

New diagnostical methods in immuology

Enzyme-Linked Immunosorbent Assay (ELISA or EIA)

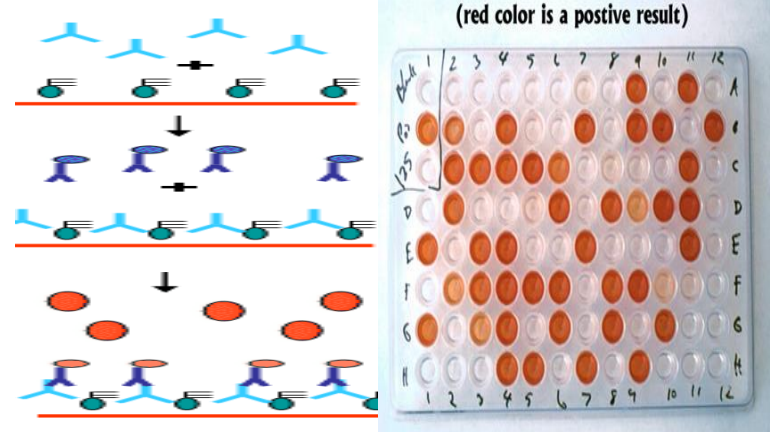
- The substances detected by ELISA tests include hormones, bacterial antigens and antibodies.

ELISA test may be used to diagnose:

- HIV, which causes AIDS
- Lyme disease
- pernicious anemia
- Rocky Mountain spotted fever (RMSF)
- rotavirus
- squamous cell carcinoma
- syphilis
- toxoplasmosis
- varicella-zoster virus, which causes chicken pox and shingles

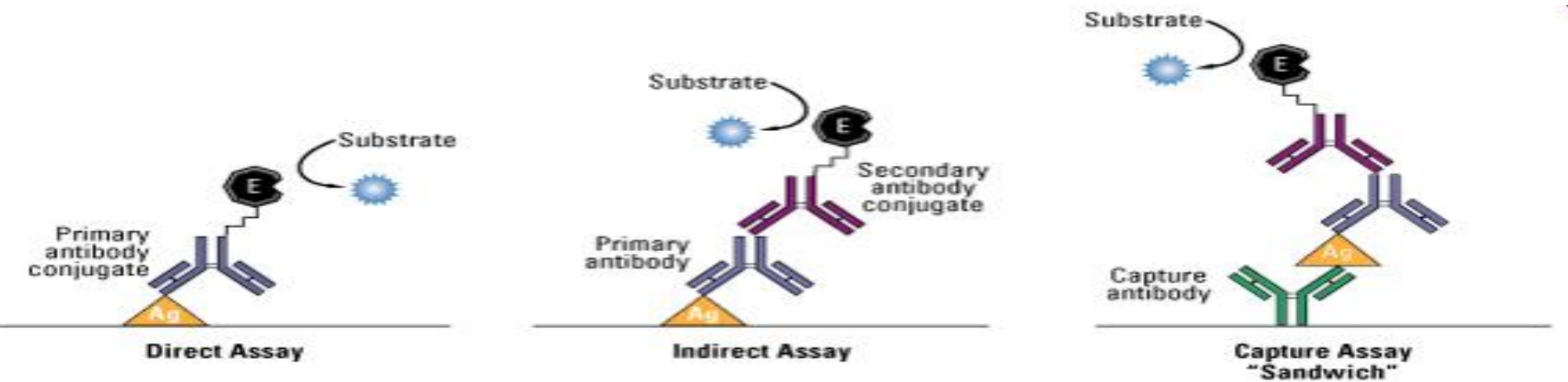
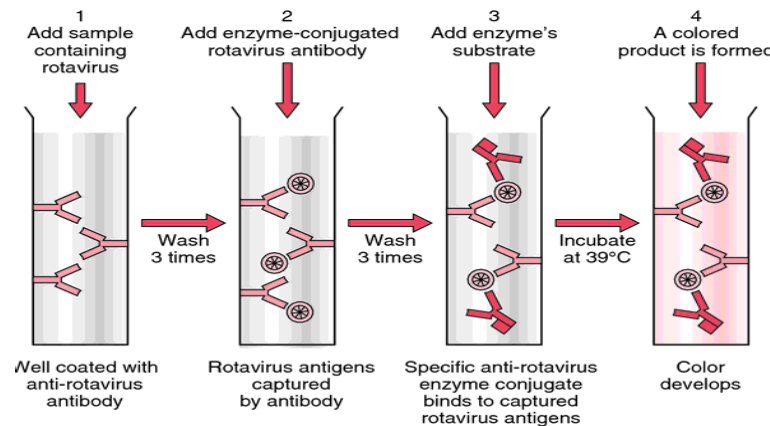
For an antibody ELISA:

