

A person wearing a white lab coat and blue nitrile gloves is using a pipette to transfer a red liquid into a conical flask. The flask already contains some red liquid. The background is a gradient of red and white.

450MBIO  
Practical lesson -3

# Methods in virus diagnosis

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# Learning outcome

- Recognize the methods that most frequently used in virus diagnosis.
- Learn how to detect viruses by direct, indirect and Serological tests.
- Compare the advantages and disadvantages of viral examination techniques



# Diagnostic Methods in Virology

1. Direct Examination
2. Indirect Examination (Virus Isolation)
3. Serology



# Direct Examination

**In direct examination the specimen is tested directly for the presence of virus particles**

## **1. Antigen Detection**

Immunofluorescence, ELISA etc.

## **2. Electron Microscopy**

Morphology of virus particles  
Immune electron microscopy

## **3. Light Microscopy**

Histological appearance  
Inclusion bodies

## **4. Viral Genome Detection**

Hybridization with specific nucleic acid probes  
Polymerase chain reaction (PCR)



# Indirect Examination

**In indirect examination the virus is detected after cultivation or isolation**

## **1. Cell Culture**

Cytopathic effect (CPE)

Haemabsorption

Immunofluorescence

## **2. Eggs**

Pocks on CAM

Inclusion bodies

## **3. Animals**

Disease or death





Direct examination

**ANTIGEN DETECTION**

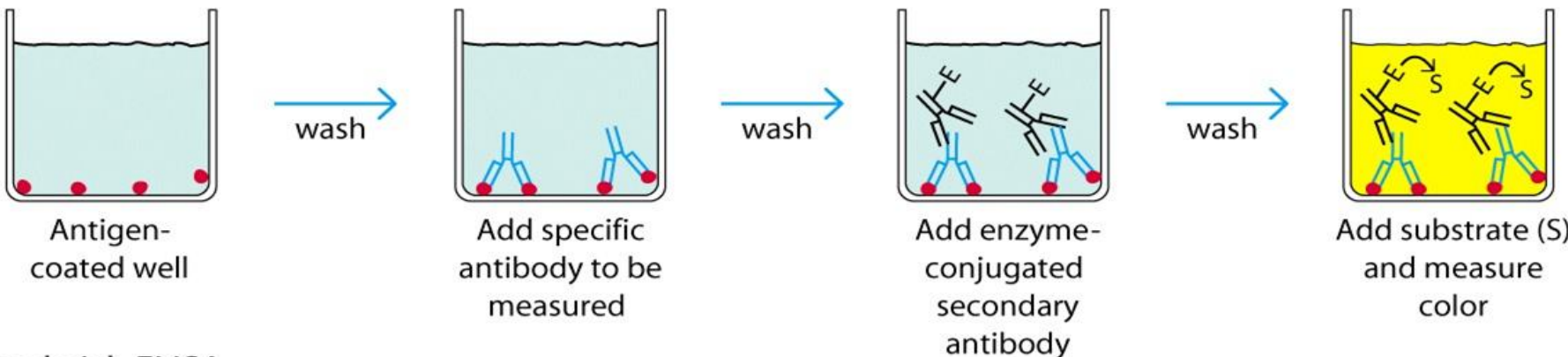
**ELISA AND IMMUNOFLUORESCENCE**



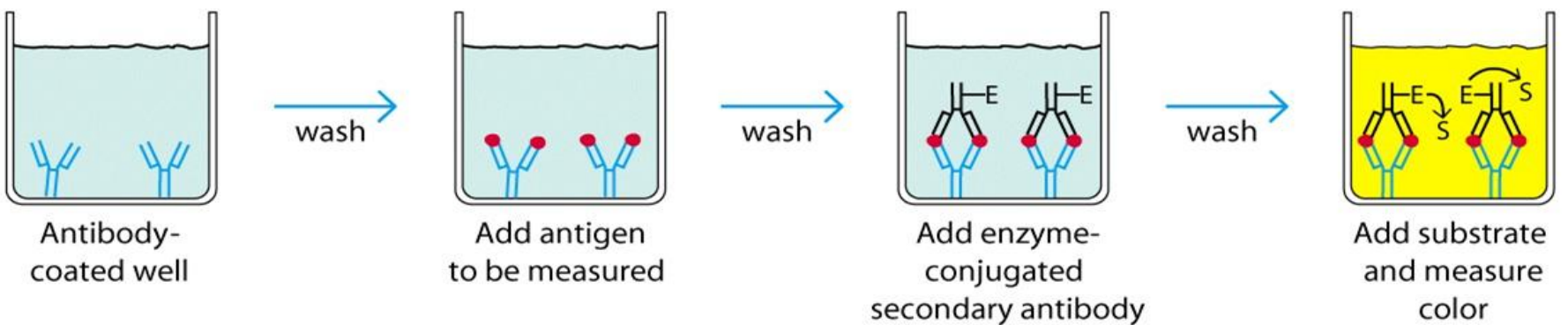
# Enzyme Linked Immunosorbent Assay (ELISA)

- Enzyme Linked Immunosorbent Assay (ELISA)
- Term Was Coined By Engvall and Pearlmann in 1971
- Can be used to detect either antigen (as a direct test) **OR** antibody (as a serology test).
- **Different Types**
  1. Sandwich
  2. Indirect
  3. Competitive

