Microbiology

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Virology – **Diagnosis 3**
JU- 2nd Year Medical Students

By

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Diagnosis 3

- Virus isolation in Cell culture
- Detection of antiviral antibodies

- **Virus isolation in cell culture:**
- not common
- gold standard for some tests as corona SARS
- 3 types of cell lines are commonly used
  - Semi continuous, continuous and lymphocytes culture

- **Semi continuous cell lines (e.g WI38, MRC9)**
- From human or animal fetal cells
- Normal karyotypes
- Can be subcultured for 50 generations i.e limited life span
- Seeds lot?

- For vaccines such as MMR: fresh low passage level e.g 4th subculture and normal karyotype

- **Continuous cell lines**

- From cells growing indefinitely such as tumor cells or normal cells that subcultured many time (will have abnormal chromosomes number and behave as tumor cells)
- For diagnostic use
- Some lines such as vero monkey kidney cells and dog kidney cells (MDKC) used for vaccines production , polio and influenza respectively

- **Lymphocytes culture :**
- Lymphocytes Immortalization by infection with EBV i.e transformation by a virus
- IL2 stimulates its growth
- HIV and HTLV culture (syncytial giant cells)

- Monolayer of cells are grown then sample added and CPEs are observed (2 days in herpes AND enteroviruses, to 14 days in CMV) sometimes IF is necessary. see FIG 36.4:

- bursters (lytic) viruses such as enteroviruses will cause rounding up and lysis of cells
- creepers (herpes and paramyxoviruses): multinucleated giant cells or syncytia.
- Some viruses will cause no CPEs but they inhibit cells superinfection
- Immune -Fluorescence
- Haemadsorption (binding the RBCs)

- HIV: special techniques since it grows only in replicating human lymphocytes, which cannot normally be maintained inculture.

- This difficulty was overcome by stimulating the cells with a plant lectin, **phytohaemagglutinin**, and **IL-2**

- Syncytial giant cells

procedure:

- cells are grown to a monolayer in growth medium oh 7.2-7.4(salt, glucose, amino acids, vitamins, antibiotics, 10-20% fetal calf serum)

- the growth medium is then replaced with a maintenance medium (2-5% fetal calf serum) to stop cells overgrowth so keep the monolayer

- cells can be detached using trypsin and suspended in growth medium at 10^5-10^6 /ml

- **Detection of antiviral antibodies**
- Virus isolation suggestive of diagnosis but not always a proof of causality
- e.g: virus shedding from a clinically normal or asymptomatic people.
- Serological testing of antibodies :

- (1) a **rising titre of antibody** to a particular virus is sought,testing paired samples of serum, the first as soon as possibleafter onset and the second, 10–14 days later.
- A fourfold or greater rise in titre of the relevant antibody is significant

- (2) serum is tested for the presence of **specific IgM antibody** more widely used:
- rapid since that specific IgM antibody is detectable a few days after the onset of illness and stays detectable for 3-9 months
- its finding is good evidence of a current or recent infection
- ELISA-type ‘capture’ methods
- Such tests are very reliable, provided that adequate controls are included and each step is followed by thorough washing to remove unbound, non-specific reagents.

- In brief, the following steps are involved in testing for IgM antibody to a virus such as rubella
- (Fig. 36.2(b)).
- 1. IgM antibody to human IgM (anti-IgM) is adsorbed to a
- solid surface, e.g. a well in a microtitre plate.
- 2. The test serum is then added; IgM molecules are ‘captured’ by the anti-IgM.
- 3. Rubella antigen is added, and attaches only to rubella specific IgM.
- 4. & 5. Enzyme-labelled antibody to rubella is added and detected by adding a substrate

- IgM antibody rises following secondary infections (e.g. reactivation of herpesviruses)

- or booster doses of polio or rubella vaccines are possible sources of error.

