M1 D²HP Development of Drugs and Health Products

OTU 01 Diagnosis of infectious diseases
Introduction class

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- 1- Direct diagnosis: antigen detection
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III- Context of diagnosis

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Conventional microbiological diagnosis

- Conventional microbiology techniques
  - Direct examination after specific staining (Gram)
  - Detection of specific antigen
  - Bacterial and fungal culture
  - Antibiogram on isolated colonies
  - Cell culture for viruses
  - Serodiagnosis
- Issues
  - Low sensitivity
  - Multiplicity of possible etiologies for the same infectious syndrome
  - Culture default (patients already under treatment, infectious agents difficult to isolate or not cultivable)
  - Important delays in obtaining partial and then complete results
    - ↳ incompatible with early adaptation of treatments

⇨ **Emergence of molecular biology techniques +++**

Biological signs and biological diagnosis of infection

- **Non-specific diagnosis**
  - Biochemical analysis
  - Hematology analysis
  - Cytopathological analysis, etc.
- **Specific diagnosis**
  - Identification of the infectious agent
  - Identification of specific antibodies

I- Samples

Principle of sampling

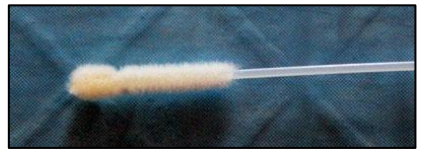
- Taking samples based on the syndromes and what we are looking for (suspected virus)
- At the site of virus multiplication
  - target organ, virus entrance door
    - if accessible
- The earliest, at the beginning of clinical signs

  = during the acute phase of the disease
    - when viral excretion is the highest

Types of samples

- Blood, serum
- Stool
- Urine
- Nasal swab, nasopharyngeal aspiration, bronchoalveolar lavage
- Pap smear
- Biopsy
- Cerebrospinal fluid
- Amniotic fluid

Collection and transportation of the sample



- For isolation, the viability of the viruses present must be preserved
- Suitable transport media
- Protected from drying, heat and pH variations

II- Virological diagnosis

Techniques for virological diagnosis

Direct diagnosis	Indirect diagnosis
Antigen detection	**Serology**
Immunofluorescence, immunoperoxidase	ELISA
Seroneutralisation	Western-Blot
IHA	Immunochromatography
ELISA	IgG avidity assay
Immunochromatography	
Genome detection	
PCR (RT-PCR, real time PCR)	
RNA amplification (NASBA, TMA)	
Branched DNA assay	
Hybridization (*in situ*, liquid phase)	
Virus isolation	
Virus isolation in cell culture (CPE, haemadsorption)	
Electron microscopy	

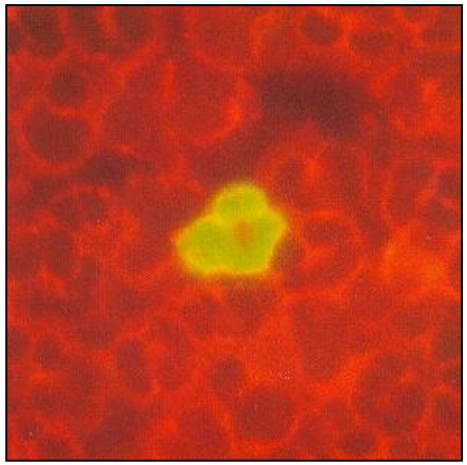
II- Virological diagnosis

1- Direct diagnosis: antigen detection

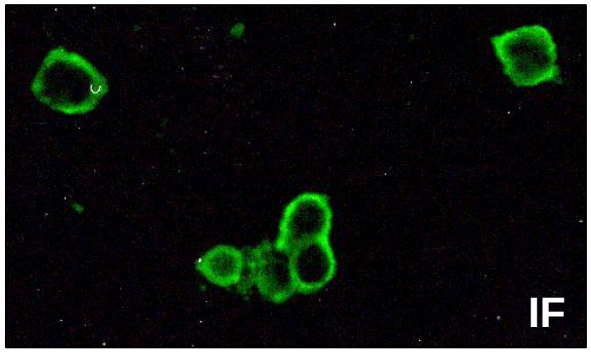
Techniques for antigen detection (1)

- **Immunofluorescence**

Antigenemia pp65 (CMV)



HSV-1 and HSV-2

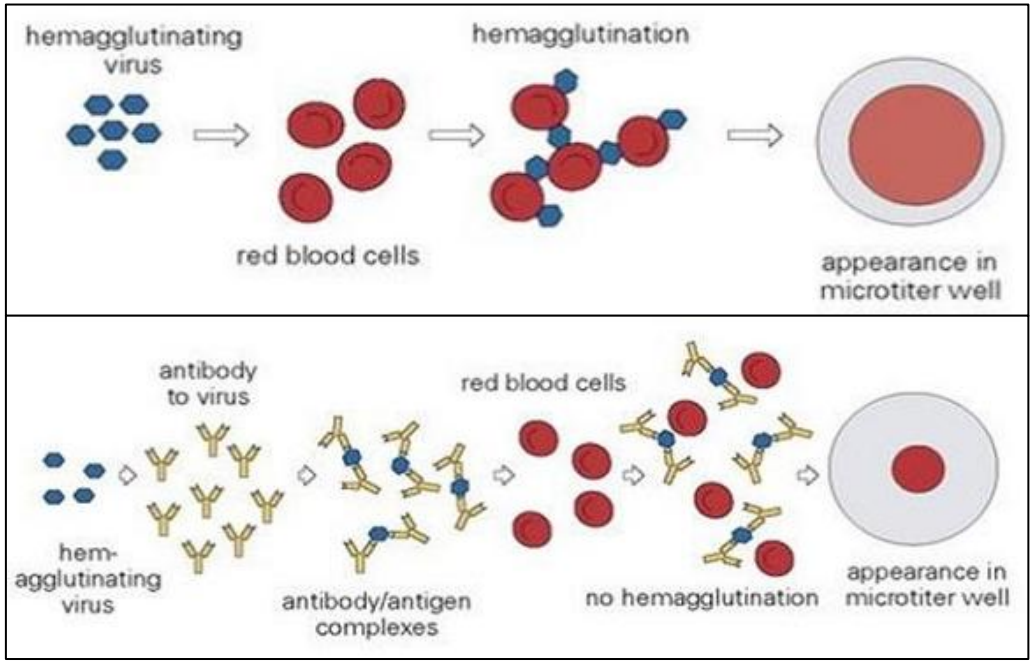


Techniques for antigen detection (2)

☞ Inhibition of hemagglutination (IHA)