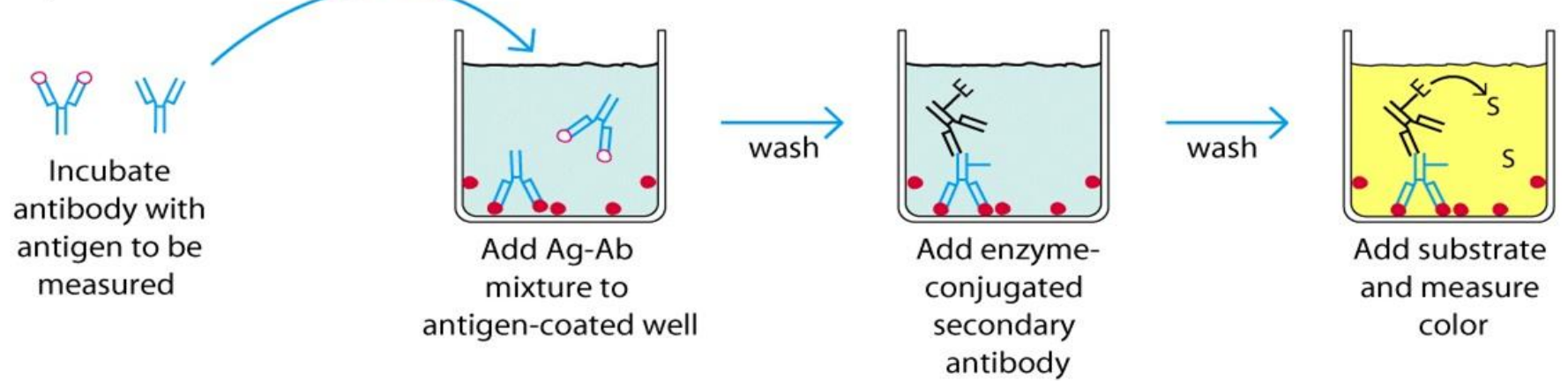
(a) Indirect ELISA



(b) Sandwich ELISA



(c) Competitive ELISA







# Rapid Diagnosis Based on the Detection of Viral Antigens

## Type of Specimens:

## Virus:

Nasopharyngeal Aspirate

RSV  
Influenza A and B  
Parainfluenza  
Adenovirus

Faeces

Rotaviruses  
Adenoviruses  
Astrovirus

Skin

HSV  
VZV

Blood

CMV (pp65 antigenaemia test)

(Enzyme Immuno Assay) EIA, also known as ELISA (for enzyme-linked immunosorbent assay)



Microplate ELISA for HIV antibody: coloured wells indicate reactivity



# Immunofluorescence (IF)

- **Immunofluorescence** is a powerful technique that utilizes fluorescent-labeled antibodies to detect specific target antigens..
- **Fluorescein** is a dye which emits greenish fluorescence under UV light. It can be tagged to immunoglobulin molecules.
- This technique is sometimes used to make viral plaques more readily visible to human eye .
- Immunofluorescent labeled tissue sections are studied using a fluorescence microscope.



## Immunofluorescence (IF)

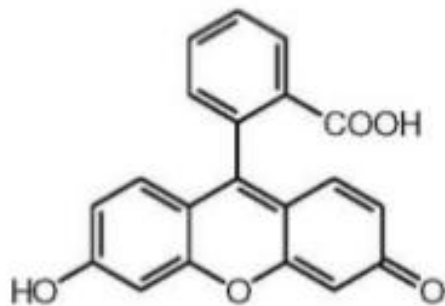
- Immunofluorescence is a technique allowing the visualization of a specific antigen in tissue sections by binding a specific antibody chemically conjugated with a fluorescent dye (also called fluorophores or fluorochromes) such as fluorescein isothiocyanate (FITC).
- The specific antibodies are labelled with a compound (FITC) that makes the glow an apple-green colour when observed microscopically under ultraviolet light.



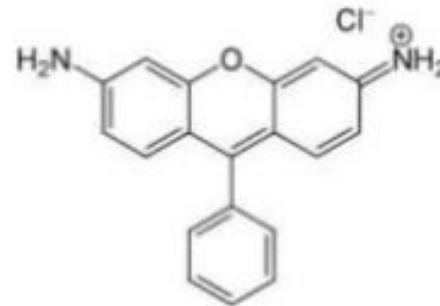
# Immunofluorescence

## Examples Of Fluorescent Dyes

Fluorescein



Rhodamine







# Immunofluorescence

There are two ways of doing IF staining :

➤ **Direct IF:**

- Antigen is fixed on the slide
- Fluorescein labeled antibodies are layered over it
- Slide is washed to remove unattached antibodies
- Examined under UV light in a florescent microscope
- The site where the antibodies attaches to its specific antigen will show apple green fluorescence
- Use : direct detection of Pathogens or their antigen in tissues or in pathological samples

