IMMUNOPEROXIDASE (IP) TEST (PEROXIDASE LINKED ANTIBODY ASSAY-PLA)

- Description:
- It is an immunocytochemical method that investigates the presence of the antigen (Ag) found in suspicious material or antibody against them by using substrate and antibody labeled with enzyme (conjugate).

Why do we use this test?

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

What do we need to perform the test?

- Cell culture
- Conjugates
- Substrate
- Virus (Known or suspected)
- Serum (known or suspected- *NOT for Direct IP)
- Invert microscope
- Test tablets and test solutions

- Conjugate: Antibody labeled with an enzyme.
    - (Marker substance + Antibody)

- Marker substance;

  is an enzyme such as, **Peroxidase, glucosoxidase, B-galactosidase, alkaline phosphatase.** PEROXIDASE is the most frequently used.

- The peroxidase enzyme is preferred because
  - ➢it is stable,
  - ➢easily purifiable and
  - ➢able to react with a large number of substrates.

- Antibody in the conjugate:
- It is specific for the virus to be detected
  - (For example, if the direct IP test is performed to investigate the presence of the "A" antigen, the antibody is formed against the "A" antigen)
    - Or
- It is specific to the primary antibody.
  - (For example, if we investigate the presence of an antibody in bovine serum, anti-bovine IgGs are in the structure of the conjugate)

The conjugate was prepared against the antigen in the direct PLA and NPLA (neutralization PLA) test

against the primary antibody in the indirect PLA test.

Substrate

- It reacts with the enzyme to reveal the presence of the immunocomplex.
- As a result of the enzyme-substrate relationship, staining occurs in regions where the viral antigen is present.

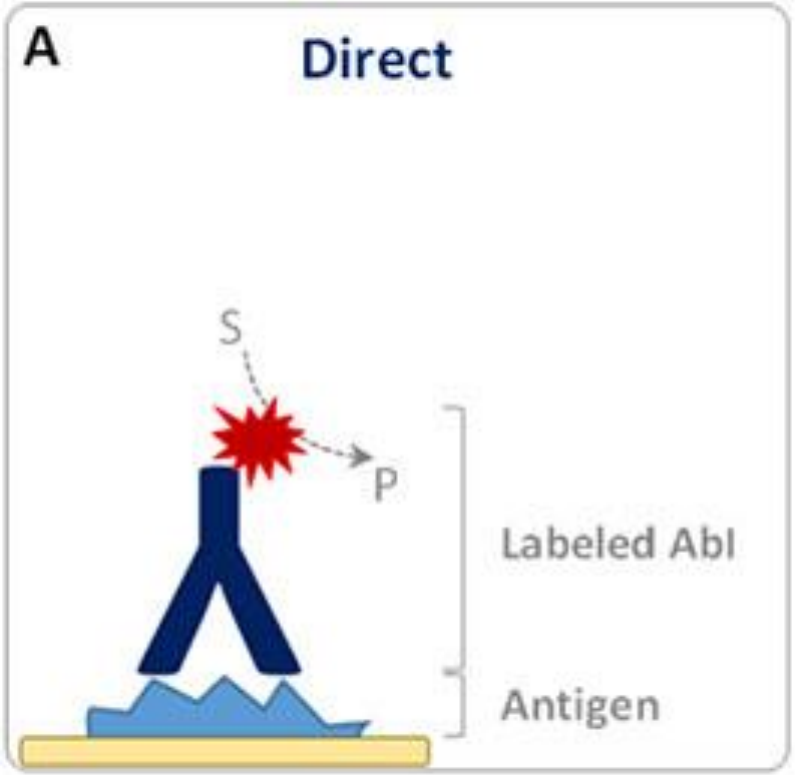
- **AEC (3-Amino-9-Ethylcarbazole)** is a frequently used perexidase-specific substrate and forms a **reddish brown** stain.

