PEROXIDASE LINKED ANTIBODY ASSAY-PLA

An immunocytochemical method of detecting the presence (or Ag) of an agent or antibody (or Ag) in a suspected material using antibodies and substrates labeled with an enzyme.

Usage areas

- 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 an enzyme. (Marker substance + Antibody)

- **Marker substance**
- **Peroxidase, glucosoxidase, B-galactosidase, alkaline phosphatase are enzymes. 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 structure in conjugate

- Detection is specific for the intended agent (eg if the direct IP 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 PLA and NPLA tests against conjugate antigens, indirect PLA test against primer antigens.

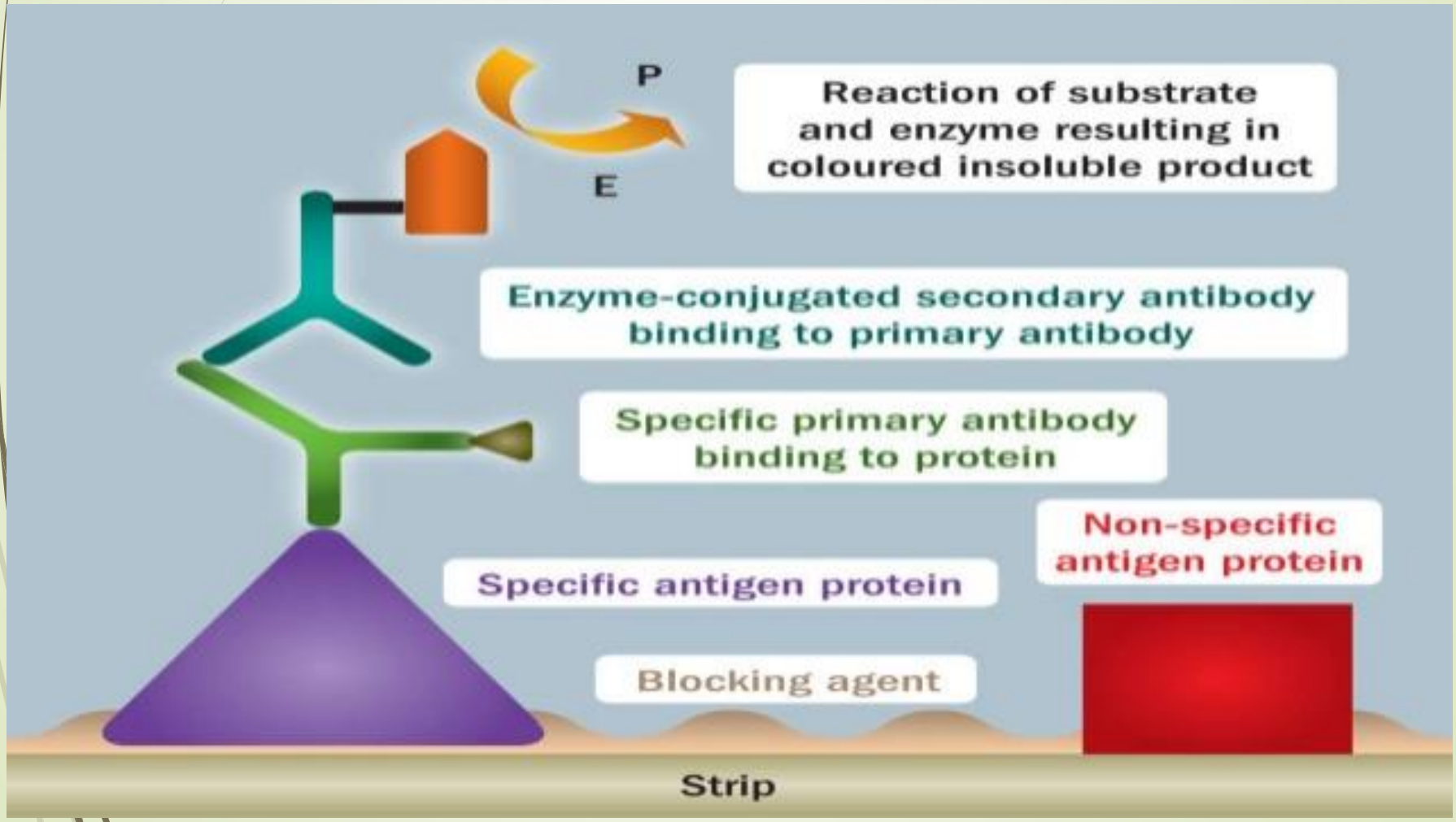
Substrate

- 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 is a frequently used perexidase-specific substrate and forms a reddish brown stain.

