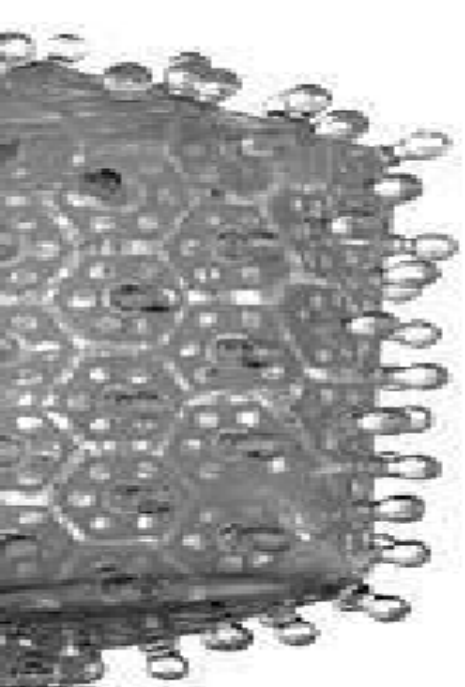


Microbiology

Slide #: Viro-7

Dr Name: Dr. Hamed

Sheet ☐ Slide ■

Mrym Ghuloom

Virology – **Diagnosis 1**
JU- 2nd Year Medical Students

By

Dr Hamed AlZoubi – Microbiology and Immunology Department – Mutah University.

MBBS (J.U.S.T)

MSc, PhD medical microbiology (UK).

FRCPath (associate, medical microbiology).

dr_alzoubi@yahoo.com

Diagnosis of viral infections

- History
- Examination
- Routines
- Microbiology (virology) investigations

Diagnosis of viral infections

- Sample collection

- no results without good quality samples from the patient

- right specimen, right time, properly taken, transported or stored

Viral transport medium (VTM)

- VTM:

- tissue culture medium containing antibacterial and antifungal antibiotics to inhibit contaminants
- a protein stabiliser (such as bovine serum albumin) to protect sensitive viruses
- a buffering solution at pH 7.0

Types of specimen

Table 36.1 Specimens required for isolation of virus or detection of antigen

Disease	Specimen
Respiratory infection	Nasal or throat swabs; postnasal washing
Gastrointestinal infection	Faeces (rectal swab not so satisfactory)
Vesicular rash	Vesicle fluid, throat swab, faeces
Hepatitis	Serum, faeces
Central nervous system	Cerebrospinal fluid, throat swab, faeces
AIDS	Unclotted blood

NB. In addition to the above, 5–10 ml of clotted blood for serological tests

Samples

- **Swabs**
- Must be adequate.
- throat or skin swabs must be taken fairly vigorously.
- into a vial of transport medium

Samples

- **Nasopharyngeal aspirates**
- For upper respiratory tract infections, of young children, e.g. RSV and influenza

- distressful to the child and requires skill and practice.

Samples

- **Vesicle fluid for EM**
- poxvirus or herpes
- Collected on the tip of a scalpel blade, spread over an area about

3–4 mm in diameter on an ordinary microscope slide, and allowed to dry.

