College of Medicine
Al-NAHRAIN UNIVERSITY

Practical Virology Lab-8- Serological Methods in Virology

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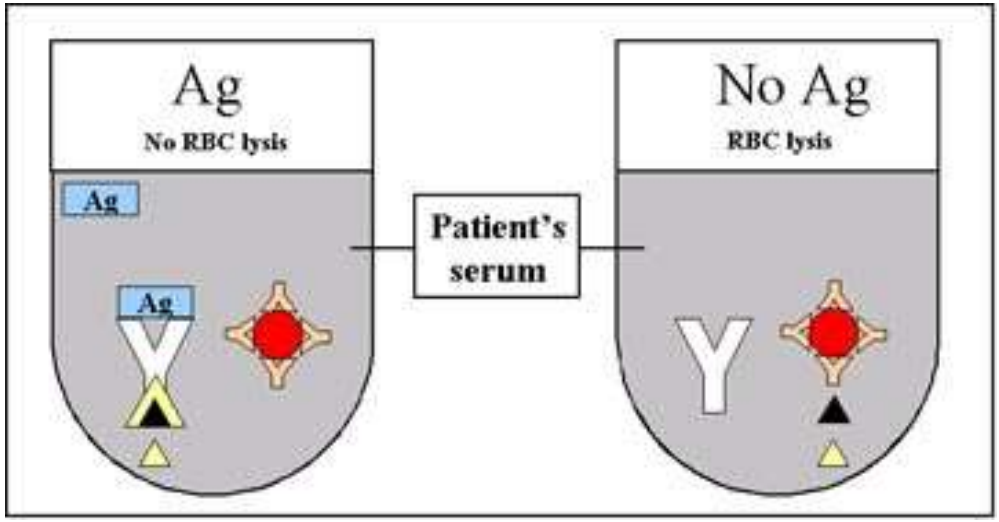
Serological Methods in Virology:

Detection of rising titres of antibody between acute & convalescent stages of infection, or the detection of IgM in primary infection.

- **Classical Techniques**
  - Complement fixation tests (CFT)
  - **Hemagglutination inhibition tests (HI)**
  - Immuno-fluorescence techniques (IF)
  - Neutralization tests (NT)
- **Newer Techniques**
  - Radioimmunoassay (RIA)
  - Enzyme linked immunosorbent assay (ELISA)
  - Particle agglutination.
  - Western blot (WB) or recombinant immunoblot assay (RIBA).
  - Immunochromatography

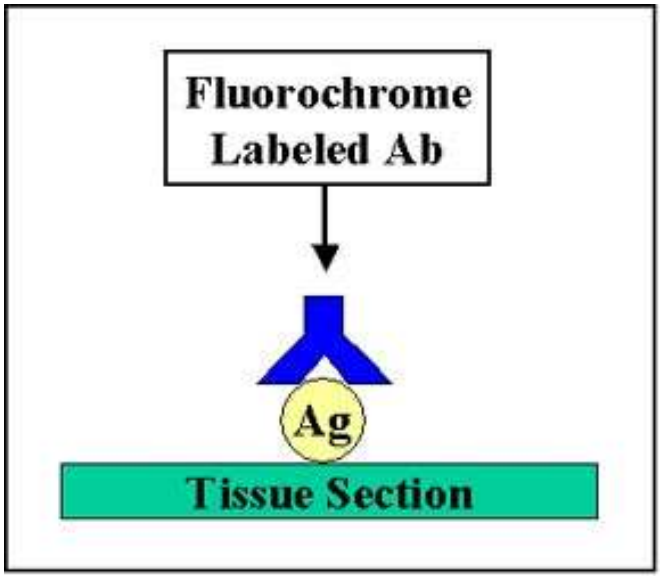
Complement fixation tests (CFT)