- **Immunoblotting methods:**
- Southern blot: DNA
- Northern blot: RNA

- Western blot:

- For protein identification
- HIV, multiple methods are necessary

- 1 viral proteins are separated as bands according to their molecular weights by
- electrophoresis through a polyacrylamide gel
- 2 The bands are eluted (‘blotted’) on to chemically treated paper, to which
- they bind tightly
- 3 The test human serum from the patient is added to the paper strip and any specific antibody attaches to the viral proteins
- 4 antihuman antibody labelled with an enzyme is added, followed by the enzyme substrate; the paper is then inspected for the presence of stained bands , which indicate the presence of complexes of specific antibody with antigen

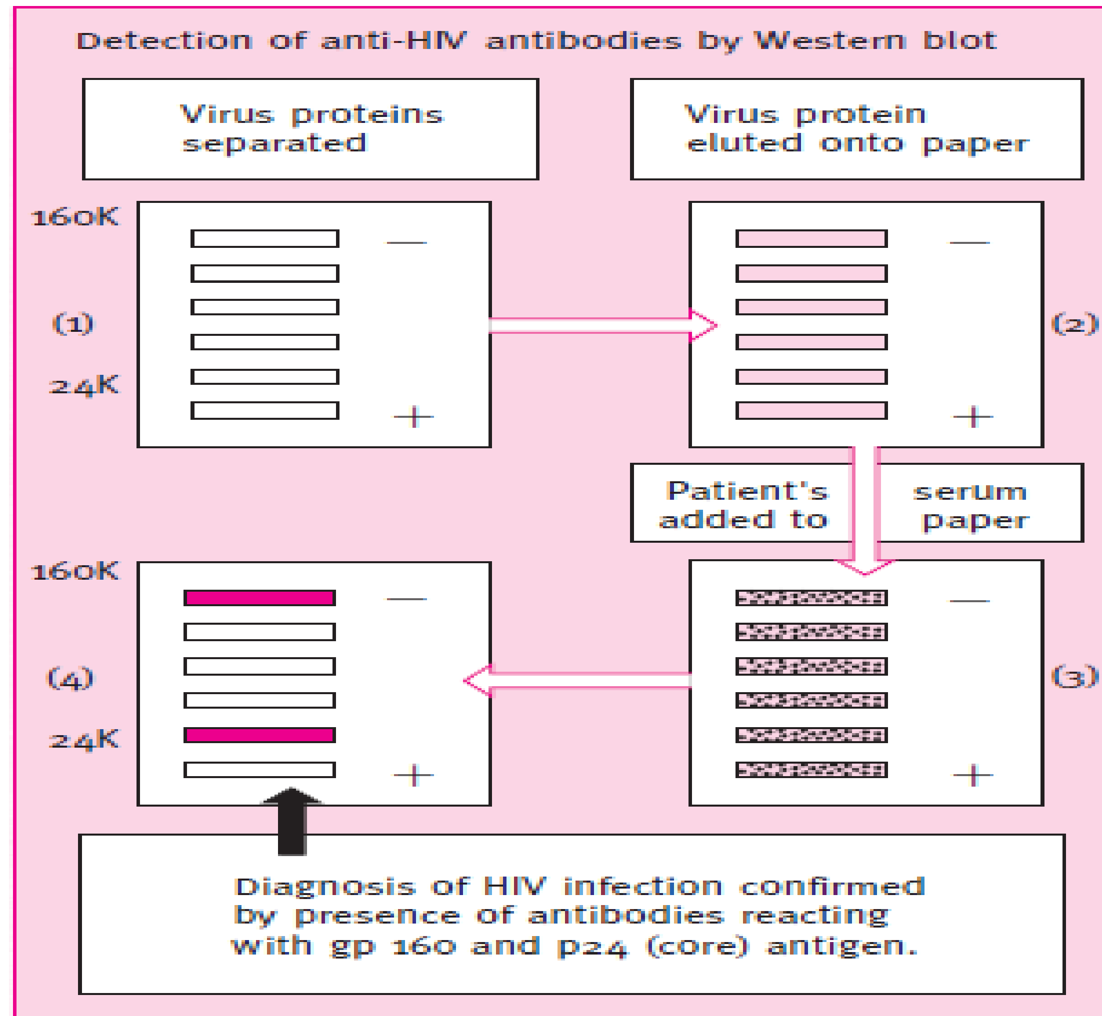


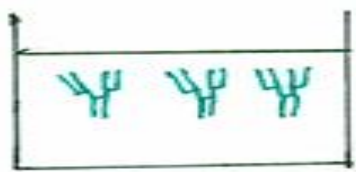
Fig. 36.5 Detection of anti-HIV antibodies by Western blot.

- **Traditional' serological tests:**

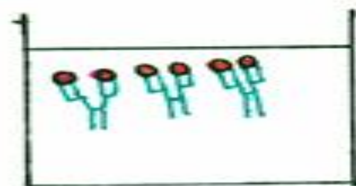
- complement fixation (many virus infections),
- radial haemolysis (screening test for rubella antibody),
- haemagglutination inhibition to detect postimmunization antibodies to influenza.

- **Complement fixation:**
- versatile test relatively insensitive and requires large amounts of antigen, which are not available for all viruses.
- in hospital practice for detecting increases in antibody titre in paired sera:
- it does not discriminate between IgM and IgG antibodies.
- Many antibodies,when reacted with their specific antigen and complement, form a complex.
- No antibody then no complex and no complement will be fixed negative result)
- or undetectable COMPLEMENT (antibody present, complement fixed: positive result)