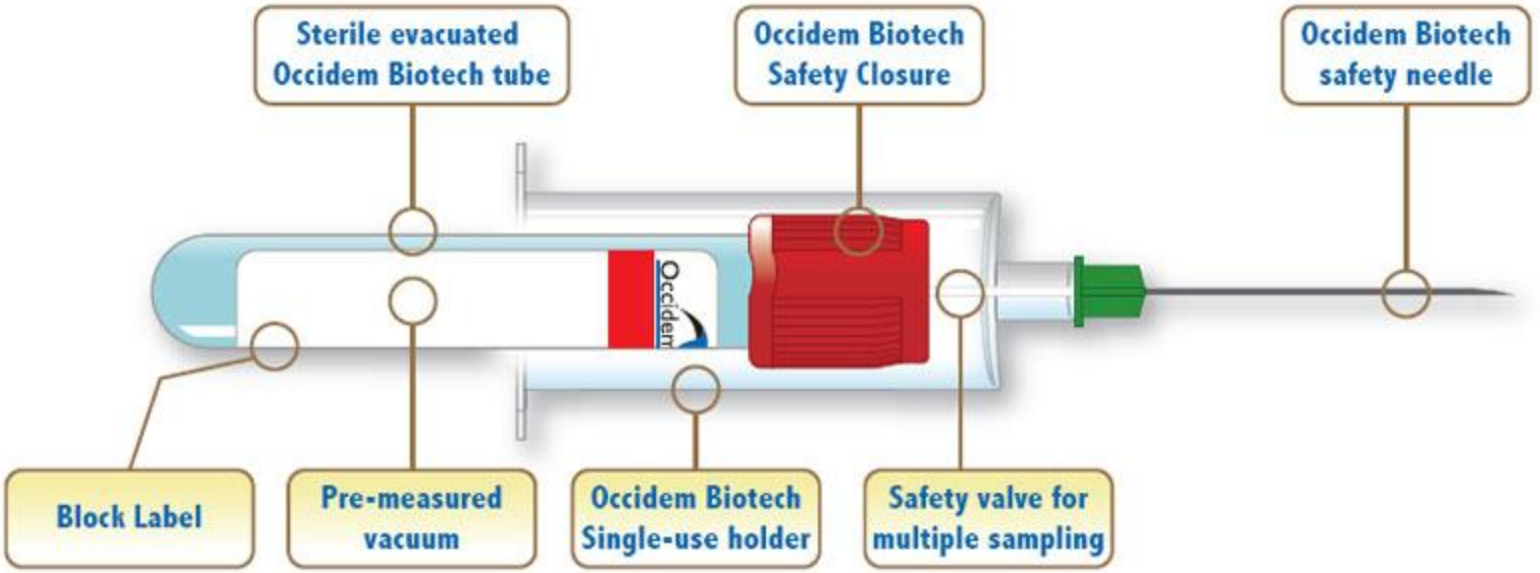
Samples

- **Faeces:**
- To identify enteroviruses or rotaviruses
- Sent in a dry sterile container
- Better than rectal swabs for virus isolation

Samples

- **Clotted blood**
- 5–10 ml blood is taken using a syringe rather than a vacuum tube
- the needle should be removed before expelling the blood to avoid haemolysis.
- EDTA (anticoagulant)blood is used for detecting various viral genomes.

VACUUM TUBE


Sterile evacuated Occidem Biotech tube
Occidem Biotech Safety Closure
Occidem Biotech safety needle
Block Label
Pre-measured vacuum
Occidem Biotech Single-use holder
Safety valve for multiple sampling

Storage and transport

- Plastic bags (details of patient, signs and suspected diagnosis)

- Send immediately or store at 4 in fridge but not frozen – enveloped viruses might be destroyed if frozen

RAPID DIAGNOSTIC METHODS

❑Immunofluorescence

✓ *Direct fluorescent antibody method:*

- *Detect viral antigen in the clinical sample or from an overnight cell culture to amplify the virus*

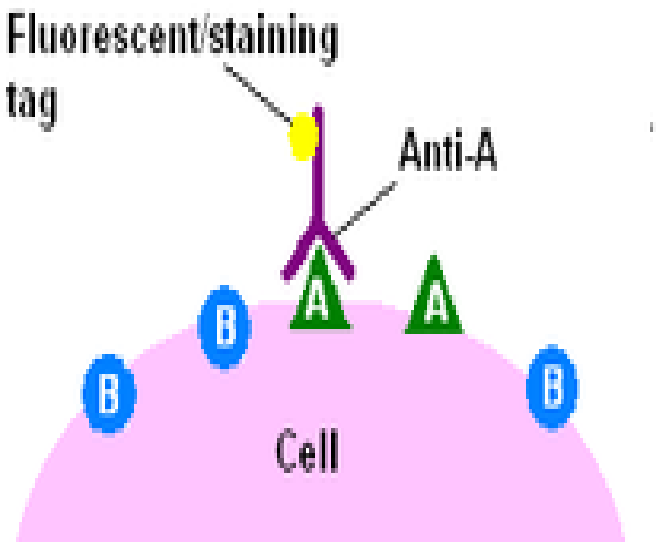
- reacted with a specific antiserum, which is