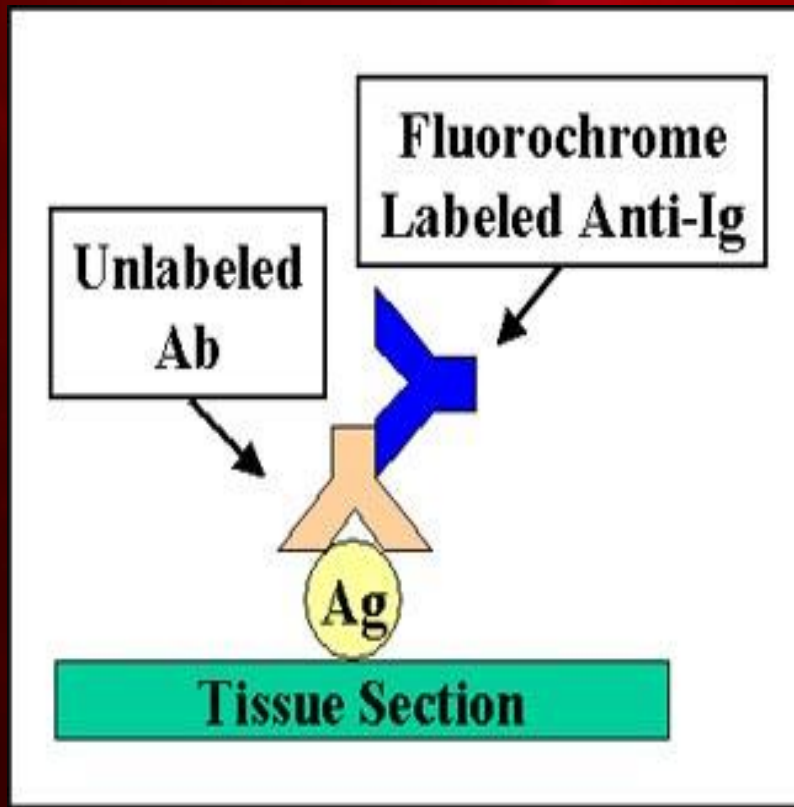


# Immunofluorescence (IF)

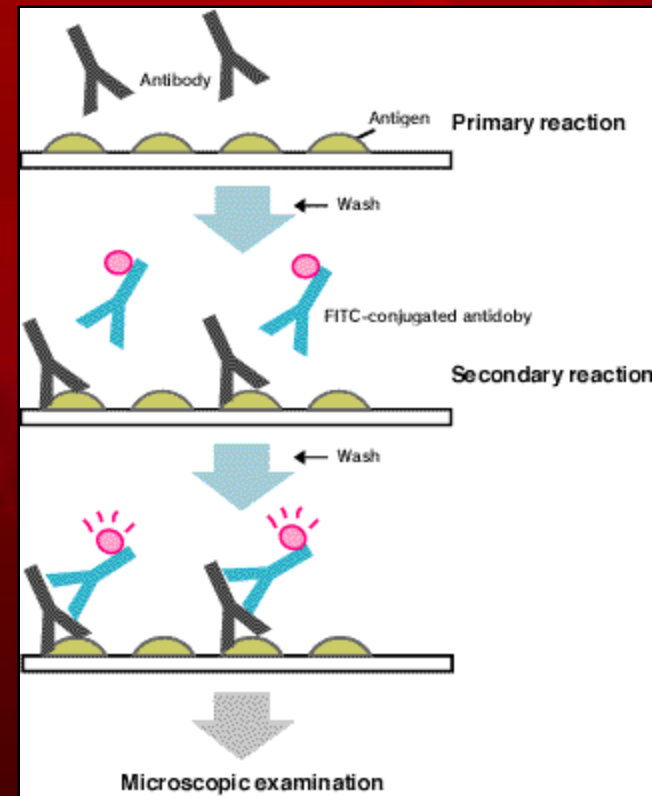
## ➤ Indirect IF:

- Indirect test is a double –layer technique
- The unlabelled antibody is applied directly to the tissue substrate
- Treated with a fluorochrome – conjugated anti-immunoglobulin serum.