↳ antiserum

Rubella virus

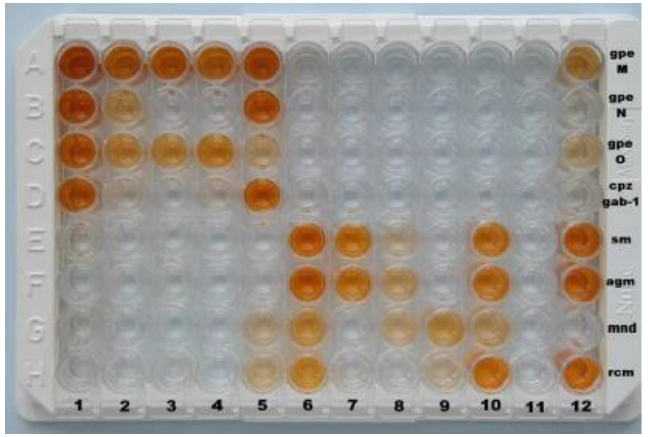


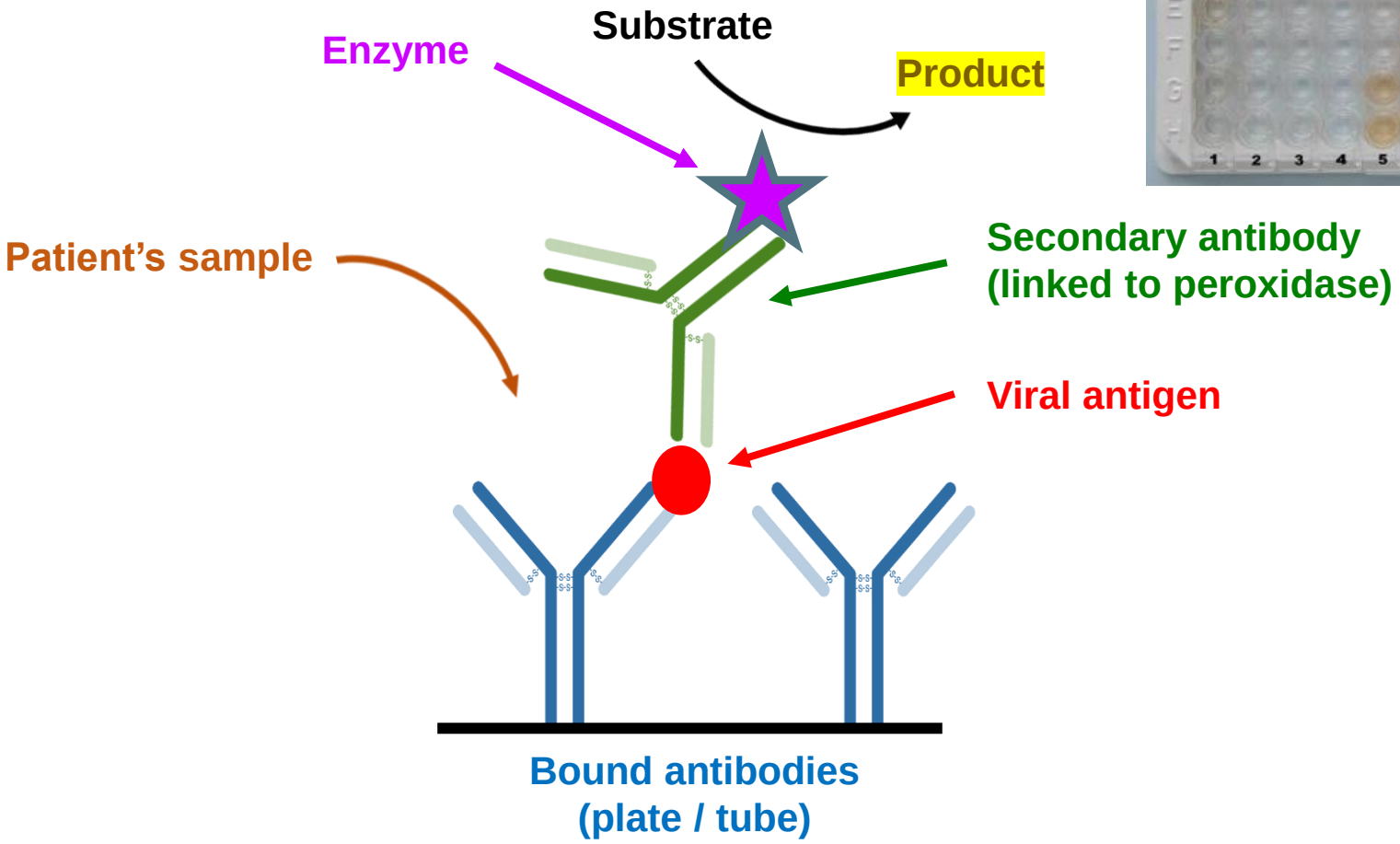
☞ Advantages

- Rapid and easy to use
- Applicable to viruses that are not cultivable or difficult to cultivate

ELISA (Enzyme-linked immunosorbent assay)

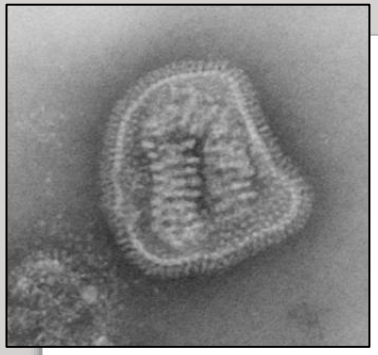
☞ **Direct ELISA (sandwich)**



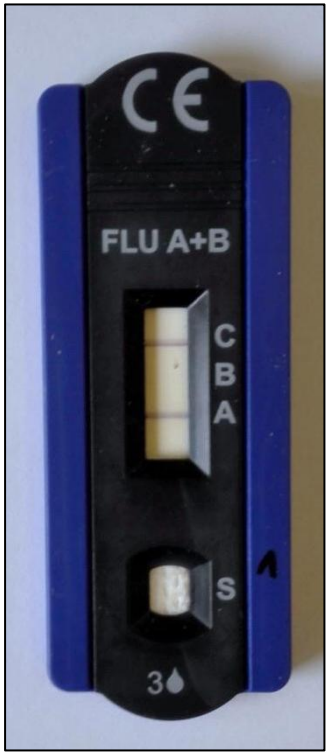


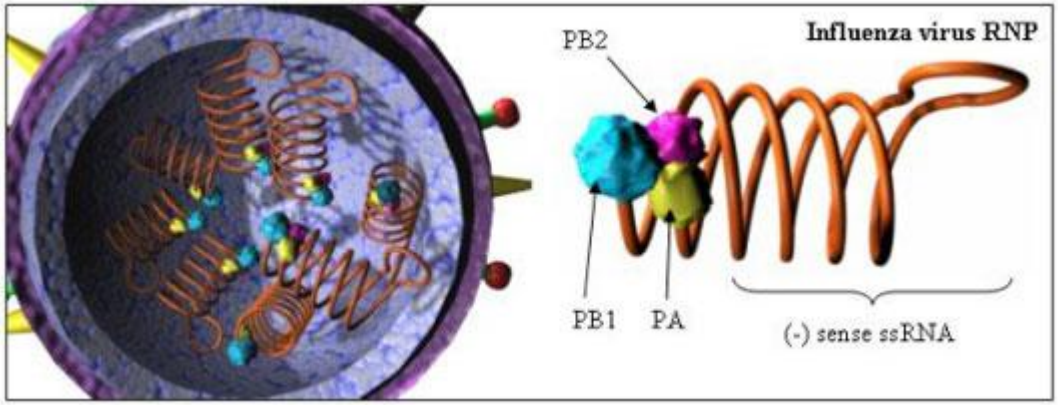
⇨ **Very sensitive method (nM or pM)**

Immunochromatography (1)