- antigens are stuck onto a plastic surface,
- sample is added
- antibodies for the disease we are testing for will bind to the antigens
- Next a second antibody with a marker is added
- positive reaction is detected by the marker changing colour when an appropriate substrate is added
- If there are no antibodies in the sample, the second antibody will not be able to stick and there will be no colour change.



For an antigen ELISA:

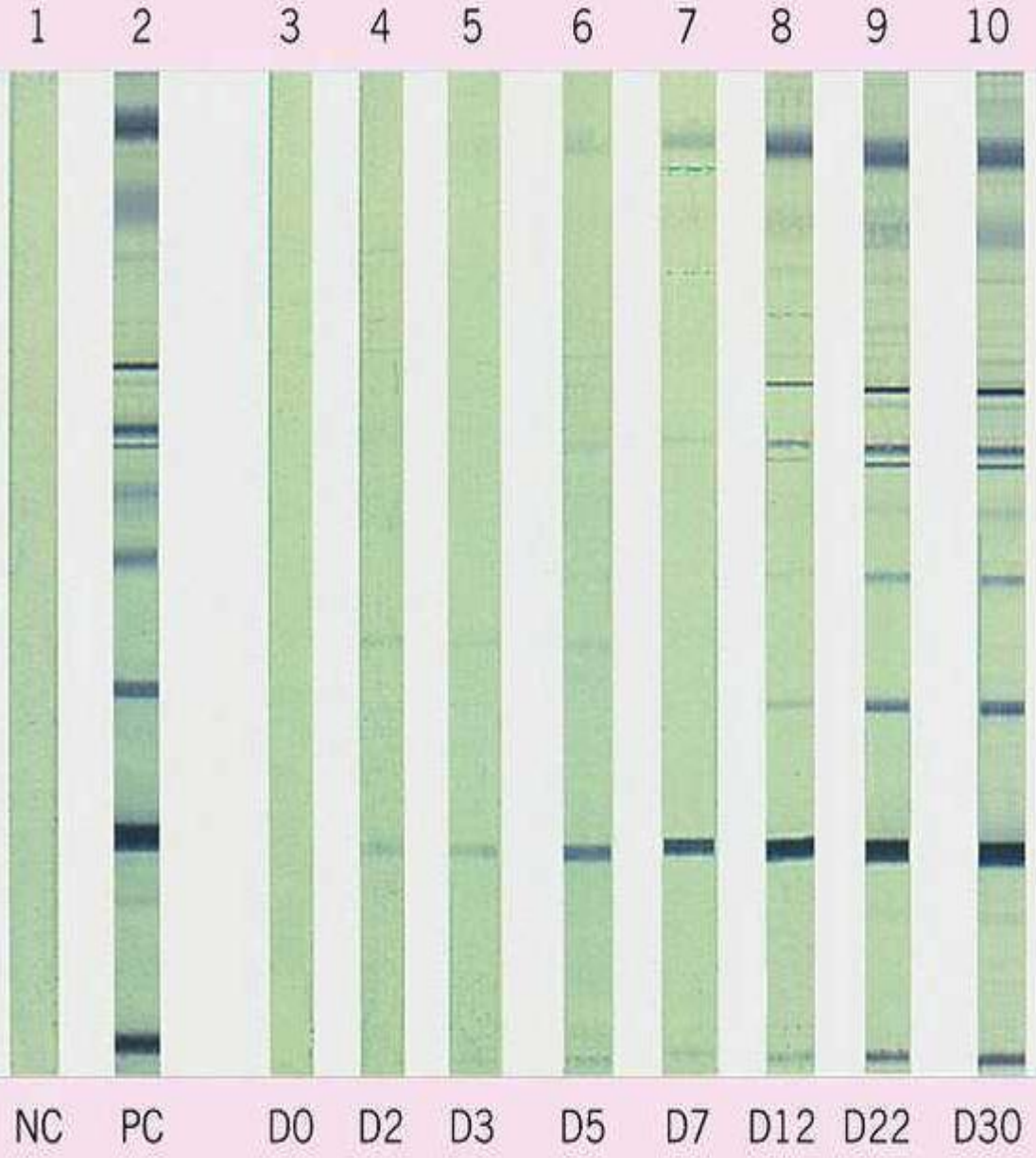
- antibodies are bound to a plastic surface
- sample is added
- if antigens from the virus we are testing for are present they will stick to the antibodies.
- positive reaction is detected by the marker changing colour when an appropriate substrate is added
- If there are no antigens in the sample, the second antibody will not be able to stick and there will be no colour change.