Methods of Assay

1. Direct IP test → for Ag detection
2. Indirect IP testing → for Ag or Ab determination
3. Neutralization IP test → for Ab test

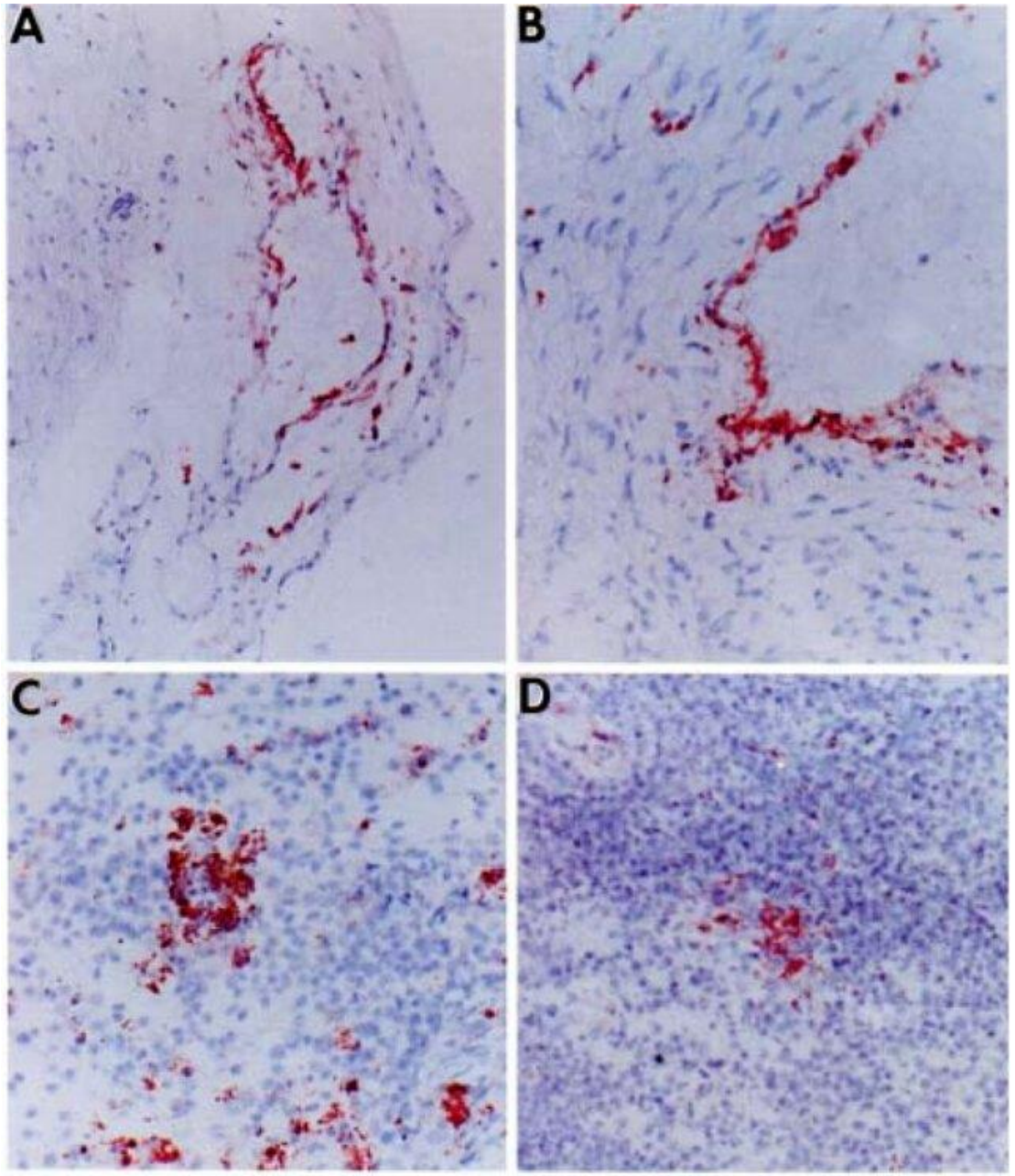
Direct IP (PLA) test

1. Suspected material was inoculated by the adsorption method to cell cultures produced as a monolayer in 24-well test tablets.
2. Following the appropriate incubation period (24-72 hours), cell surfaces is WASHED.
   - Incubation in 37 C with 5% CO_2
3. CONJUGATE is added onto the cell cultures. After incubation (usually 1 hour) the cell surfaces are re-washed.
4. Then, the substrate is placed on the cells and after 10-15 minutes the test is checked under an invert microscope.



https://www.enzolifesciences.com/science-center/technotes/2019/august/what-are-the-different-detection-methods-for-ihc?/

- Staining (+) PLA (+).
- Staining (-) PLA (-).