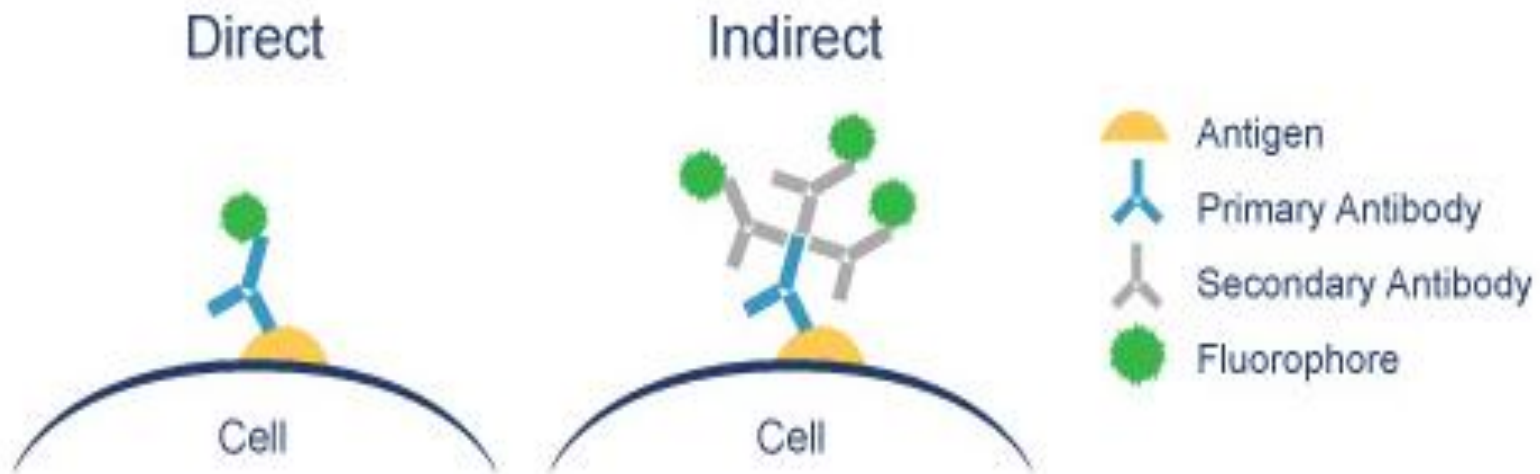
## Direct IF



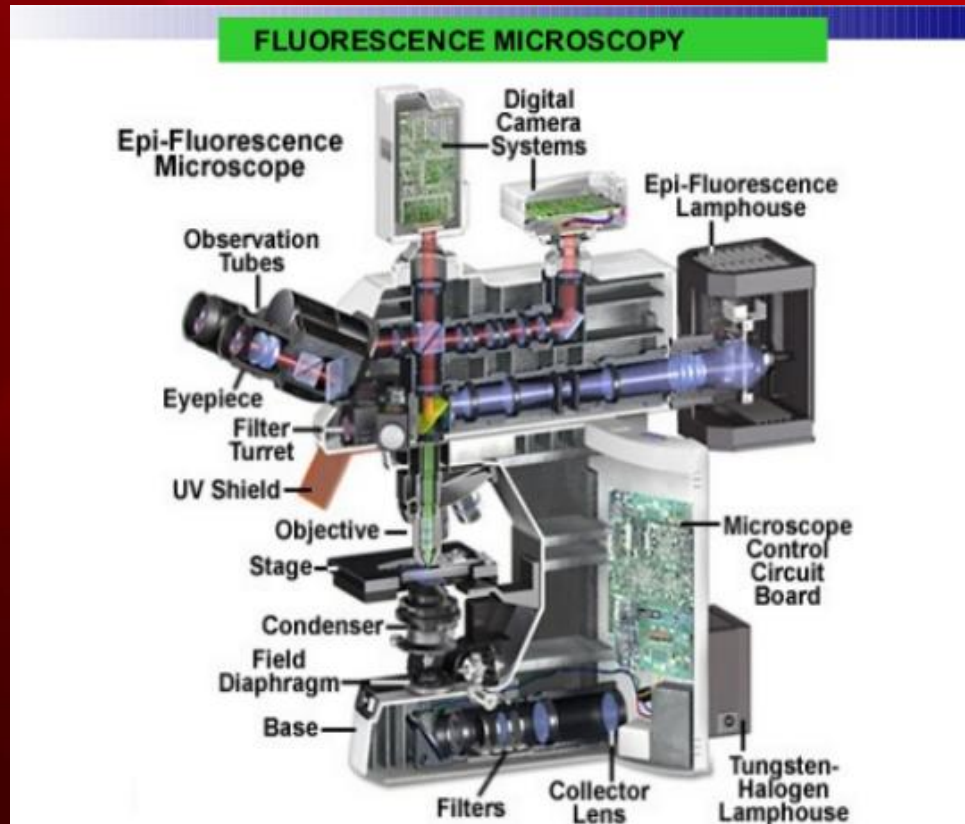
## Indirect IF



# Immunofluorescence (IF)

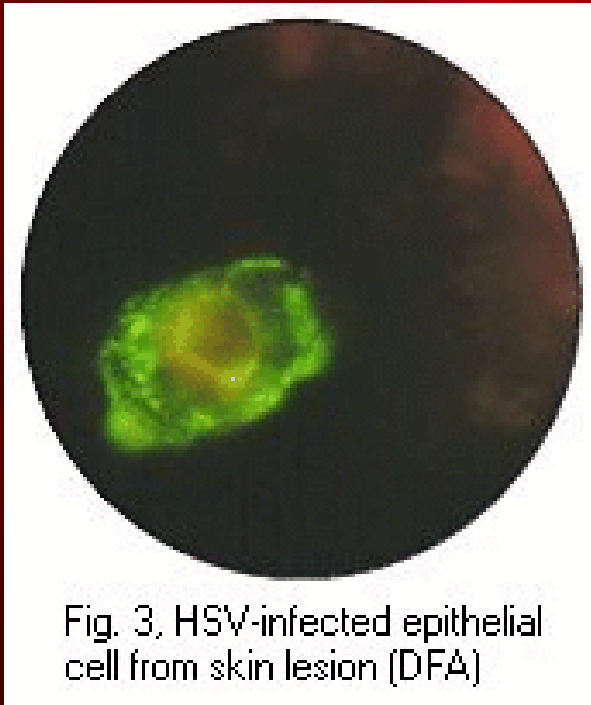


# Immunofluorescence (IF)

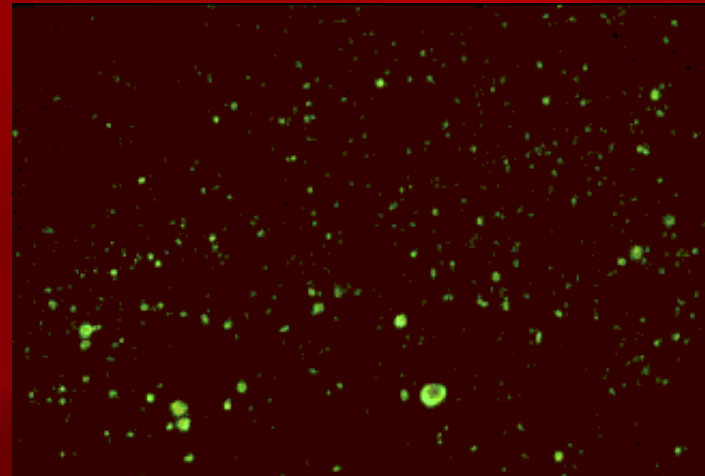




# Immunofluorescence



(Virology Laboratory, Yale-New Haven Hospital)



Positive immunofluorescence test for rabies virus antigen. (Source: CDC)

# CMV pp65 antigenaemia test

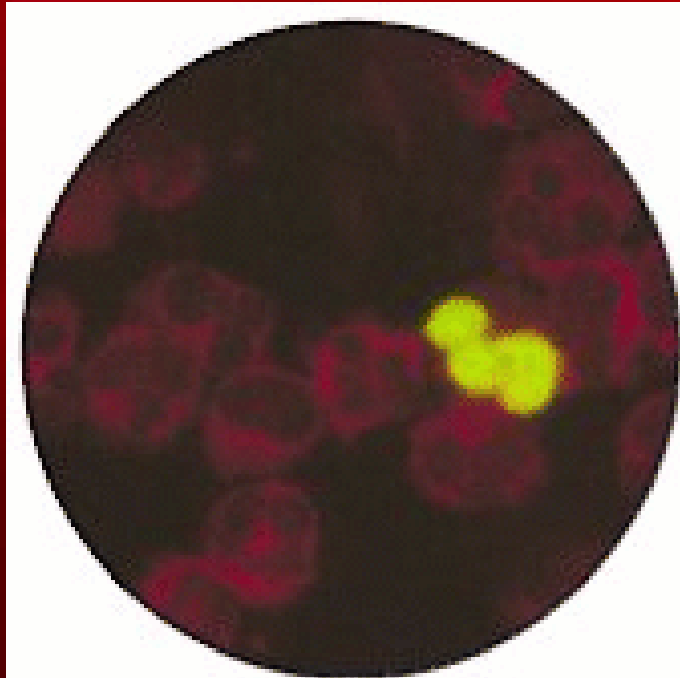


Figure 4 CMV pp65 antigens detected in nuclei of peripheral blood neutrophils

(Virology Laboratory, Yale-New Haven Hospital)



# Advantages and Disadvantages

## Advantages

- Result available quickly, usually within a few hours.

## Potential Problems

- Often very much reduced sensitivity compared to cell culture, can be as low as 20%. Specificity often poor as well.
- Requires good specimens.
- The procedures involved are often tedious and time-consuming and thus expensive in terms of laboratory time.



Direct examination

# **ELECTRON MICROSCOPY**



# Electron Microscopy

- It is a type of microscope that uses electrons to illuminate a specimen and create an enlarged image.
- Have much greater resolving power than light microscopes and can obtain much higher magnifications
- uses electrostatic and electromagnetic lenses in forming the image by controlling the electron beam to focus it at a specific plane relative to the specimen in a manner similar to how a light microscope uses glass lenses to focus light on or through a specimen to form an image.





# Electron Microscopy

- The morphology of most viruses is sufficiently characteristic to allow assigning many viruses to the correct family by appearance in the electron microscope
- $10^6$  virus particles per ml required for visualization,  $\times 50,000 - 60,000$  magnification normally used. Viruses may be detected in the following specimens.