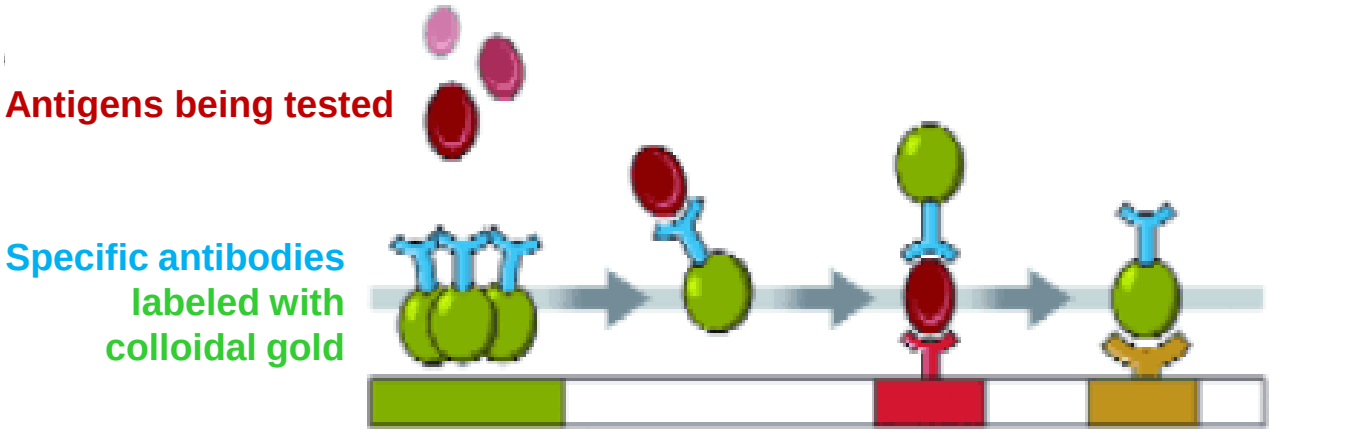


- Rapid diagnostic test (RDT)
  - Immunochromatography for influenza virus
    - Rapid and easy to use
    - Results in 15-30 minutes
    - Visual reading
    - Detection of the nucleoproteins of the influenza viruses A and B
    - Detection of all subtypes of influenza A virus
      - H1N1, H5N1





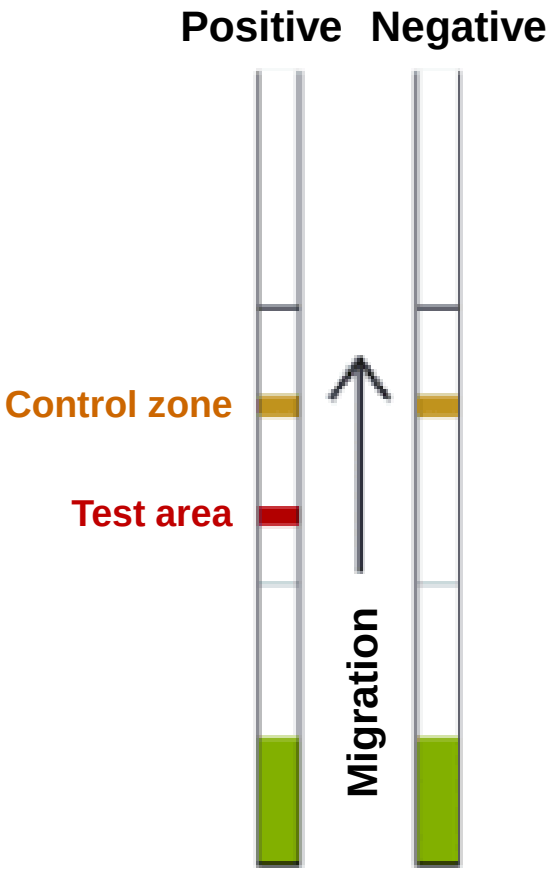
Immunochromatography (2)



Nitrocellulose membrane: antigen-antibody mixture migration by capillarity

Test zone: capture of antigen by a second specific antibody

Control zone: capture of excess gold by a specific antibody



Rapid diagnosis

- Indications
  - Specific and timely patient management in certain circumstances
    - Respiratory infections: influenza virus, SRV (Syncitial Respiratory virus), SARS-CoV-2, etc.
    - Infant diarrhea: adenovirus, rotavirus

- Advantages
  - Speed of rendering result
- Limitations
  - Lack of sensitivity

II- Virological diagnosis

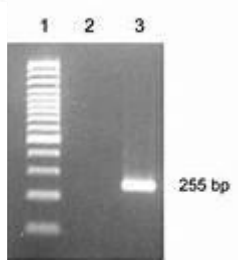
2- Direct diagnosis: genome detection

PCR / RT-PCR

Sample (plasma, blood, urine) → Nucleic acid extraction → RT in some cases + PCR amplification → Results in a few hours