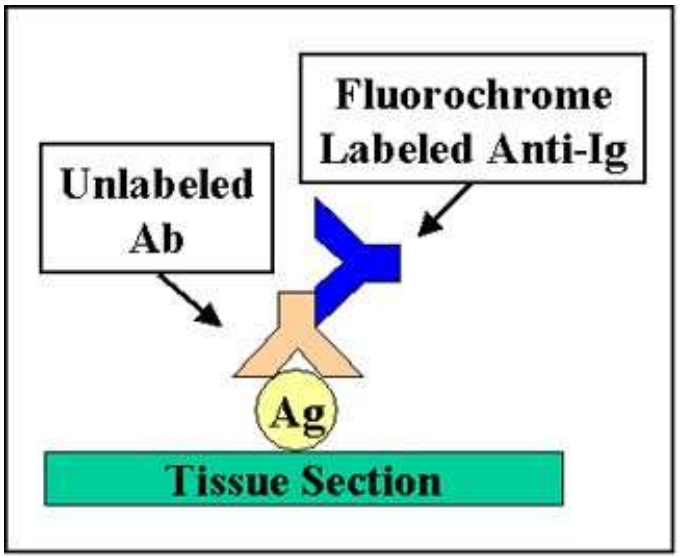
Tests for antigen/antibody complexes that rely on the consumption of complement are termed complement fixation tests and are used to quantitate antigen/antibody reactions. This test will only work with complement fixing antibodies (IgG and IgM are best).



Immuno-fluorescence techniques (IF) (1)

Immunofluorescence is a technique whereby an antibody labeled with a fluorescent molecule (fluorescein or rhodamine or one of many other fluorescent dyes) is used to detect the presence of an **antigen** **in** or **on** a cell or tissue by the fluorescence emitted by the bound antibody.





Immuno-fluorescence techniques (IF) (2)

Principle:

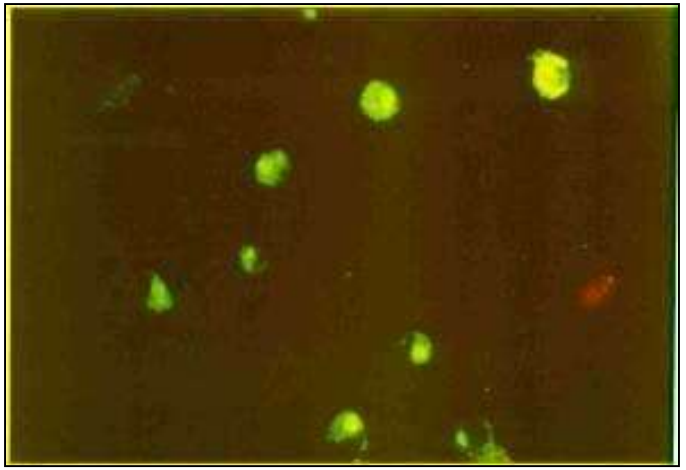
- Use fluorescein isothiocyanate labeled-immunoglobulin to detect **antigens** or **antibodies** according to test systems.
- Requires a fluorescent microscope.
- e.g. Herpes virus IgM, Dengue virus, Rabies virus.

Advantages:

- Sensitive & specific
- Can be used for discrepant analysis

Limitations:

- Expensive (Reagents & equipment)
- Cross reactivity
- Non-specific immuno-fluorescence
- Time taken (1 day)



Cell infected with Dengue virus

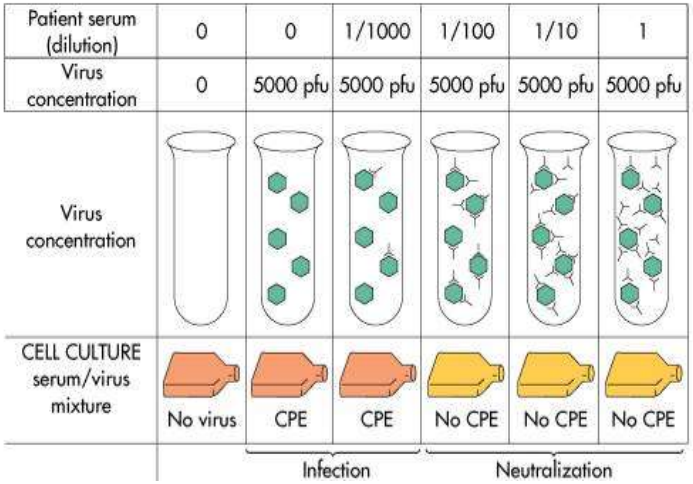
Virus Neutralization Test (VNT)