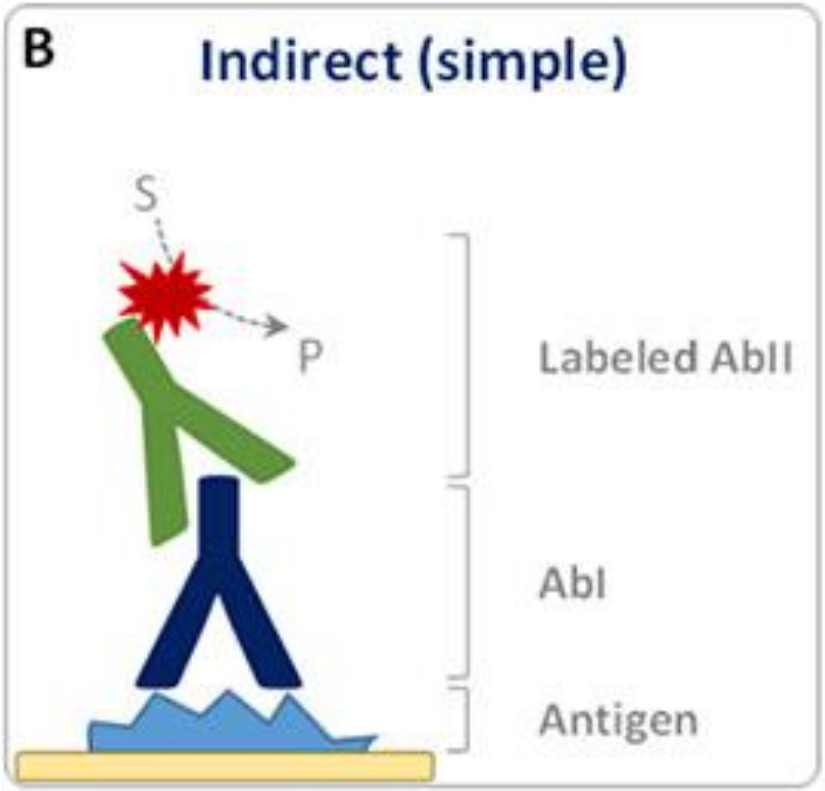
MacLachlan, N. J., Balasuriya, U. B. R., Rossitto, P. V., Hullinger, P. A., Patton, J. F., & Wilson, W. D. (1996). Fatal experimental equine arteritis virus infection of a pregnant mare: immunohistochemical staining of viral antigens. *Journal of Veterinary Diagnostic Investigation*, *8*(3), 367-374.

Indirect PLA

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

Method for indirect PLA

- Virus is inoculated into monalayer layered cell cultures.
- After the appropriate incubation period, the cell surface is WASHED and placed in suspected serum, incubated at 37 ° C for 1 hour.
- Following the incubation, the cell surfaces are washed again and Conjugate is added to the cells.
- İncubation step (37 ° C-1 h) and WASH again,
- Substrate is added to the cells and after 10-15 minutes the results are evaluated under an inverted microscope.