## Faeces

Rotavirus, Adenovirus  
Norwalk like viruses  
Astrovirus, Calicivirus

## Vesicle Fluid

HSV  
VZV

## Skin scrapings

papillomavirus, orf  
molluscum contagiosum



The most widely used procedure is:

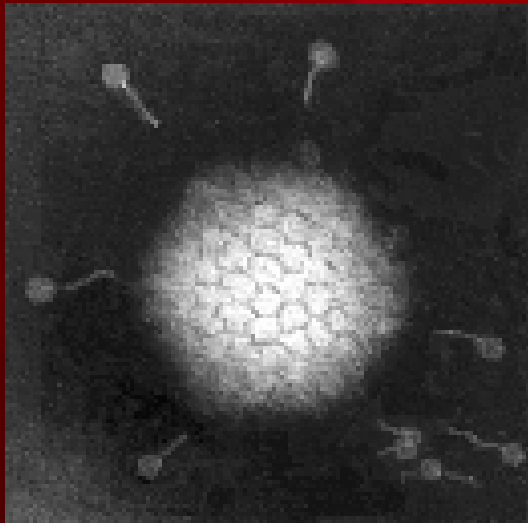
### **Negative staining**

- Virus-containing fluid is placed on a carbon grid
- Virions adhere to the surface and become “negatively stained” when an electron-dense fluid is added and surrounds the virions

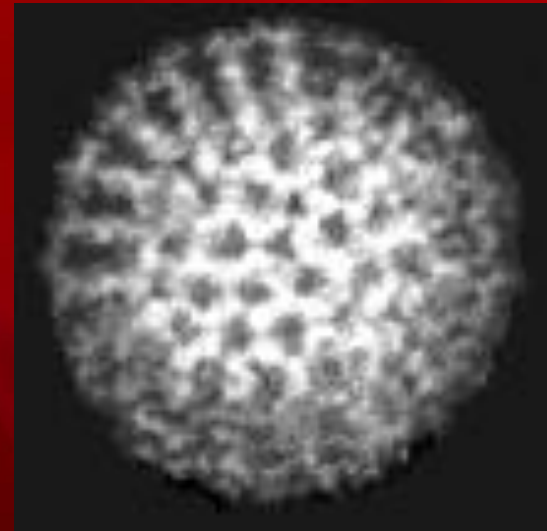
### **immune electron microscopy**

- Virus-specific antibody is used to agglutinate virus particles together and thus making them easier to recognize, or to capture virus particles onto the EM grid.

# Electronmicrographs



Adenovirus



Rotavirus

(courtesy of Linda Stannard, University of Cape Town, S.A.)



# Immune Electron Microscopy

The sensitivity and specificity of EM may be enhanced by immune electron microscopy. There are two variants:-

**Classical Immune electron microscopy (IEM)** - the sample is treated with specific anti-sera before being put up for EM. Viral particles present will be agglutinated and thus congregate together by the antibody.

**Solid phase immune electron microscopy (SPIEM)** - the grid is coated with specific anti-sera. Virus particles present in the sample will be absorbed onto the grid by the antibody.

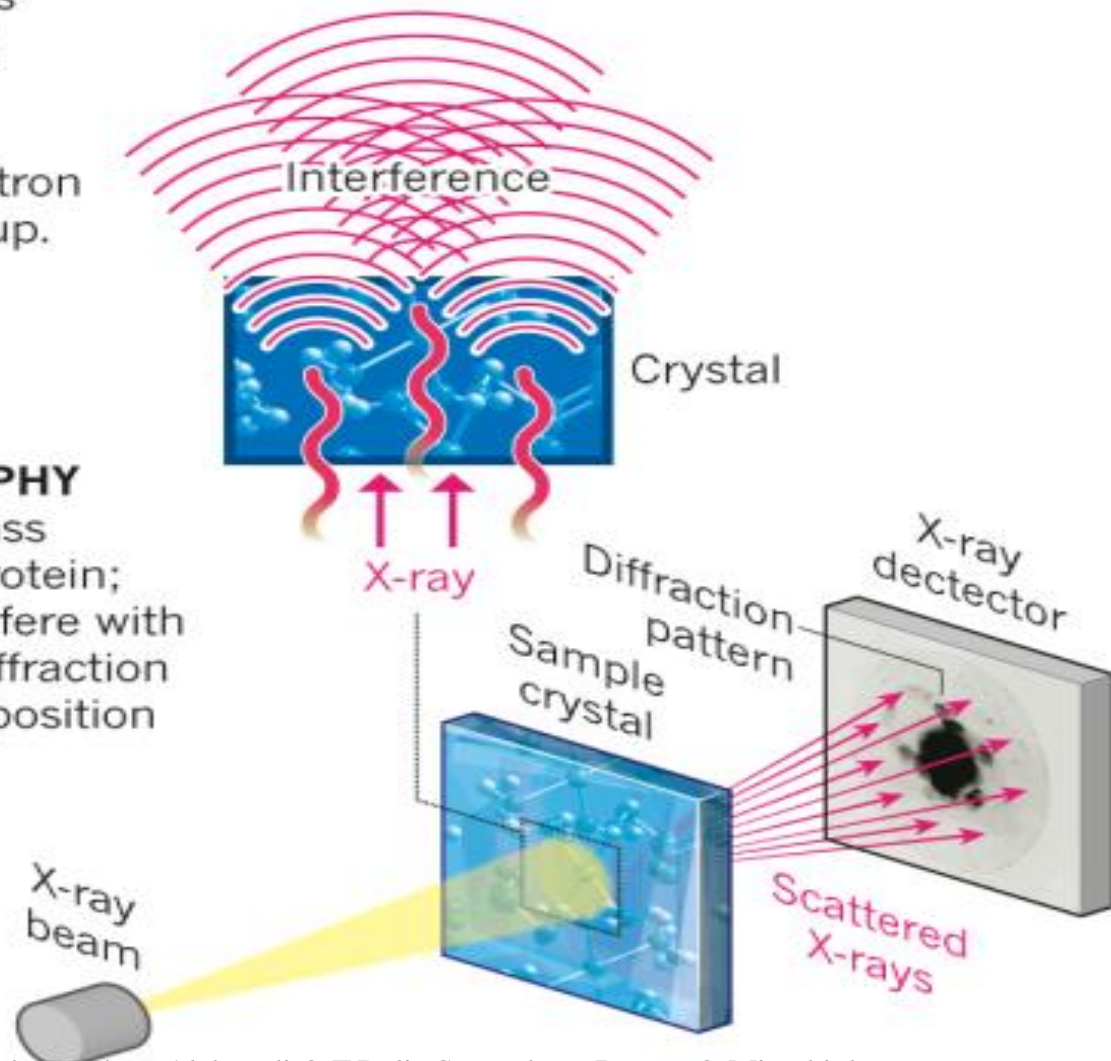


# X-ray crystallography

X-ray crystallography has long been the dominant method for deducing high-resolution protein structures, but cryo-electron microscopy is catching up.