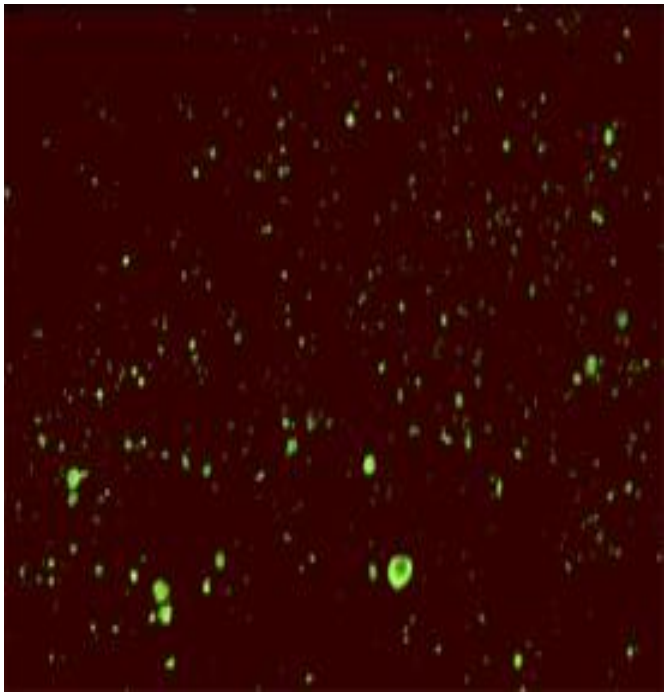
coupled with a fluorescent dye (fluorescein isothiocyanate, FITC).

RAPID DIAGNOSTIC METHODS

- washing to remove unattached serum and dye
- the specimen is viewed in an ultraviolet microscope.
- The FITC on serum specifically attached to virus or viral antigen becomes visible as a green fluorescence
- Disadvantage: many specific sera must be labelled in order to test for a range of viruses

RAPID DIAGNOSTIC METHODS





RAPID DIAGNOSTIC METHODS

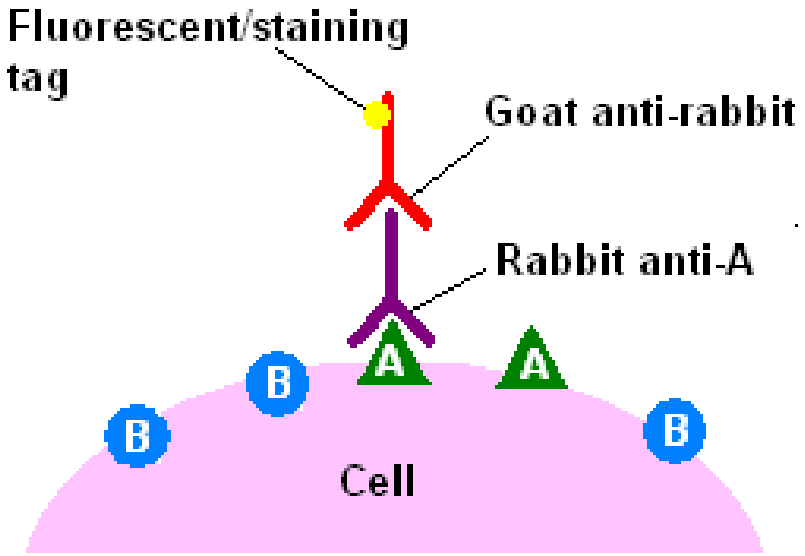
✓ *Indirect fluorescent antibody method*

As direct but:

- the dye is attached to a second serum prepared against globulins from the species in which the specific serum was made For example, antibodies to human immunoglobulins are often made in rabbits or goats (e.g rabbit antihuman IgG)
- RSV, Influenza directly in swabs or 12-18 hrs post culture
- CMV 48 hrs post culture (faster than CPEs)

RAPID DIAGNOSTIC METHODS

✓ *Indirect fluorescent antibody method*



RAPID DIAGNOSTIC METHODS

✓*Indirect fluorescent antibody method*

- called a ‘sandwich’ method, because there are three layers:
- 1. The specimen being tested for a specific virus.
- 2. The specific antiviral serum, prepared in (say) rabbits.
- 3. FITC-labelled antirabbit antibody.

RAPID DIAGNOSTIC METHODS

- *Indirect fluorescent antibody method*
- great advantage that only one labelled (anti-species) serum is needed to test for many viruses.
- Similar to ELISA but immunoperoxidase instead of FITC is used, which is then reacted with a substrate to give a precipitate visible by ordinary light microscopy

RAPID DIAGNOSTIC METHODS

- ✓ **Enzyme-linked immunosorbent assay and radioimmunoassay**
- Very common quantitative method for antigens or antibodies
- Commercially available e.g capture antibodies on beads
- Detect antigens or antibodies
- Positive and negative control is necessary

ELISA

- As indirect IF but:
- the label is either an enzyme (ELISA) or radioactive iodine (RIA, less used) not FITC
- binding of the labelled antibody (or antigen) is detected by reacting the enzyme with a substrate which then produces a visible colour in the reaction mixture (ELISA) or by counting radioactive emissions (RIA)
- The reaction takes place in a multiwell plastic plate or tube,read by photometry (ELISA) or by a gamma counter (RIA) and printed out automatically