2 methods of detection

- Agarose gel



- Real-time PCR

⇨ Very sensitive method

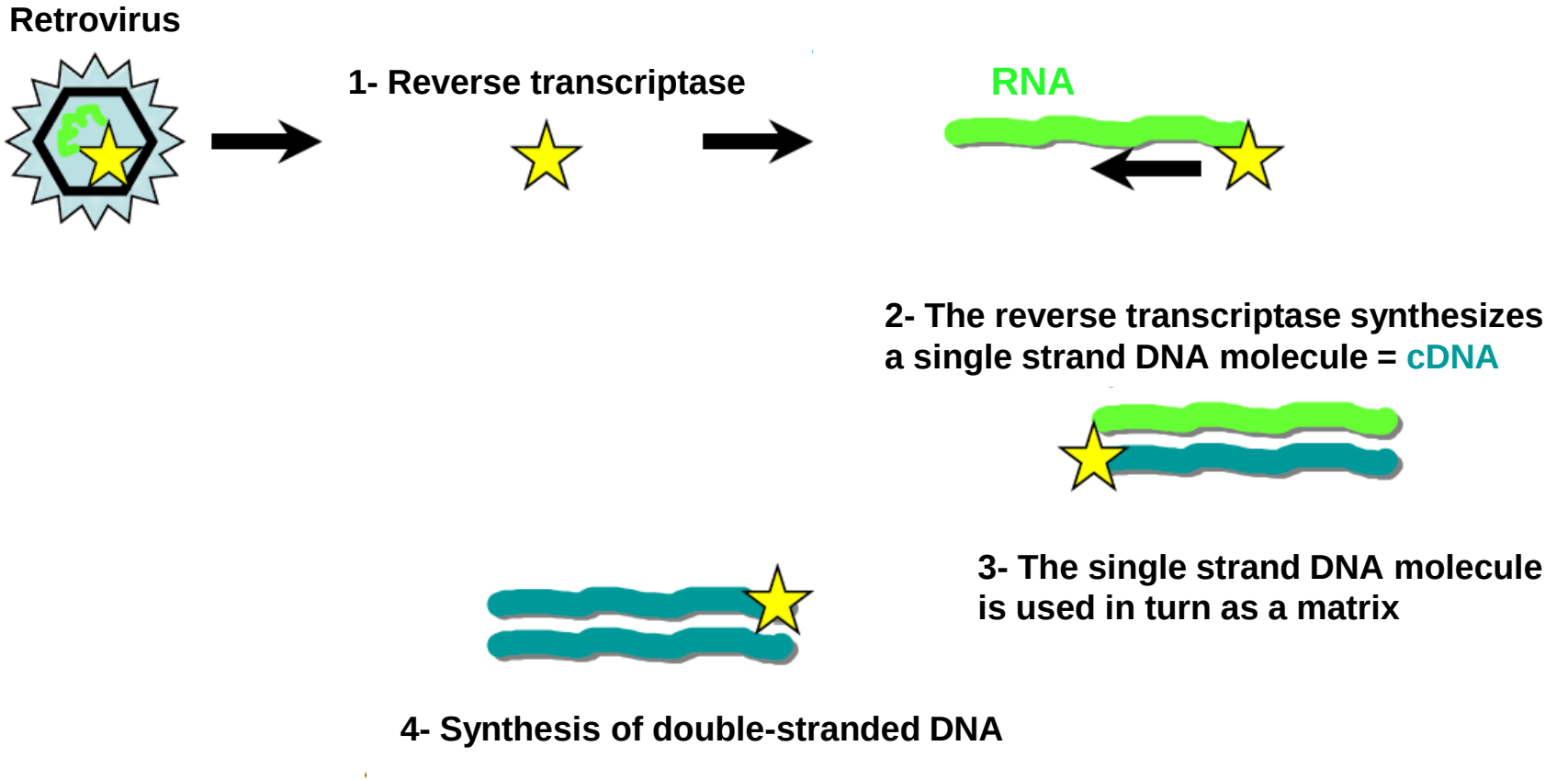
⇨ But beware of false negatives

↳ highly variable viral genomes

Reverse transcription (RT)

☞ Reverse transcriptase synthesizes single strand DNA

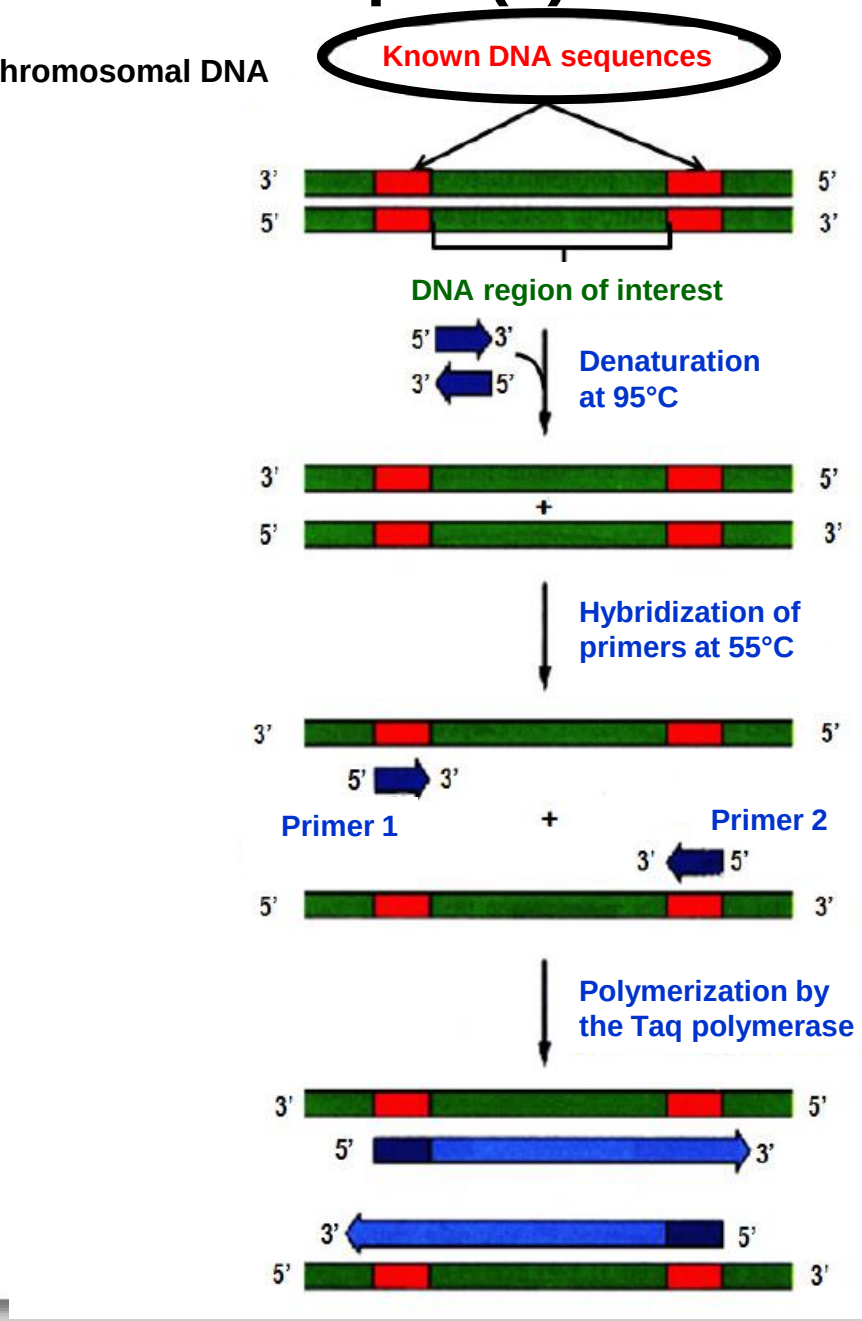
↳ using an RNA as a matrix



⇨ The complementary DNA (cDNA) is then amplifiable by PCR

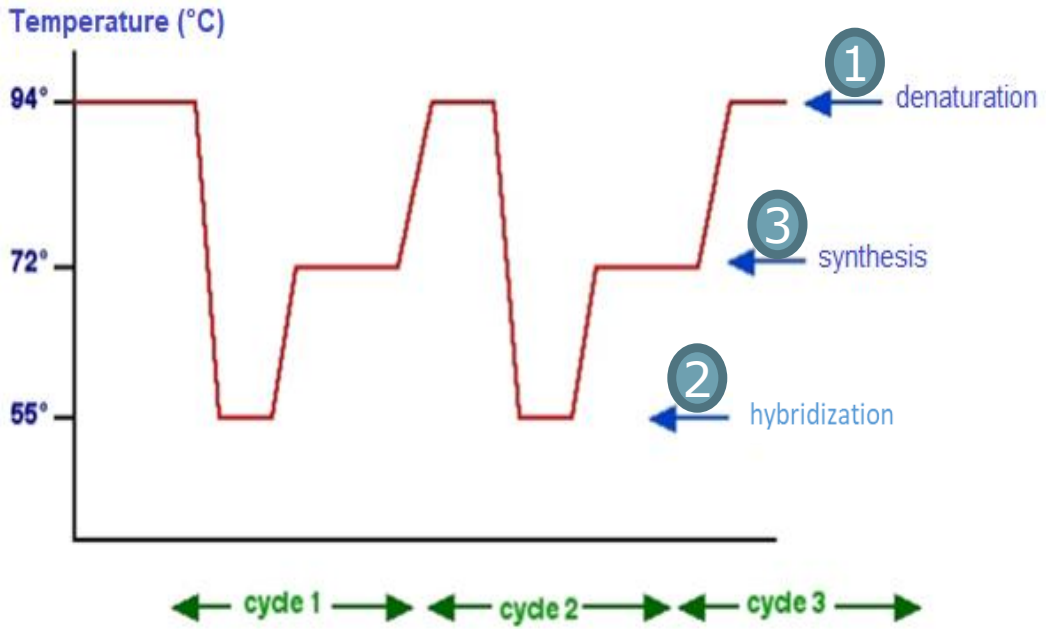
Gene amplification by PCR technique (1)