VNT are conducted by:

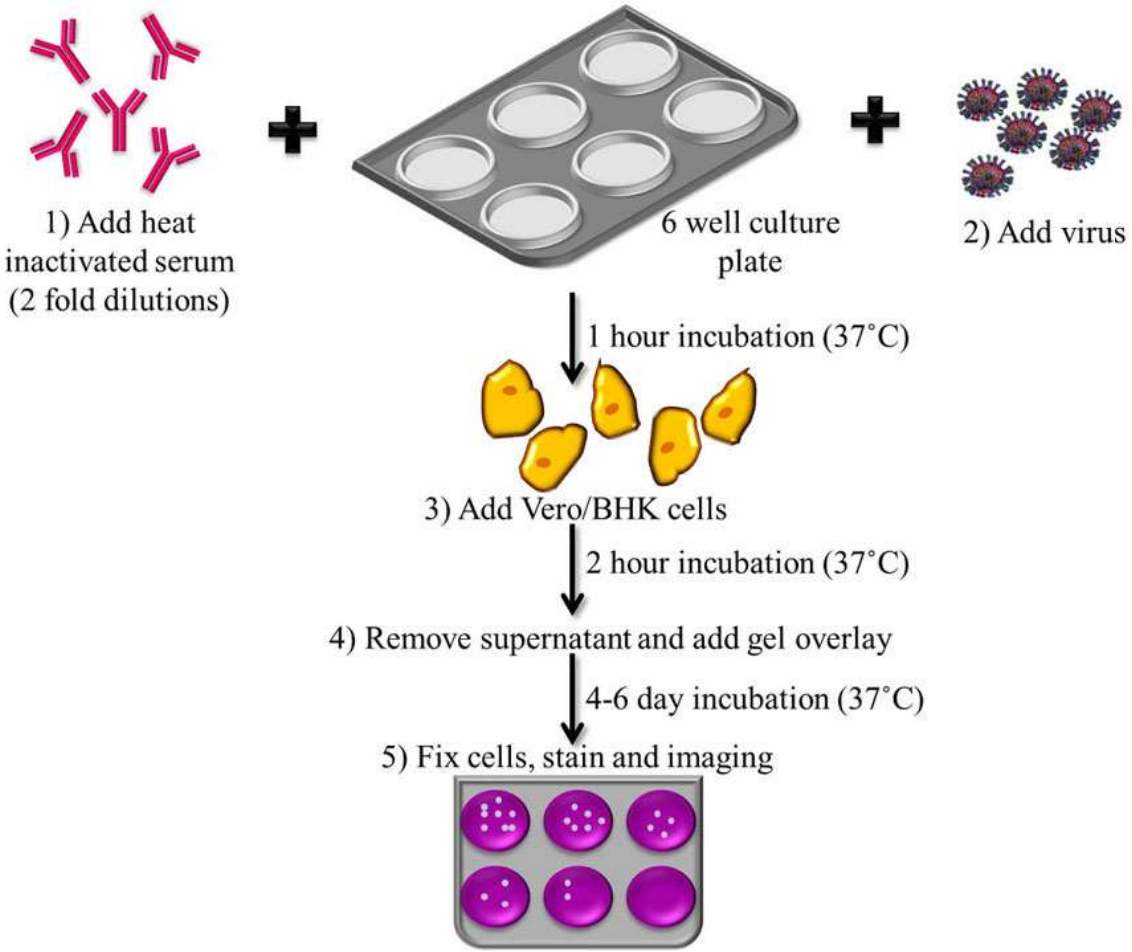
- ❑ **Mixing dilutions of antibodies with standardized amount of virus, incubating them and cultured into cells, eggs or animals to have a clear cytopathic effect observation**