ELISA

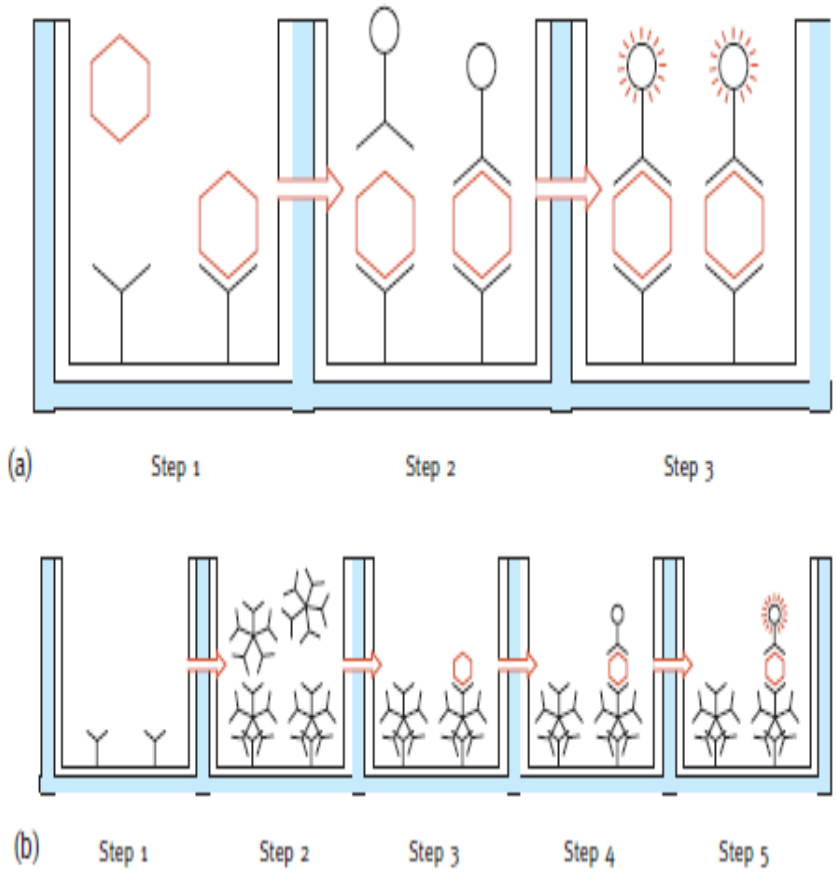
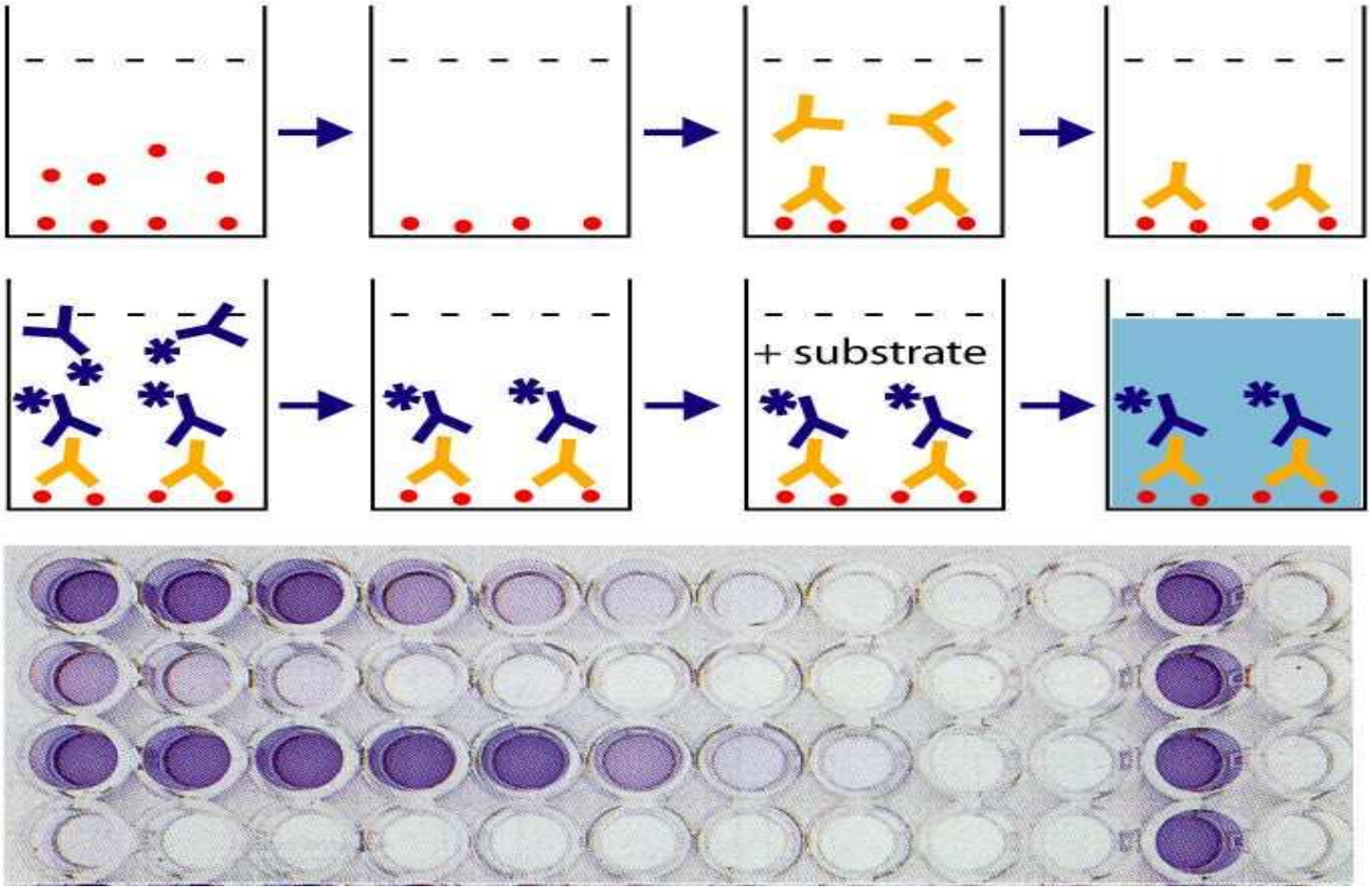