## X-RAY CRYSTALLOGRAPHY

X-rays scatter as they pass through a crystallized protein; the resulting waves interfere with each other, creating a diffraction pattern from which the position of atoms is deduced.

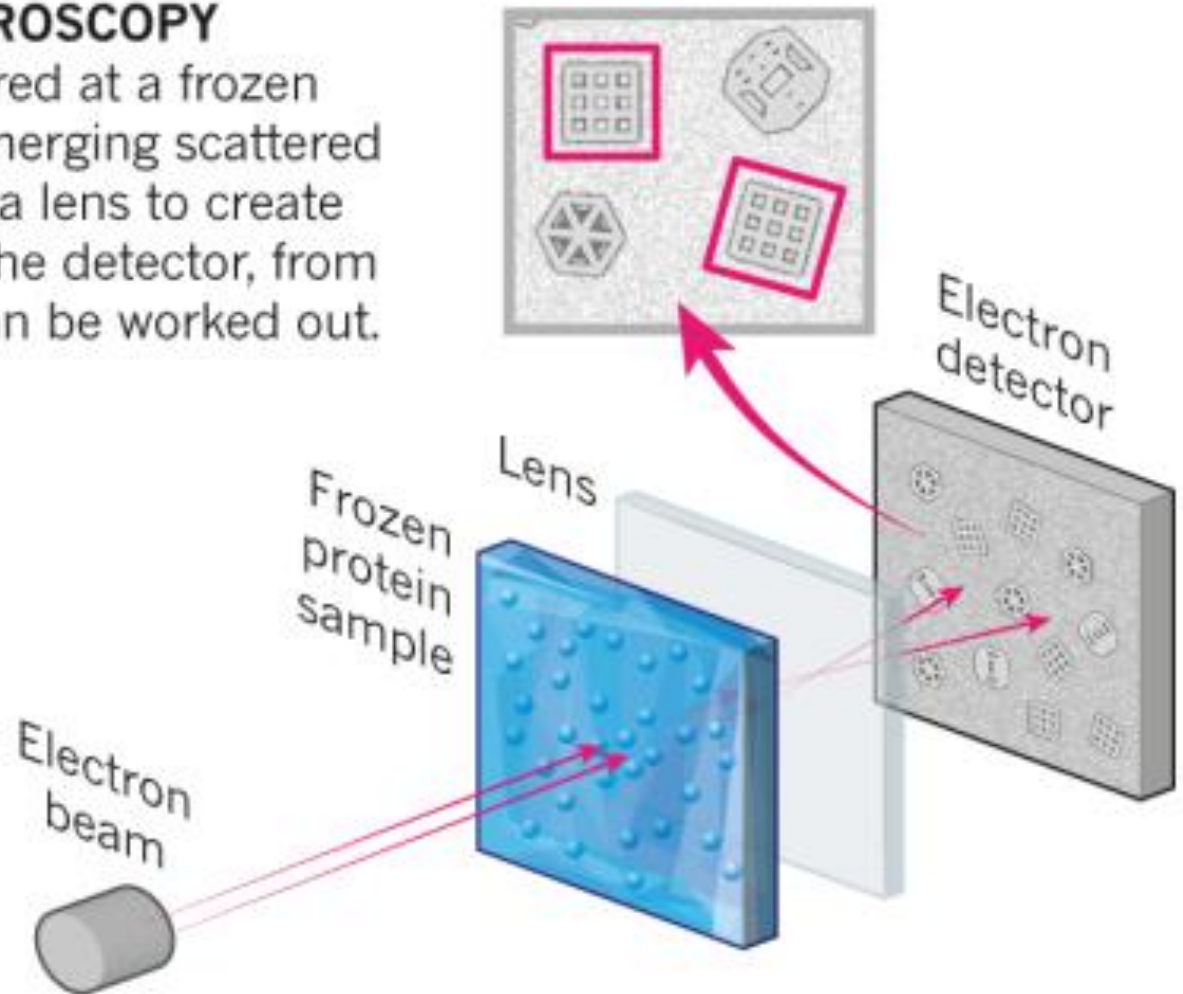




# Cryo-electron microscopy

## CRYO-ELECTRON MICROSCOPY

A beam of electron is fired at a frozen protein solution. The emerging scattered electrons pass through a lens to create a magnified image on the detector, from which their structure can be worked out.





# Problems with Electron Microscopy

- Expensive equipment
- Expensive maintenance
- Require experienced observer
- Sensitivity often low



Other examinations

# **SEROLOGY**



# Serology

- Serology forms the mainstay of viral diagnosis. Detection of rising titres of antibody between acute and convalescent stages of infection, or the detection of IgM in primary infection.
- This is what happens in a primary humoral immune response to antigen. Following exposure, the first antibody to appear is IgM, which is followed by a much higher titre of IgG.



# Serology

## Criteria for diagnosing Primary Infection

- 4 fold or more increase in titre of IgG or total antibody between acute and convalescent sera
- Presence of IgM
- Seroconversion
- A single high titre of IgG (or total antibody) - very unreliable

## Criteria for diagnosing Reinfection

- fold or more increase in titre of IgG or total antibody between acute and convalescent sera
- Absence or slight increase in IgM





- Newer techniques such as EIAs and radioimmunoassay (RIA) offer better sensitivity, specificity and reproducibility than classical techniques such as Complement Fixation Test (CFT) and Hemagglutination Inhibition Test (HAI).