Methods of Assay

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

https://www.google.com.tr/imgres?imgurl=https%3A%2F%2Fimage.slidesharecdn.com%2Fsyphilisppt-150814202230-lva1-app6892%2F95%2Fsyphilis-serology-ppt-syphilis-laboratory-diagnosis-of-syphilis-vdrl-ftaabs-64-638.jpg%3Fcb%3D1439583907&imgrefurl=https%3A%2F%2Fwww.slideshare.net%2FSanjaysingh07%2Fsyphilis-serology-ppt-syphilis-laboratory-diagnosis-of-syphilis-vdrl-ftaabs&docid=si1LgJNPZKAPQM&tbnid=KhzKkyHHZDakfM%3A&vet=10ahUKEwjemfHjgYraAhVqJpoKHe3qBRsQMwg-KAEwAQ..i&w=638&h=359&bih=735&biw=1536&q=immunoperoxidase%20assay%20principle&ved=0ahUKEwjemfHjgYraAhVqJpoKHe3qBRsQMwg-KAEwAQ&iact=mrc&uact=8



Materials

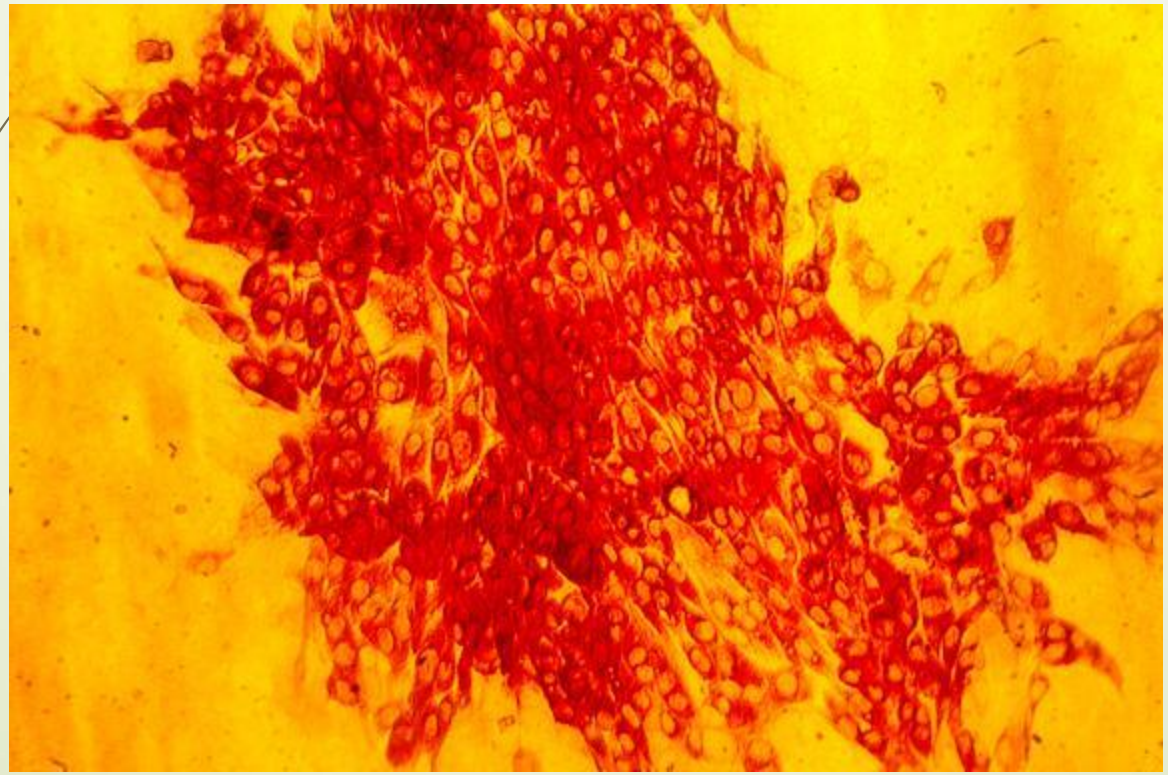
- Cell culture
- conjugates
- substrate
- Virus (suspect in known area)
- Serum (known or suspected - excluding direct IP)
- Invert microscope
- Test tablets and test solutions

Direkt PLA testi:

- In 24-well test tablets, culturing is done by adsorption-dependent method from monolayer-suspected cell cultures.
- Following the appropriate incubation period (24-72 hours), cell surfaces WASH.
- CONJUGATE is added onto the cell cultures. After incubation (usually 1 hour) the cell surfaces are re-worn.
- SUBSTRAT is placed on the cells and after 10-15 minutes the test is controlled in the inverted microscope.

Direct PLA assay result

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



Indirect Immunoperoxidase Assay

- Test Ag is used to detect the presence of antibodies in the body. It is often applied to investigate the presence of Ab.
- Antibodies in the conjugate construct used in the test are primer specific to the antibody.

Method

- The virus is inoculated into cell cultures of tablets monolayers layered.
- After appropriate incubation, the cell surface is washed and incubated at 37 ° C for 1 hour.
- Following incubation, the cell surfaces are washed and CONJUGATE is added onto the cells.
- After incubation (37 ° C-1 h) and washing the substrate is added to the cells and after 10-15 minutes the results are evaluated on an inverted microscope.

Result

- If staining in cells (+) is Indirect IPT (+). (There is known virus-specific antibody in the suspected serum).
- If staining in cells (-) is Indirect IPT (-). (There is no known virus-specific antibody in the suspect serum)