Fig. 36.2 **(a)** Direct identification of antigen by captures and ELISA. **Step 1:** Addition of specimen containing antigen that combines with the specific 'capture' antibody on a plastic surface. **Step 2:** Addition of enzyme-labelled specific antibody. **Step 3:** Substrate is added, reacts with bound enzyme, and undergoes colour change. **(b)** Identification of specific IgM antibody by capture and ELISA. **Step 1:** Plastic surface coated with antibody to IgM. **Step 2:** Patient's serum added; IgM molecules are captured by the anti-IgM. **Step 3:** After washing to remove unattached IgM, test antigen is added and combines with any captured IgM of the same specificity. **Steps 4 and 5:** as steps 2 and 3 in (a). Note that the captured IgM molecule on the left, having no specificity for the test antigen, does not react.


+ substrate

✓Latex agglutination tests

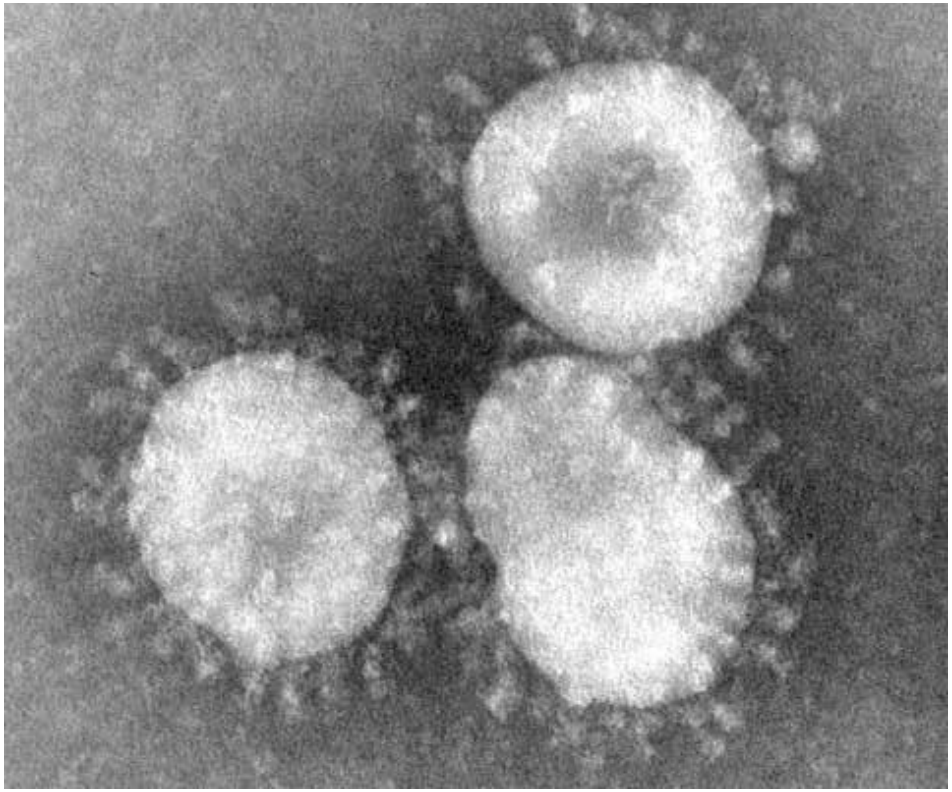
- Rapid, easy, no complicated devices
- Latex with viral antigen mixed with sample has antibody : agglutinate forming particles
- liable to prozone effects, giving false negative results at low dilutions of serum


Reagent
Positive Control
Reagent
Staph
Reag
1
2
3
HARDY
DIAGNOSTICS
Copyright 2009 All rights reserved.