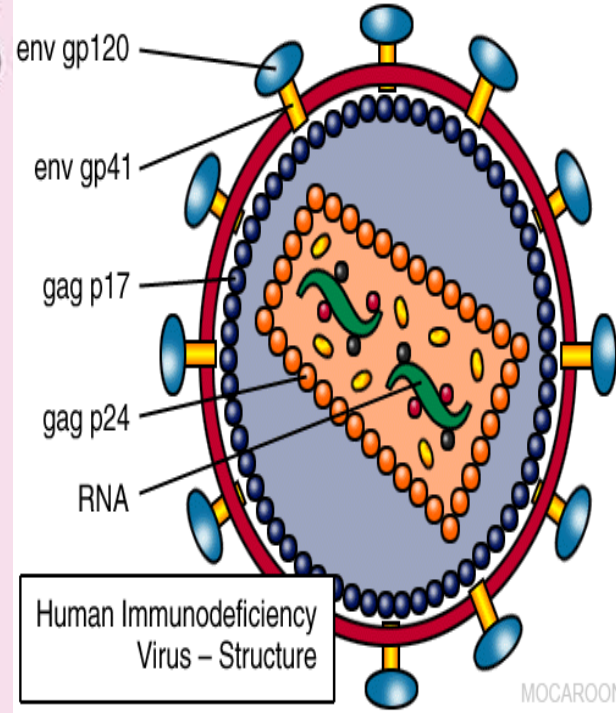
Western blot

- Agens is divided by electrophoresis to separate antigens that are immobilised on the nitrocellulose strip.
- The serum of patient is added on the strip to react with antigens, specific antibodies to the antigen or epitope is reacting on the place where the appropriate antigen is bound and the complex ag-ab is formed.
- Next steps are identical as in ELISA – conjugate labelled with enzyme, substrate. Color reaction is produced for positive reaction (presence of antibody against an epitope or antigenic fragment).
- **Detection of specific antibodies against individual antigens**

WESTERN BLOT REACTIVITY IN ONE HIV-1 SEROCONVERTER



MW
gp160
gp120
p68
p55
gp41
p40
p34
p24
p17



ELISPOT – Enzyme Linked Immunosorbent Spot Assay

- To measure even low quantities of activated lymphocytes
- Rapid information about specific cell immunity on the base of minimal invasivity – detection from periferal blood sample lymphocytes
- Detection of low quantities of cytokines produced by stimulsed T cells
- ELISpot assays employ the sandwich enzyme-linked immunosorbent assay (ELISA) technique.

PCR – polymerase chain reaction

- Every organism – microbes included contain specific sequence of amino acids
- With the help of enzymes this sequence can be „cut out“ and millions of copies can be synthesised in the infectious material if present there.
- These can be then identified via „antibodies“ against this sequence – labelled by enzymes or fluorochrome ...
- The purpose of a PCR is to make a huge number of copies of a gene. This is necessary to have enough starting template for sequencing.