- **PCR = Polymerase Chain reaction**
  - production of a large number of copies of a specific DNA sequence
    - N cycles of PCR
    - 2^N copies of initial DNA
    - General case: N = 30 cycles
- **Synthesis in direction 5' to 3'**
- **Reaction mix**
  - Taq polymerase
  - DNA matrix
  - 2 primers forward and reverse
  - Buffer
  - dNTPs
  - $MgCl_2$

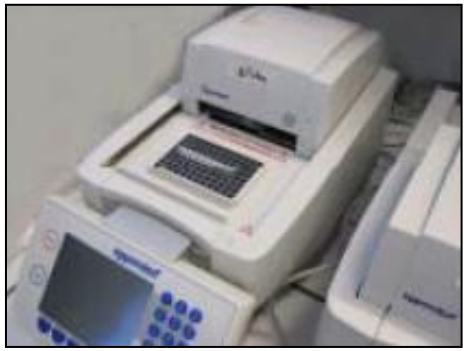


Gene amplification by PCR technique (2)

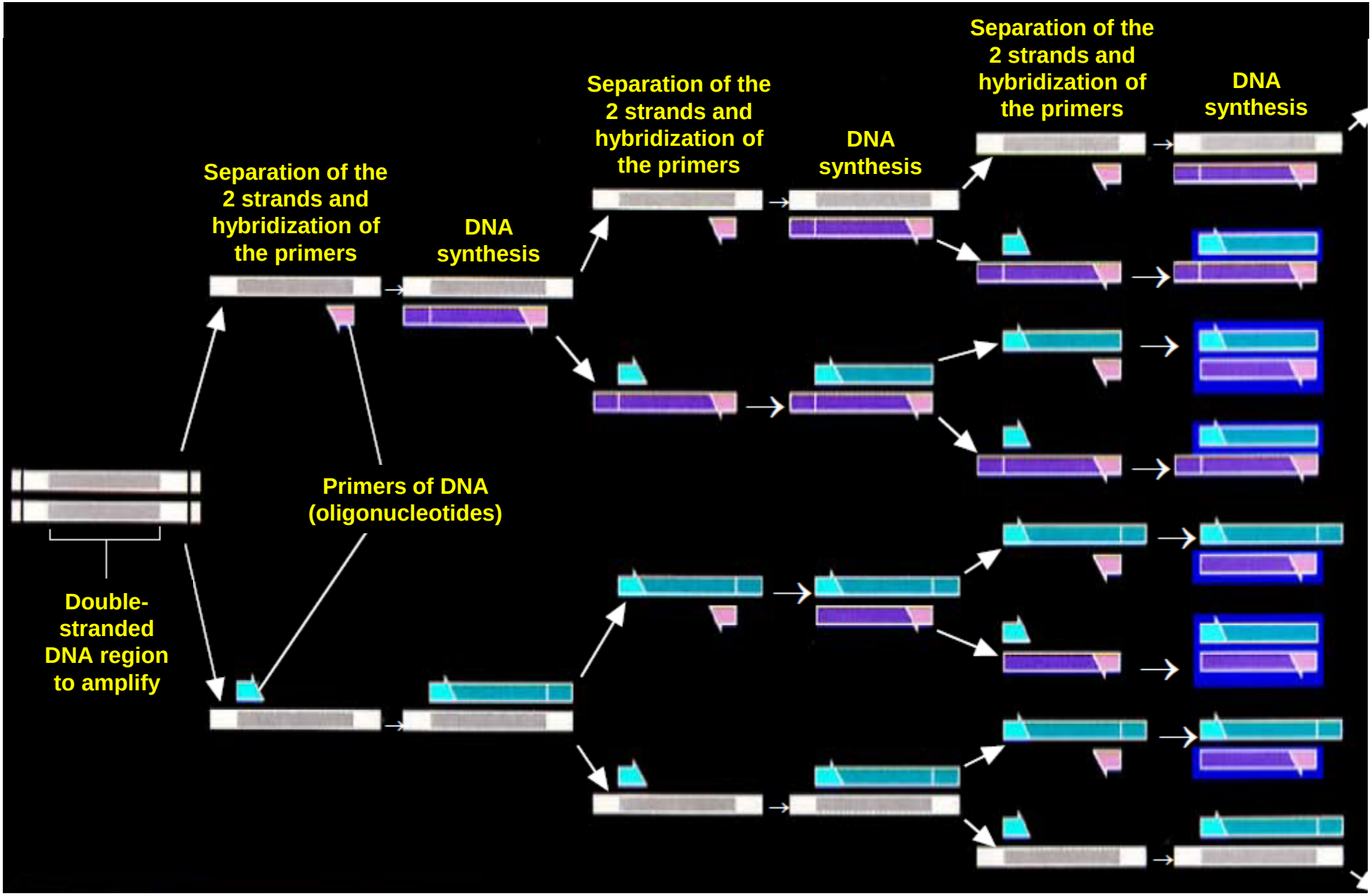
☞ **N cycles of PCR**



Thermocycler



Gene amplification by PCR technique (3)