✓Electron microscope , less used

- PTA stain
- Negative staining
- White particles ? on a black background
- It needs an expert microscopist and at least 10^6/ml virus particles (might need concentration)

Coronavirus under EM



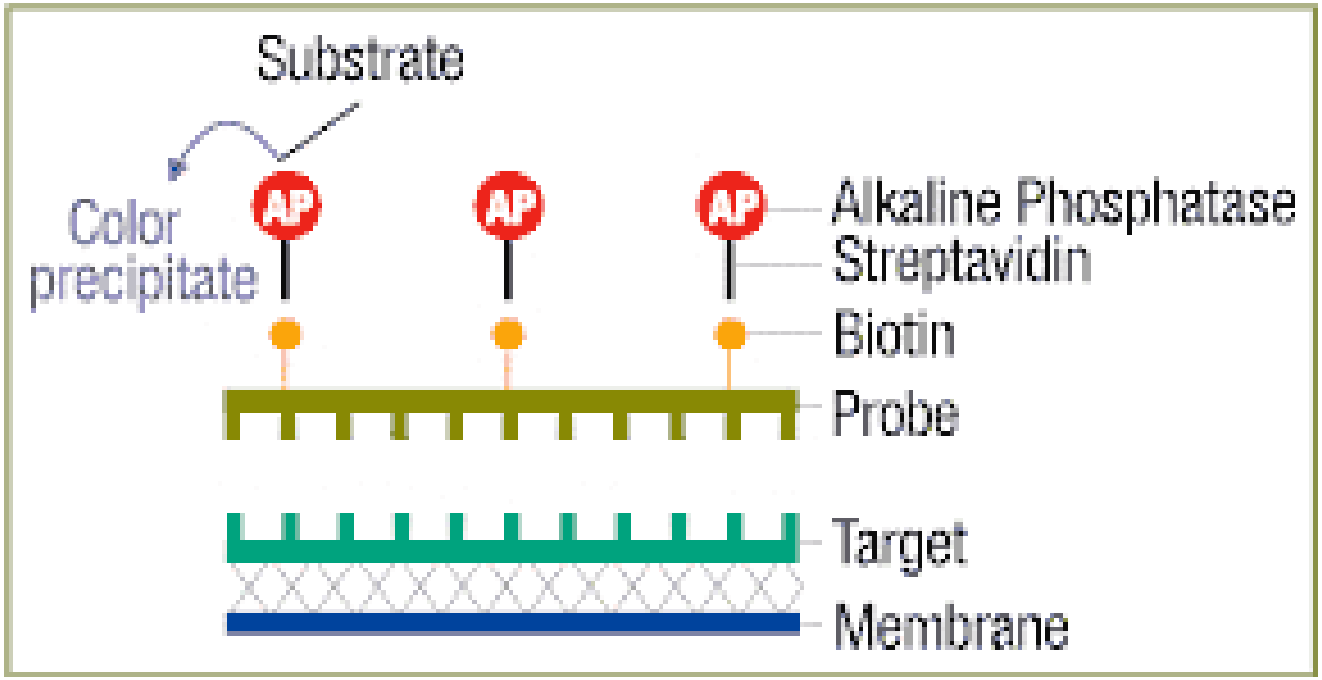
✓Electron microscope , less used

- SARS coronavirus
- HSV and VZV
- HBV and gastroenteritis viruses (Can't be cultured)
- N.b: Immuno EM:

Addition of specific labelled antiserum to increase specificity

Detection of viral genome by nucleic acid hybridization

- Sensitive and common e.g for HPV, HSV, enteroV
- Dot blot hybridization:
- Extract and denature DNA
- Place on nitrocellulose paper
- Treat with a probe consisting of a labelled (fluorescent dye or a radioisotope) stretch of DNA or RNA complementary in sequence to the specific region being sought in the specimen
- Hybridization *in situ* *is similar, except that it is used directly in* tissue sections.


Substrate
Color precipitate
AP
AP
AP
Alkaline Phosphatase
Streptavidin
Biotin
Probe
Target
Membrane

✓ **Detection of viral genomes by nucleic acid amplification methods**

END