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### **Classical Techniques**

1. Complement fixation tests (CFT)
2. Haemagglutination inhibition tests
3. Immunofluorescence techniques (IF)
4. Neutralization tests
5. Counter-immunoelectrophoresis

### **Newer Techniques**

1. Radioimmunoassay (RIA)
2. Enzyme linked immunosorbent assay (EIA)
3. Particle agglutination
4. Western Blot (WB)
5. RIBA, Line immunoassay



# Hemagglutination Inhibition Test (HAI).

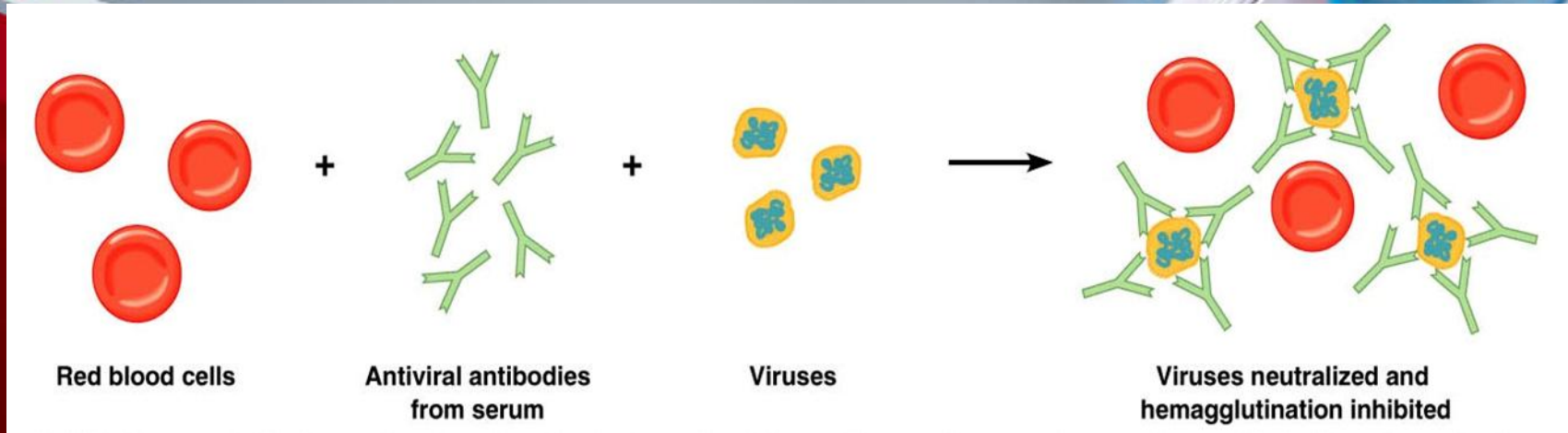
## Materials and Reagents:

- Red cells from an appropriate species (Chicken, goose, guinea pig, trypsinized human O) collected in Alsever's solution or heparin
- Diluent (e.g. Bovine albumin veronal buffer) at appropriate pH
- Solutions to remove nonspecific hemagglutinins from serum
- Infected cultural fluid or standard antigen (e.g preparation of influenza virus) for serology



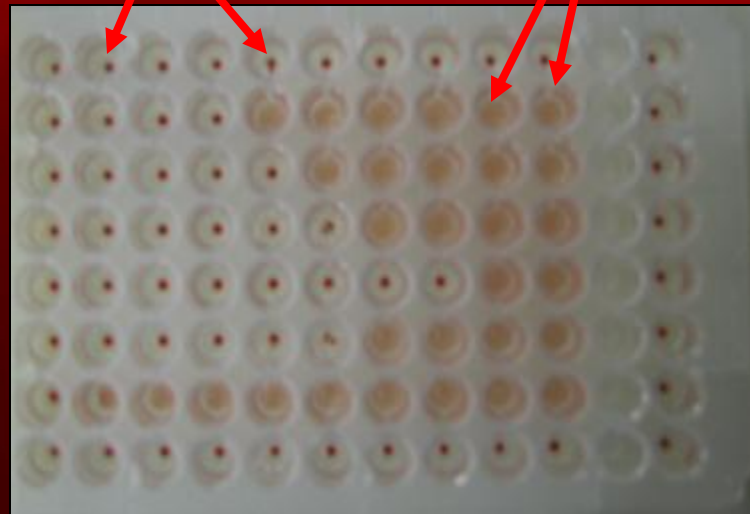
## Procedure

- Obtain a preparation of virus (e.g. influenza viruses) with known HA titer or determine its HA titer
- Prepare two-fold dilutions of patient/test serum to be tested e.g. from 1:4 to 1:1024.
- Add a fixed amount of virus to every well of a 96-well plate, equivalent to 4 HA units (varies according to virus), except for the serum control wells.
- The plate is then allowed to stand at room temperature for 60 minutes (time varies according to specific requirements).
- Add red blood cells (RBC) and incubate at 40C for 30 minutes.
- Read the wells.