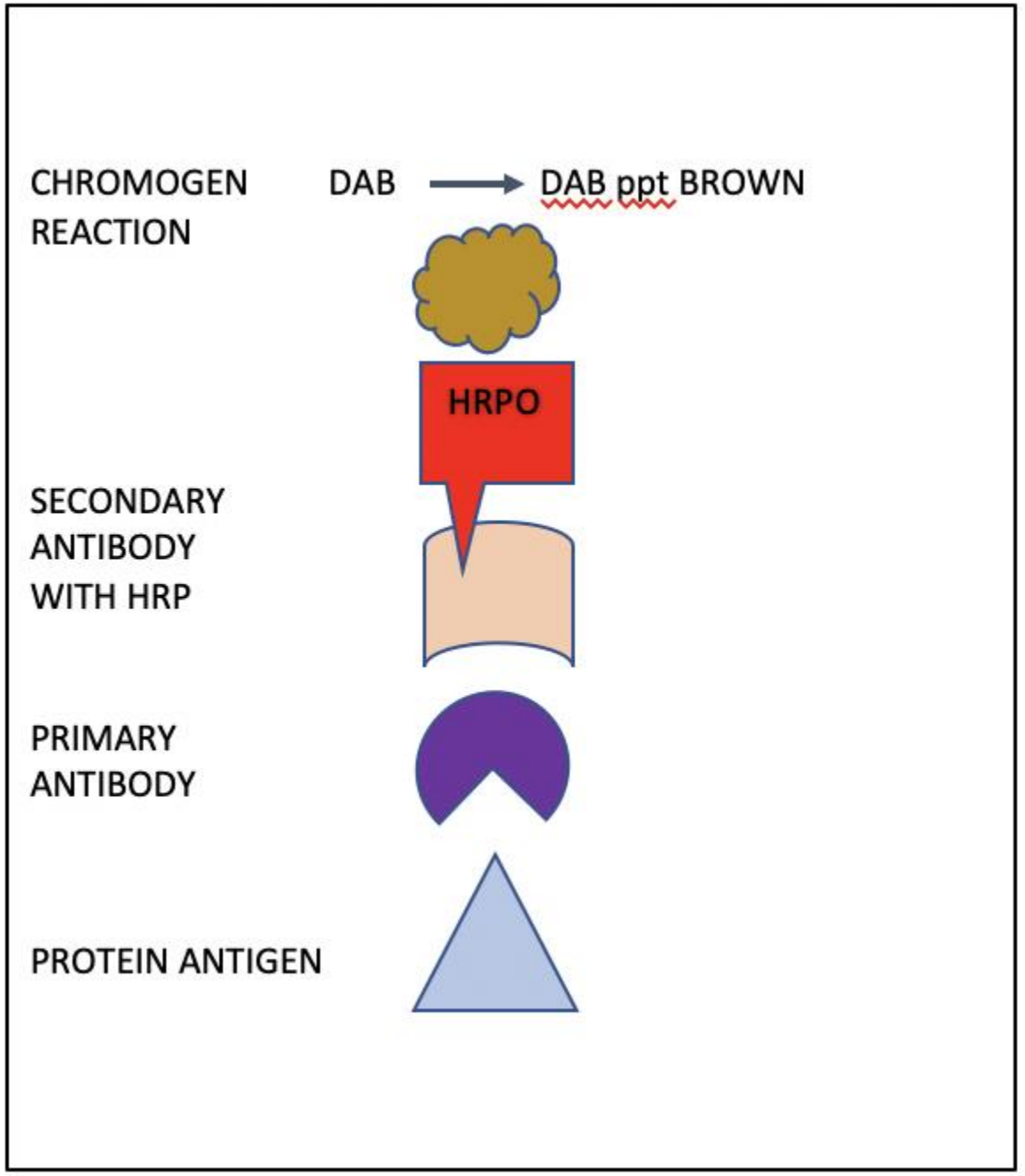
https://www.enzolifesciences.com/science-center/technotes/2019/august/what-are-the-different-detection-methods-for-ihc?/

If the staining is positive in the cells means Indirect IPT (PLA) POSITIVE.