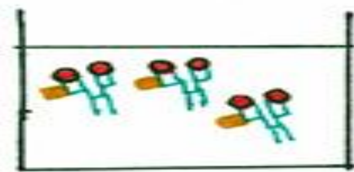
Comp. fix



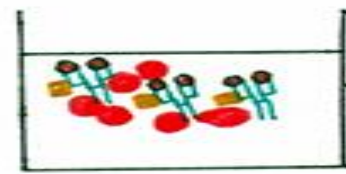
Serum with antibodies



Binding of antigen-antibody (Ag) (Ab)



Binding of complement with Ag/Ab complex

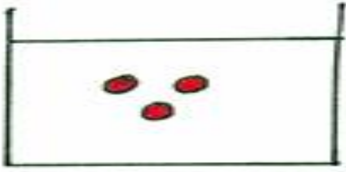


SRBCs serve as an indicator

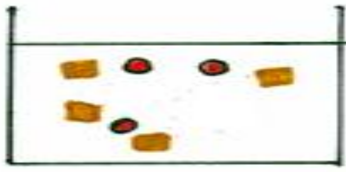
RBCs settle into a pellet no lysis

Reactive

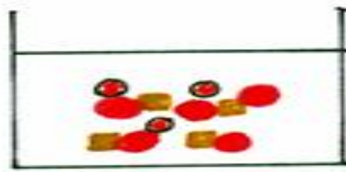
Serum without antibodies



unbound antigen



Unbound compliment



Unbound compliment lysing the SRBCs

Pink colour solution Lysis

Non-reactive

- **The radial haemolysis test:**
- is a variant of the complement fixation test
- the virus is linked to red blood cells by chromium chloride. THEN mixed
- with molten agarose, and poured into a plate and left to cool down.
- small wells are then punched in the agarose,
- each well filled with a serum sample (antibodies)
- incubated overnight to allow diffusion of antibody into the agarose and combination
- with the antigen on the red cells

- a solution of complement is poured over the plate, and lyses those red cells in which both antigen and antibody are present.

- A well containing antibody is thus surrounded by a clear zone of lysis,

- the diameter of the zone is related to the amount of antibody in the sample.

- commonly used as a screening test for rubella antibodies in females.
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- It is not accurate enough for assessing the antibody status of individual patients, but can be
- used to screen large numbers of sera, e.g. from antenatal clinics.
- used also to test for influenza antibody also

END