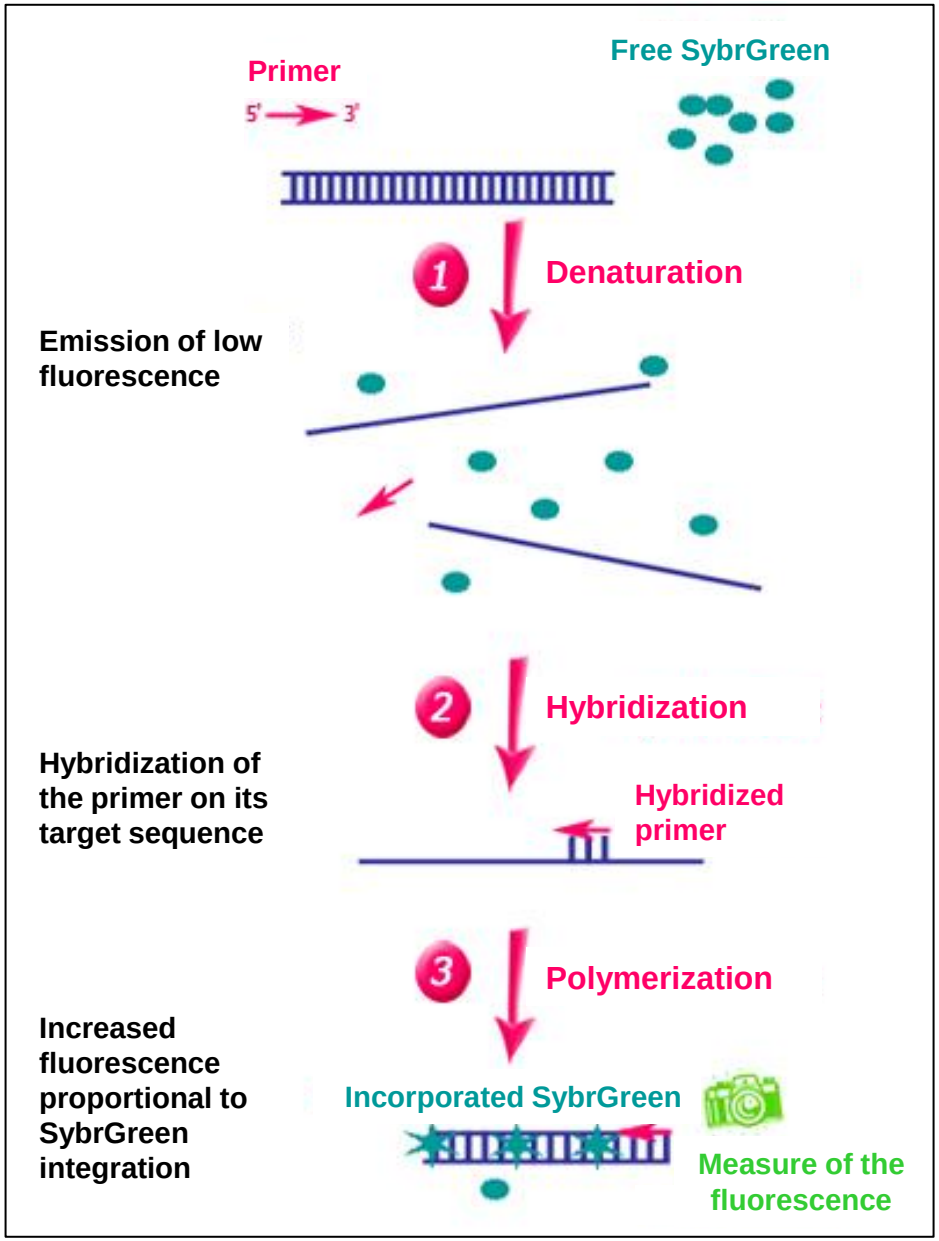
Real-time PCR

- **Principle**
  - **real-time measurement of quantities of PCR products synthesized during amplification**

- **Requirement**
  - **- emission of a signal proportional to the amount of amplicon produced**
  - **- signal measurement tool**
  - **- computer tools for analysis**

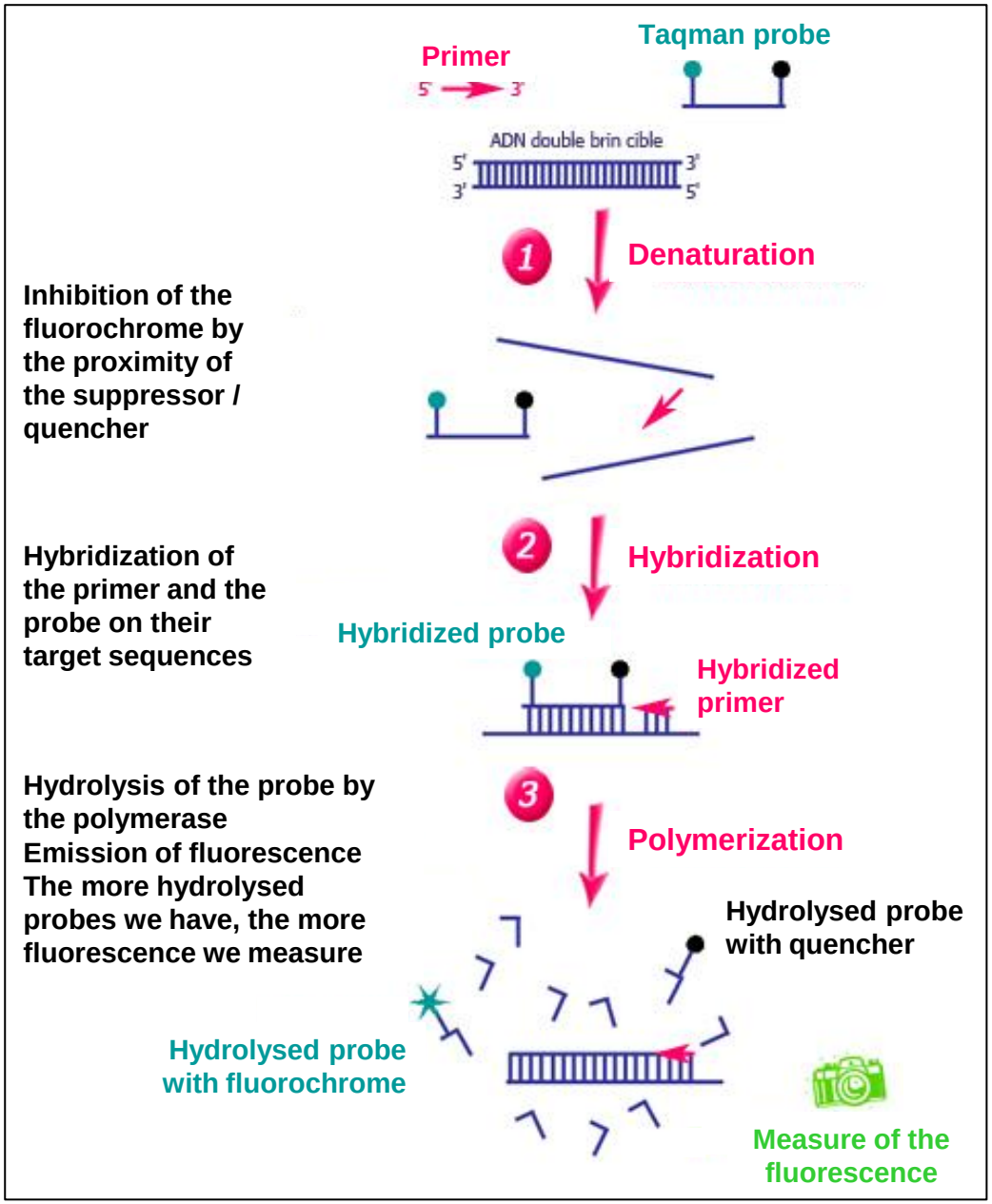
Real-time PCR and SybrGreen

- **SybrGreen**
  - ⇨ **double-stranded DNA binding agent**
    = intercalant of DNA