Expectations:

- **No Ab (in serum) + virus → no neutralization → CPE**
- **Ab (in serum) + virus → neutralization → No CPE (cells remain intact**



Virus Neutralization Test (VNT)



Virus Neutralization Test (VNT)

Advantages:

- Can be specific
- Sensitive
- Vaccine production/immunological studies

Disadvantages:

- Very slow(days)
- Intensive
- Require skilled people
- Depend on cell line-use of wrong cell lines may assume that the antibodies have neutralization ability when not, or may seem to be ineffective when they actually have the neutralization abilities.

Enzyme-linked immunosorbant assay (ELISA)

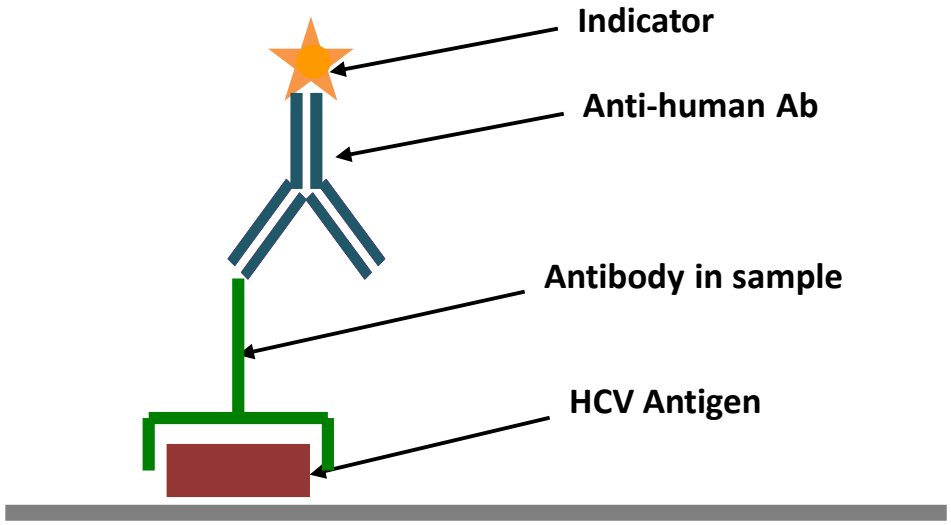
Principle of the test:

Use of enzyme-labelled immunoglobulin to detect antigens or antibodies. Signals are developed by the action of hydrolyzing enzyme on chromogenic substrate. Optical density measured by micro-plate reader.

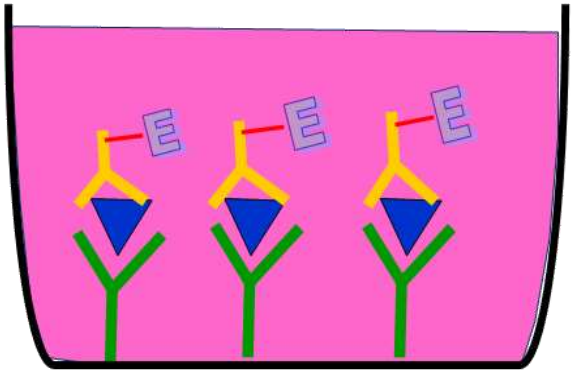
- **Advantages**
  - Automated, inexpensive
  - Small quantities required
  - Class specific antibodies measurable
- **Limitations**
  - Expensive initial investment
  - Variable sensitivity/specificity of variable tests
  - Cross contamination

Enzyme-linked immunosorbant assay (ELISA)

Types of the test:



Indirect ELISA (e.g. HCV)

