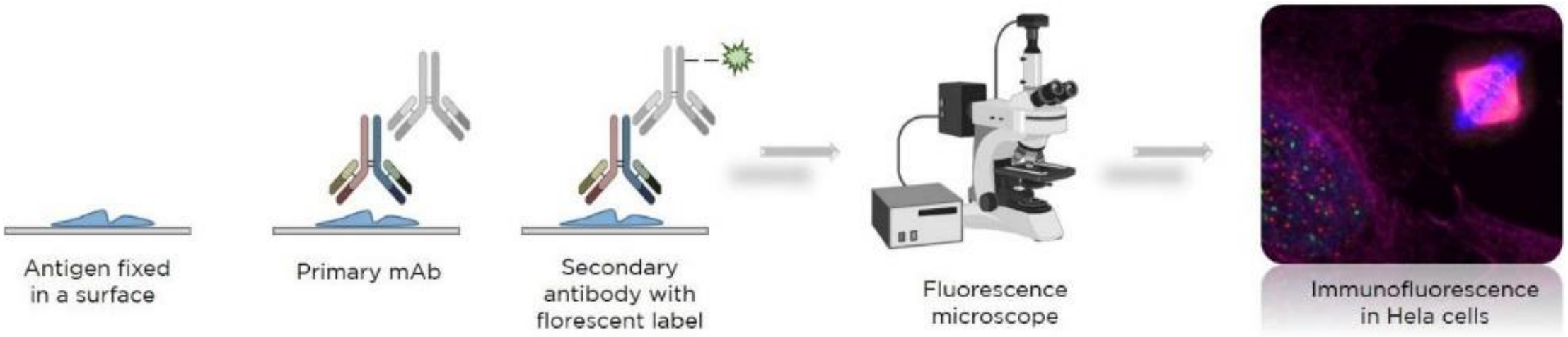
- The test is used to detect antibody or Ag presence. It is often applied to investigate the presence of Ab.
- Antibodies in the conjugate used in the test are specific to the primer antibody.



https://blog.addgene.org/introduction-to-immunofluorescence Image created with BioRender.com

To detect Ab,

- Known virus is inoculated into monolayer cell cultures.
- After the appropriate incubation period, the suspected serum is added to the medium and incubated for 1 hour at 37°C.
- Following incubation, cell surfaces are washed, and ddd fixative material for fixation.
- Conjugate is added and kept at 37°C for 1 hour.
- Following incubation and washing, cells are examined under a fluorescence microscope.



https://www.euromabnet.com/protocols/immunofluorescence-for-culture-cells.php

- 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

Neutralization IF test (NIF)

- Definition: It is based on the principle of keeping the suspected serum sample with known virus for neutralization and then checking whether neutralization has occurred by using the direct IF method.

Reminding:

- The test is applied to investigate the presence of antibodies.
- The conjugate contains known virus-specific Ab.

- The virus, diluted according to 100$TCID_{50}$, is incubated with an equal volume of suspect serum for 1 hour at 37°C.
- At the end of the period, 0.05 ml of cell suspension is dropped into the wells of the plate.
- Then, incubate at 37°C for 2-3 days.
- After incubation, cell surfaces are washed and added fixative.
- Then, conjugate (specific to a known virus) is added to the cells. The test is evaluated following incubation for 1 hour at 37°C.

Evaluation of NIF test result

- If fluorescence is (+), NIF is (-).

  There is no known virus-specific antibody in the suspect serum

- If fluorescence is (-), NIF is (+).

  There is a known virus-specific antibody in the suspect serum.