Real-time PCR and Taqman probe

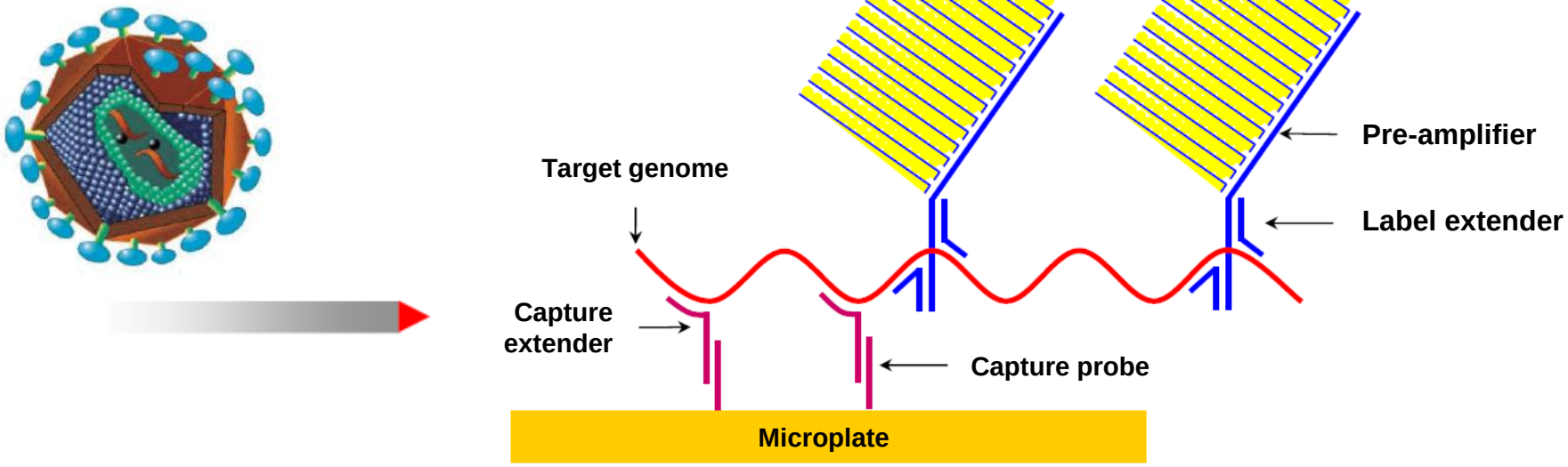
☞ Taqman hydrolysis probe



Branched DNA Assay

- Numerous complementary probes to different regions
  - detection of highly variable viruses

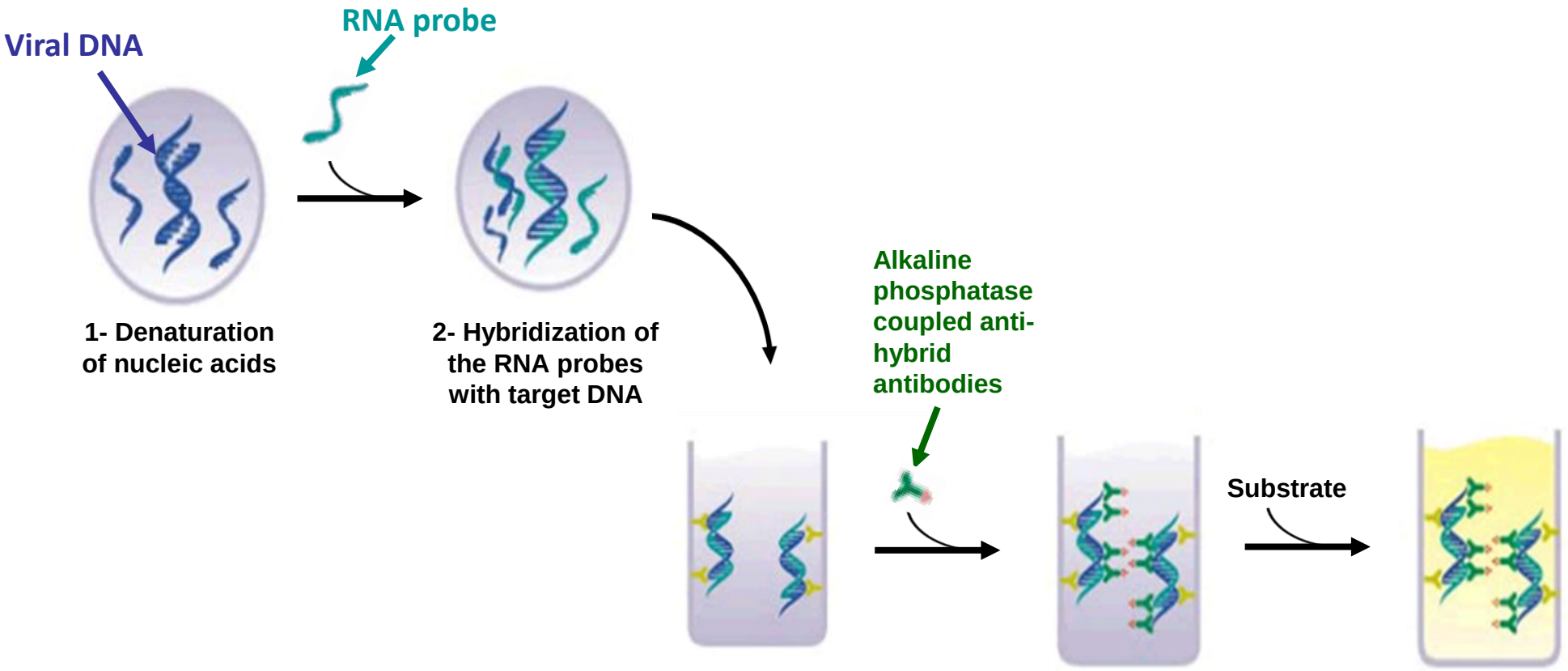
    HIV RNA, HCV RNA, HBV DNA



Liquid phase hybridization

☞ Capture of hybrids on solid phase with anti-hybrid antibodies

Digene HPV test ⇨ detection of 13 high-risk HPV types



Molecular diagnosis (1)

- **Qualitative techniques**
  - **Show the presence of a virus**
- **Quantitative techniques**
  - **Follow-up of patients treated with antivirals (HIV, HBV)**
- **Sequencing**
  - **Characterization of viral genotypes (HCV)**
  - **Detection of nucleotide mutations associated with antiviral resistance (HCV, HIV)**
  - **Characterization of viral tropism (HIV)**
  - **Phylogenetic analysis to trace the origin of an infection**

Molecular diagnosis (2)

- **Advantages**
  - **High sensitivity of available tests**
  - **Automation +++**
  - **Result rendering time shortened (emergencies)**
- **Limitations**
  - **No evidence of the infectivity of the virus**
  - **Equipment and experienced personnel**
  - **Relatively high cost**