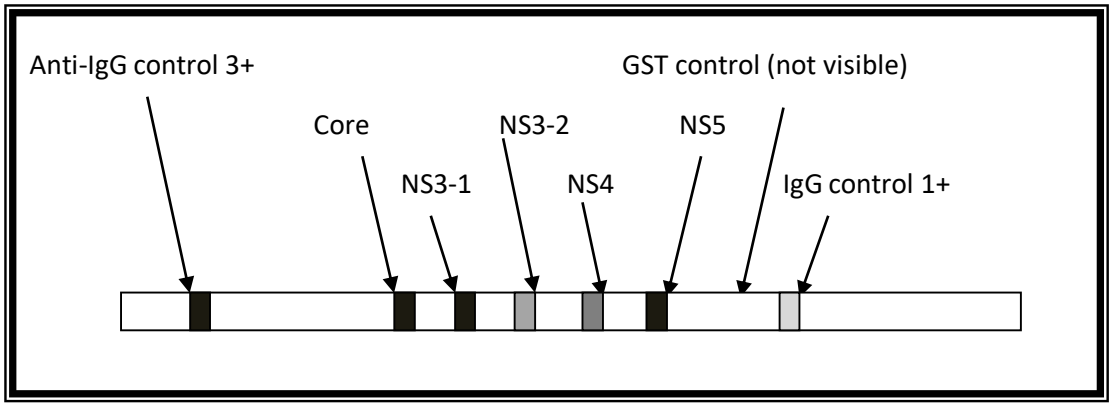


Sandwich ELISA (e.g. HBS Ag)

ELISA as a screening for HBV, HCV, & HIV (e.g. Blood banks), For HCV & HIV (ELISA test first, if positive, do immunoblotting as confirmatory test). Also for HCV & HIV (PCR is diagnostic)

Confirmatory test (e.g. HCV): Enzyme immunoblot assay (EIBA):

- Viral protein antigens are separated by electrophoresis and blotted onto nitrocellulose paper strips.
- The strip is incubated with patient antibody, washed to remove the unbound antibody, and then reacted with enzyme-conjugated antihuman antibody and chromophoric substrate.
- Serum from an viral-infected person binds and identifies the major antigenic proteins of virus.



Enzyme immunoblot assay (EIBA) or Western-blot analysis

- Samples: Serum, saliva, urine can be tested
- Kits are commercially available
- Recombinant immuno-blotting assays (RIBA) uses recombinant proteins