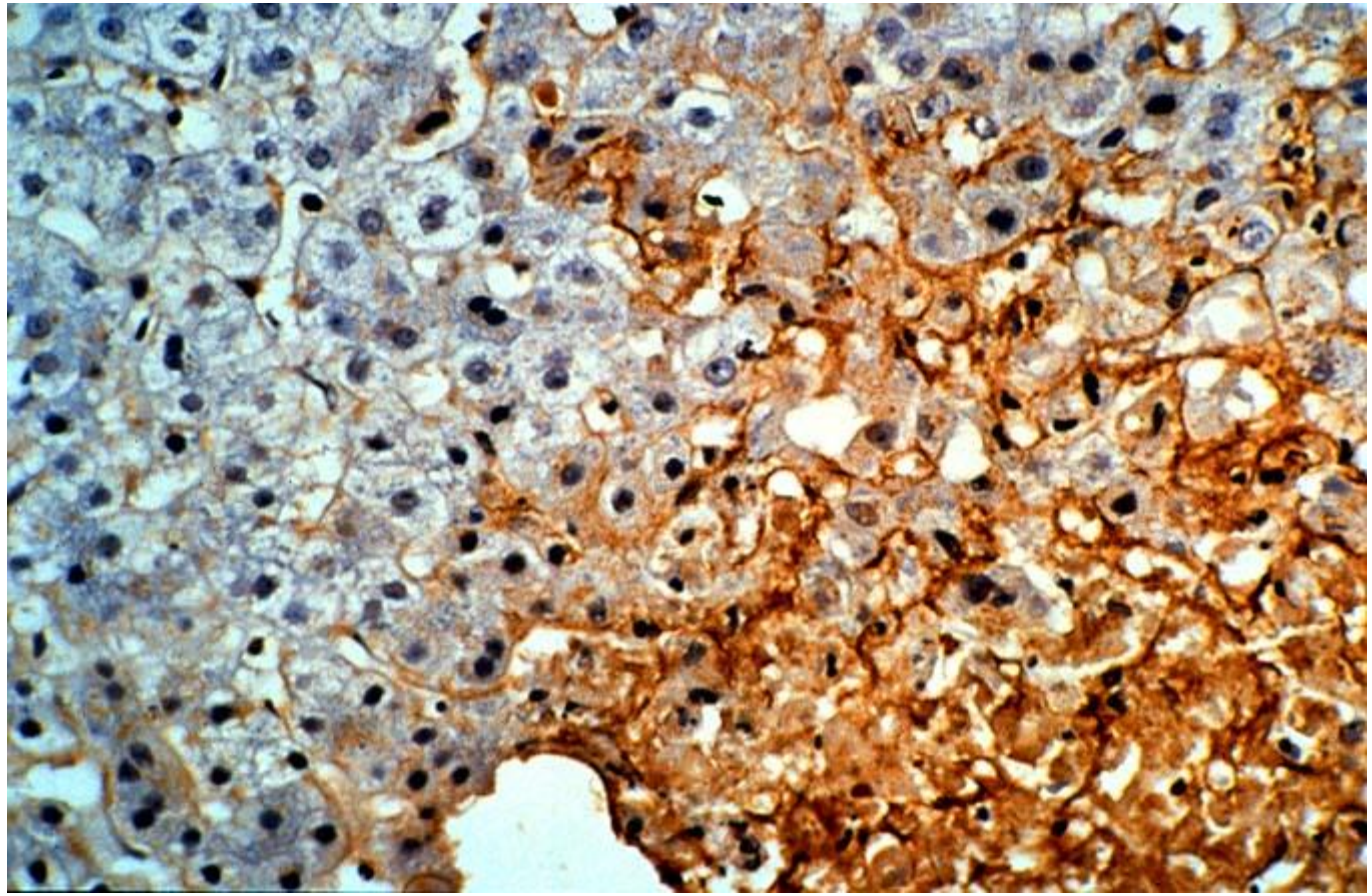
There are antibodies against virus-specific in suspected serum.

If the staining is negative in the cells means Indirect IPT (PLA) NEGATIVE.

There are NO antibodies against virus-specific in suspected serum.



https://rankinbiomed.com/2019/10/07/immunohistochemistry/



https://tpis.upmc.com/changeBody.cfm?url=/tpis/HB/H00034a.jsp

Neutralisation IP test (NPLA)

- Description: 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 IP method.

Reminding:

The test is used to investigate the presence of antibodies.

Conjugate contains virus-specific Ab.

Method

- The virus diluted at the rate of 100$TCID_{50}$ incubated for 1 hour at 37 °C with an equal volume of suspected serum.
  - For this purpose, mostly 96-wells plates are used.
- At the end of the period, 0.05 ml cell suspension is added to the wells of the plate and they are incubated at 37 ° C for 2-3 days.
- After incubation, cell surfaces are washed and added fixative.
- Conjugate (Conjugate virus specific) is added to the cells.
- Following incubation at 37 ° C for 1 hour, the cell surfaces are washed again and SUBSTRATE is added.
- The test is evaluated under an invert microscope after 10-15 minutes.

- If stain is (+), NPLA (NIP) is (-).

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

- If stain is (-), NPLA (NIP) is (+).

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