Positive

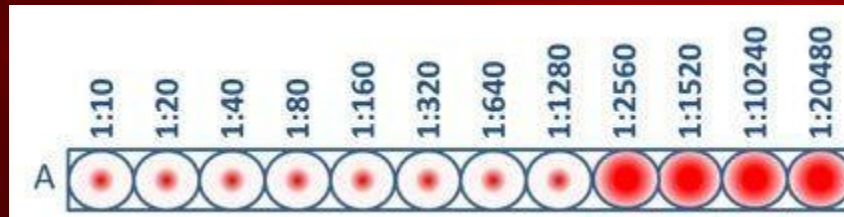
Negative



Hemagglutination inhibition for detection of Dengue antibodies

## Results/interpretation

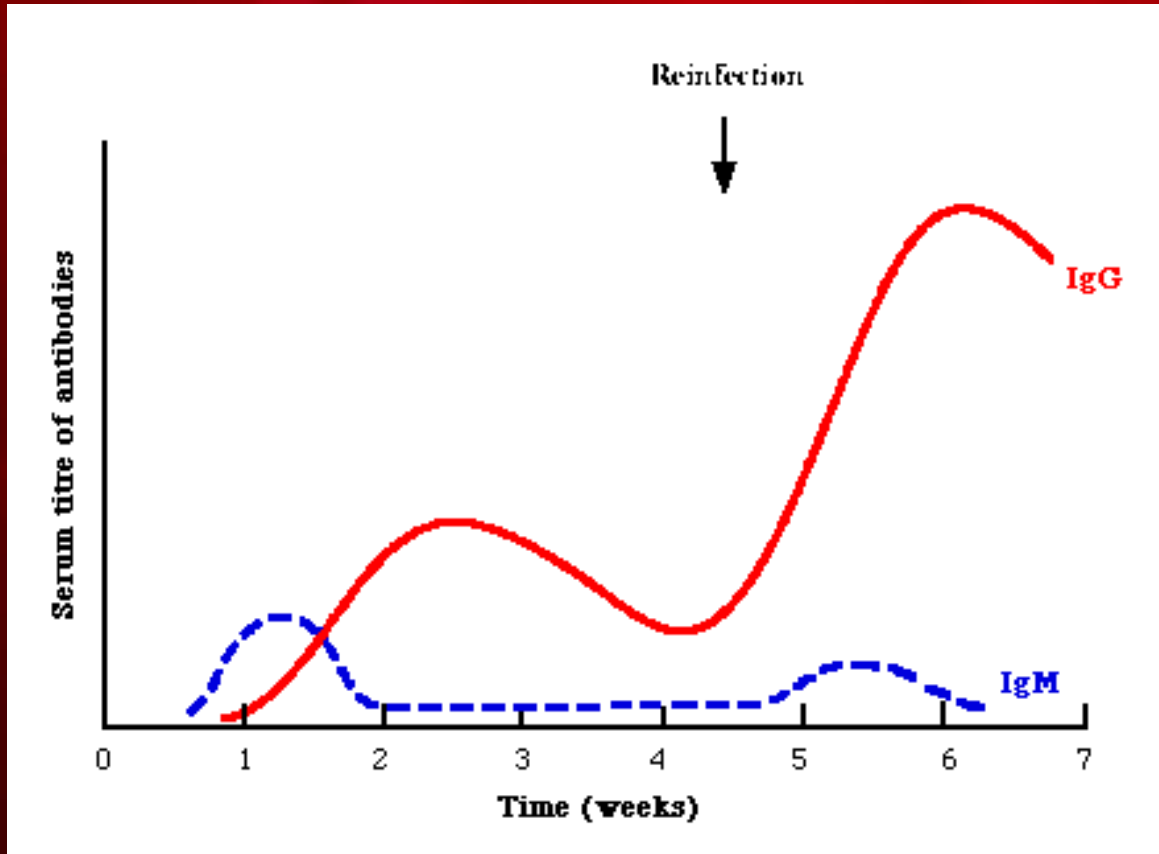
The highest dilution of serum (Ab) that prevents hemagglutination is called the HAI titer of the serum.. A smooth or jagged shield of cells or an irregular button indicates agglutination. Observation of movement of the button of red cells when the plate is tilted may help to clarify the end point.



This virus sample has an HAI titer of 1280, which means that the greatest dilution of antibody that still blocked hemagglutination from occurring was at 1280 dilution. At this dilution, the antibodies were still capable of recognizing and binding to the antigens on the virus.



# Typical Serological Profile After Acute Infection

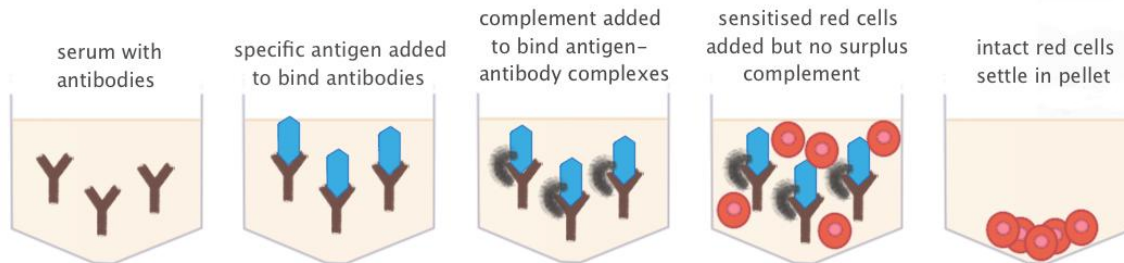


**RIA assay** (In cases of reinfection, the level of specific IgM either remain the same or rises slightly. But IgG shoots up rapidly and far more earlier than in a primary infection. Many different types of serological tests are available)

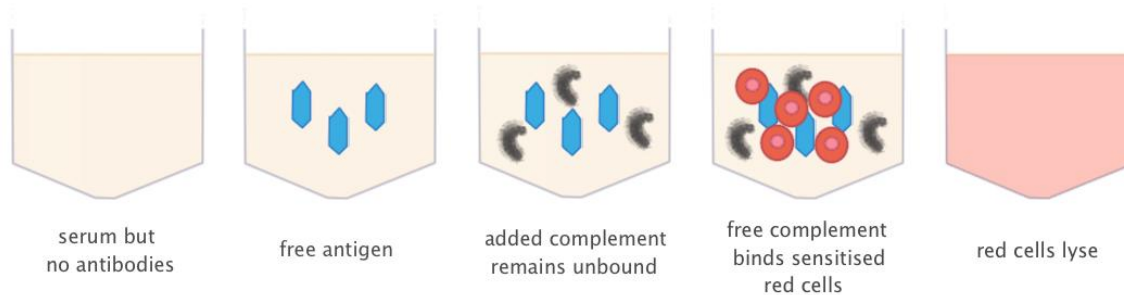
# Complement Fixation Test (CFT)

## complement fixation test

reactive



nonreactive



tji 2013. Creative Commons



# Usefulness of Serological Results

- How useful a serological result is depends on the individual virus.
- 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.
- However, many viruses often produce clinical disease before the appearance of antibodies such as respiratory and diarrhoeal viruses. So in this case, any serological diagnosis would be retrospective and therefore will not be that useful.
- 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

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



## CSF antibodies

- Used mainly for the diagnosis of herpes simplex and VZV encephalitis
- CSF normally contain little or no antibodies
- presence of antibodies suggest meningitis or meningoencephalitis

$\frac{\text{CSF antibody titre}}{\text{Serum antibody titre}} > \frac{1}{100}$  is indicative of meningitis



# Specimens for Routine Tests

Clinical Category	Blood	Throat swab	Faeces	CSF	Other
1. Meningitis	+	+	+	+	
2. Encephalitis	+	+	+	+	Brain biopsy
3. Paralytic disease	+	+	+	+	
4. Respiratory illness	+	+			Nasopharyngeal aspirate
5. Hepatitis	+				
6. Gastroenteritis			+		
7. Congenital diseases	+				Urine, saliva
8. Skin lesions	+		+		Lesion sample e.g. vesicle fluid, skin scrapping
9. Eye lesions					Eye swab
10. Myocarditis	+				Pericardial fluid
11. Myositis	+		+		
12. Glandular fever	+				
13. Post Mortem	+				Autopsy

After use, swabs should be broken into a small bottle containing 2 ml of virus transport medium. Swabs should be sent to the laboratory as soon as possible without freezing. Faeces, CSF, biopsy or autopsy specimens should be put into a dry sterile container.