❑ **Advantages**

- Used for discrepant analysis
- Highly specific
- Rapid kits available

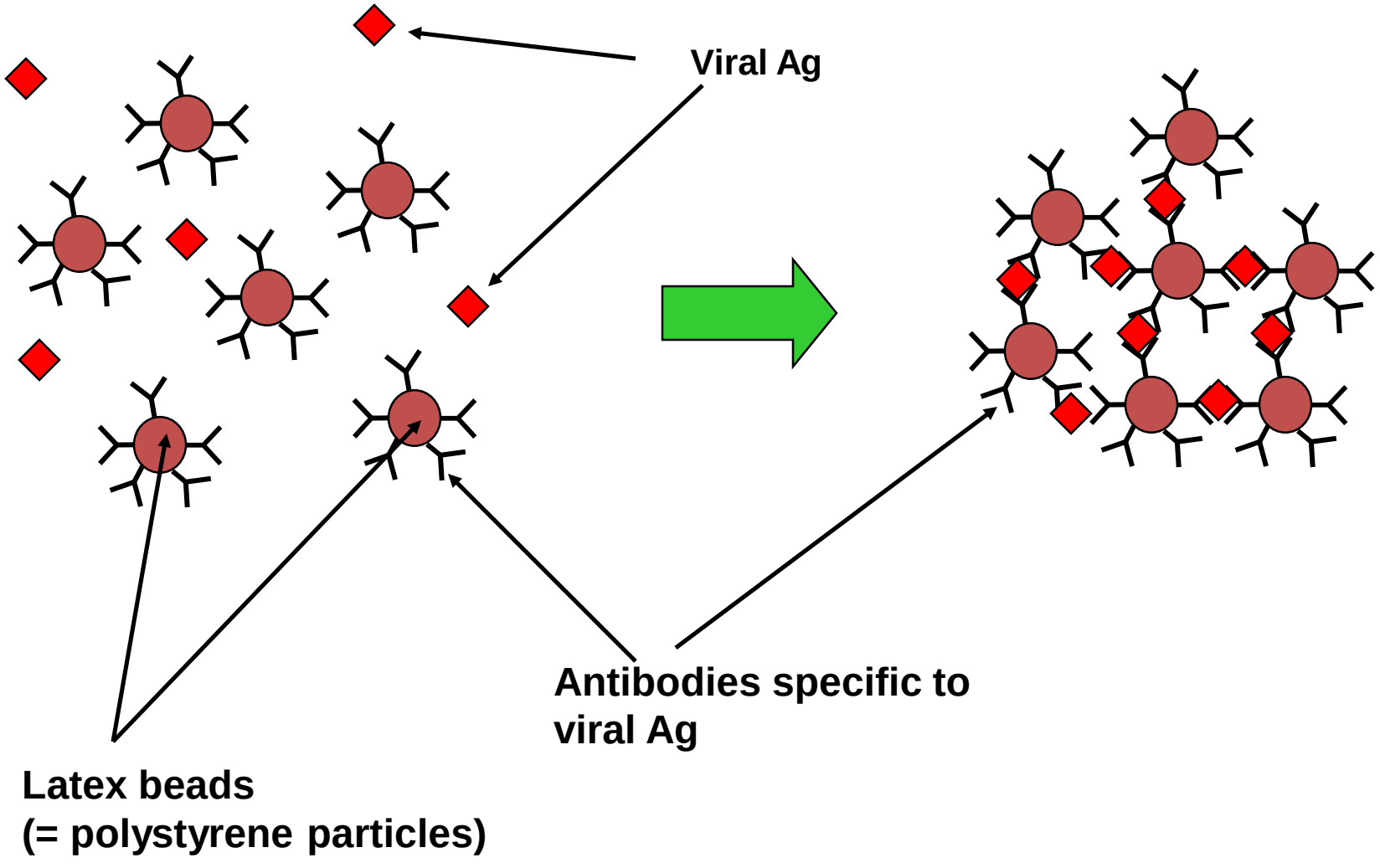
❑ **Limitations**

- Cost
- Concern validated data
- Time taken (1 day)

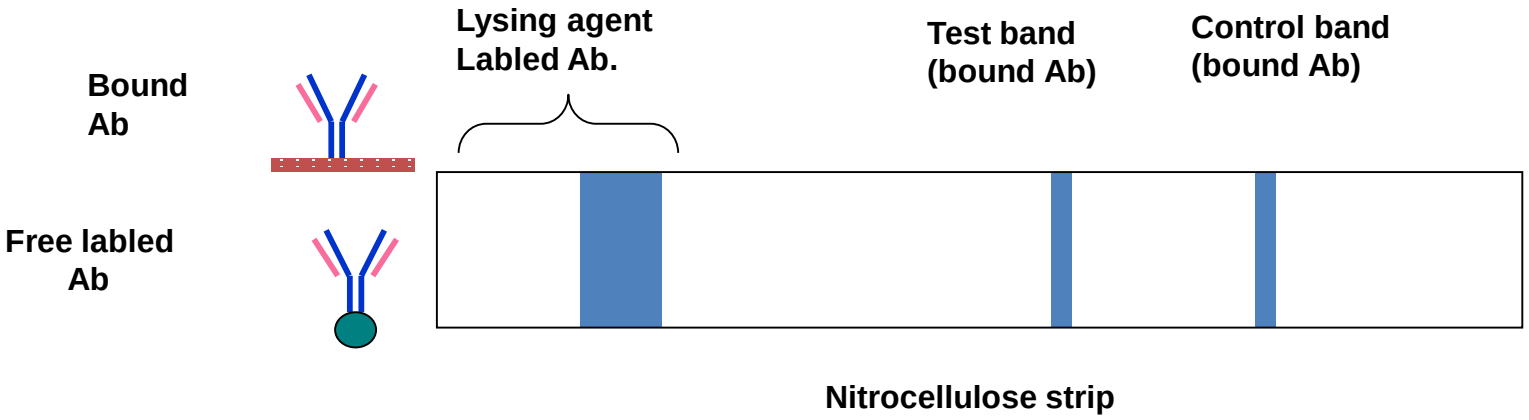
Latex agglutination test

- The **latex agglutination test** is a laboratory method to check for certain antibodies or antigens in a variety of body fluids including saliva, urine, cerebrospinal fluid, or blood.
- A simple and rapid latex agglutination test (LAT) has been developed for detecting rabies virus (RABV) antigens in saliva. Latex particles are coated with anti-rabies antibody.
- The agglutination is evident macroscopically within minutes.

Latex agglutination test

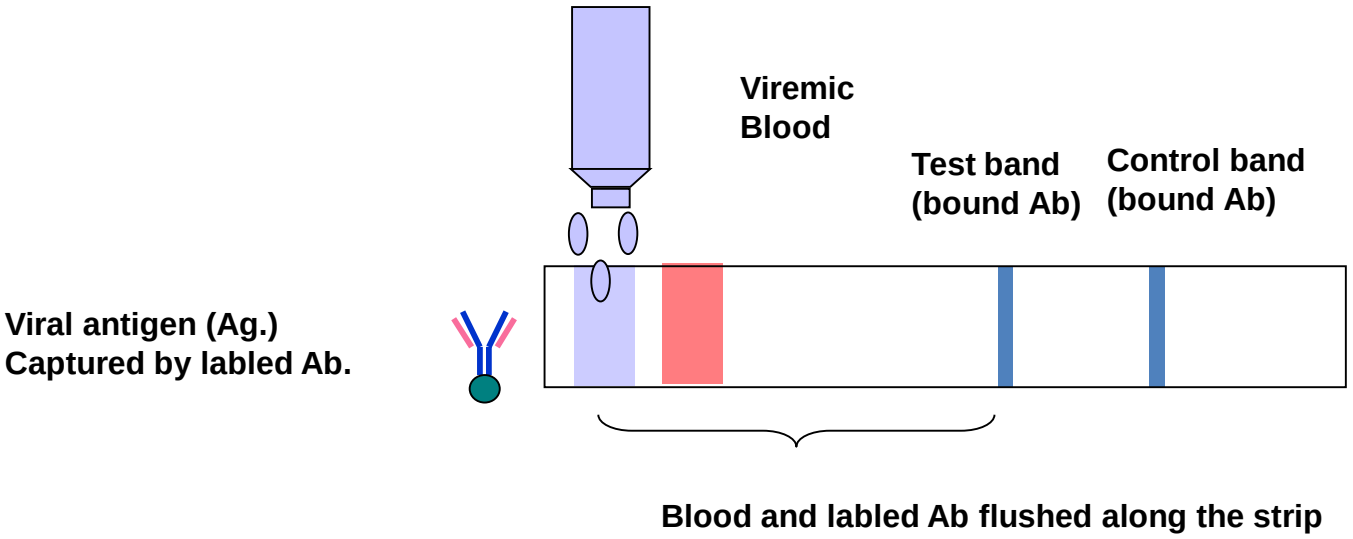


Immunochromatography



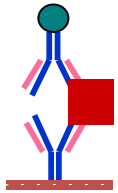
- Dye-labelled antibody, specific for target antigen, is present on the lower end of nitrocellulose strip or in a plastic well provided with the strip.
- Antibody, also specific for the target antigen, is bound to the strip in a thin (test) line, and antibody specific for the labelled antibody is bound at the control line.

Immunochromatography

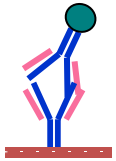


- Blood and buffer, which have been placed on strip or in the well, are mixed with labelled antibody and are drawn up strip across the lines of bound antibody.

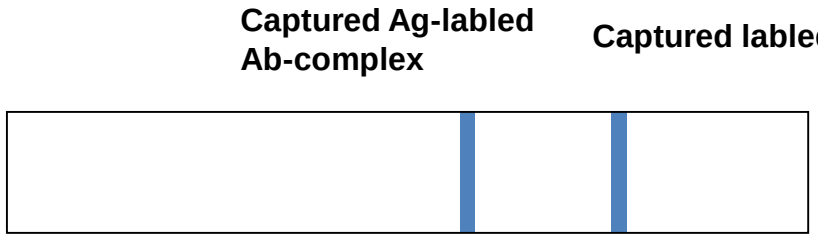
Immunochromatography



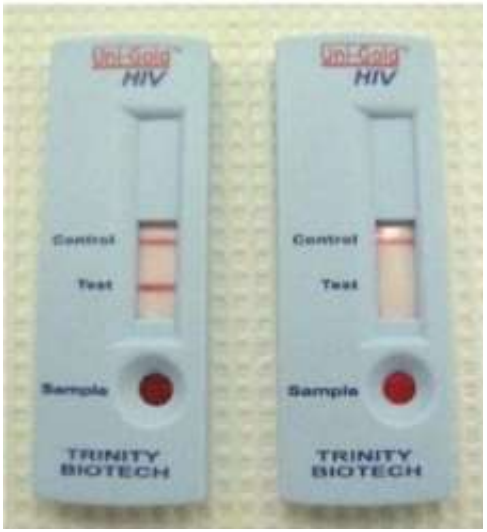
Labled Ab-Ag-complex Captured by bound Ab of test band



Labled Ab-bound Ab-complex Captured by bound Ab of control band



If antigen is present, some labelled antibody will be trapped on the test line.
Excess-labelled antibody is trapped on the control line.



Advantages & disadvantages

❑ Advantages

- **Easy to use, minimal training, cheap (7-12 $).**
- **Rapid – same day results possible (20 min)**
- **Shelf life up to 1-2 years without refrigeration**
- **Limited/no instrumentation; can be performed at the periphery of health systems without laboratory or electricity .**
- **Some tests as accurate as reference-level laboratory tests**

❑ Disadvantages

- **Cost per test could be more than traditional tests**
- **Mainly produce only "yes/no" answers**
- **Could require subjective interpretation (reader variation)**
- **Rapid tests can be less sensitive or less accurate compared to existing tests**

Usefulness of Serological Results

- How useful a serological result is depends on the individual virus.

1. For example, for viruses such as rubella and hepatitis A, the onset of clinical symptoms coincide with the development of antibodies. The detection of IgM or rising titres of IgG in the serum of the patient would indicate active disease.
2. However, many viruses often produce clinical disease before the appearance of antibodies such as respiratory and diarrheal viruses. So in this case, any serological diagnosis would be retrospective and therefore will not be that useful.
3. There are also viruses which produce clinical disease months or years after seroconversion e.g. HIV and rabies. In the case of these viruses, the mere presence of antibody is sufficient to make a definitive diagnosis.

Problems with Serology

1. Long period of time required for diagnosis for paired acute and convalescent sera.
2. Mild local infections such as genital HSV may not produce a detectable humoral immune response.
3. Extensive antigenic cross-reactivity between related viruses e.g. HSV and VZV, may lead to false positive results.
4. Immunocompromised patients often give a reduced or absent humoral immune response.
5. Patients with infectious mononucleosis and those with connective tissue diseases such as SLE may react non-specifically giving a false positive result.
6. Patients given blood or blood products may give a false positive result due to the transfer of antibody.


THE END