II- Virological diagnosis

3- Direct diagnosis: virus isolation

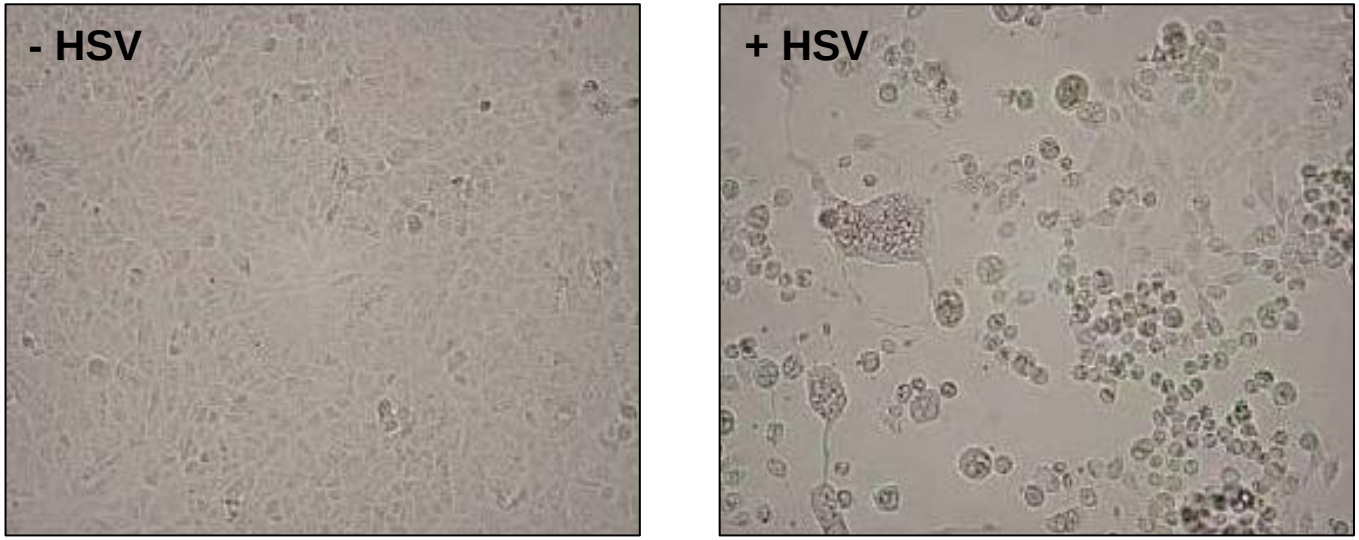
Virus culture

- Infection of cells in culture
- Long-standing reference technique
- Use of permissive cells
- Cytopathic effect (CPE)
- Haemadsorption

Cytopathic effect (1)

- **Changes in cell morphology**
  - **rounding**
  - **size increase**
  - **support detachment**
  - **lysis of infected cells**

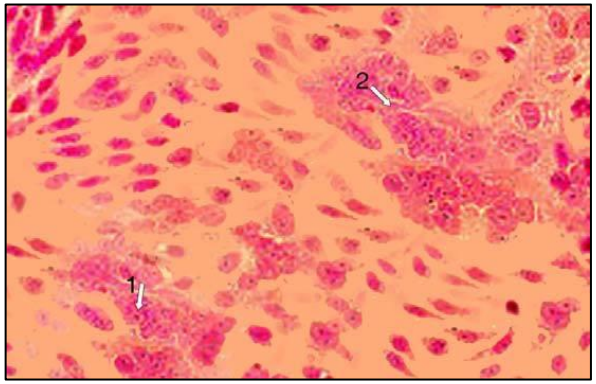
HSV



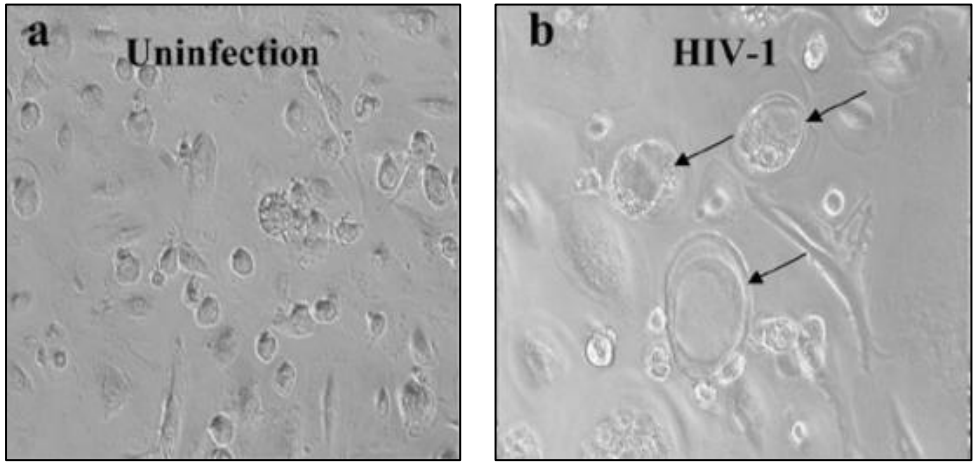
Cytopathic effect (2)

- Changes in cell architecture
  - syncitium formation
    - giant cell formed by membrane fusion of several cells

RSV infection on Hep-2 cell culture

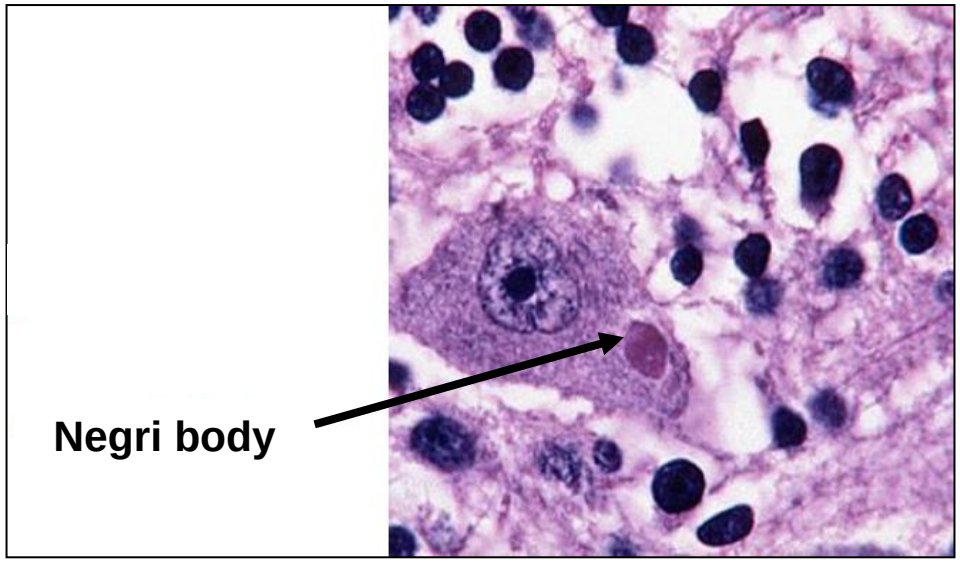


HIV-1



Cytopathic effect (3)

- **Formation of inclusions**
  - **visible after accumulation of viral material**
- **Cytoplasm**
  - **Poliovirus**
  - **Rabies virus: Negri bodies**
- **Nucleus**
  - **Herpesvirus**
  - **Adenovirus**



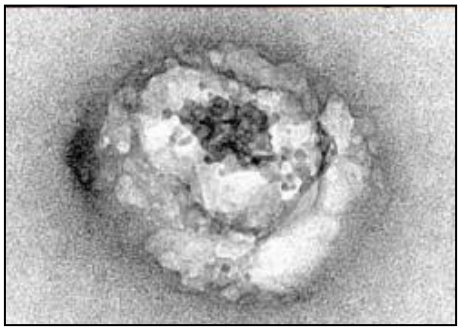
Virus isolation

- **Indications**
  - **Proof of infectivity**
  - **Characterization of the phenotype of a viral strain for antiviral resistance (HIV) or tropism (HIV)**
  - **Epidemiological studies**
  - **Vaccine production (flu)**
- **Advantages**
  - **Very good sensitivity = reference method**
  - **Essential for the availability of strains used in vaccine development (flu vaccine)**
  - **Reasonable cost**
- **Limitations**
  - **Time to result: 48 h to 10 days**
  - **Possible subjectivity of the reading**
  - **Non-cultivable virus problems: HPV**

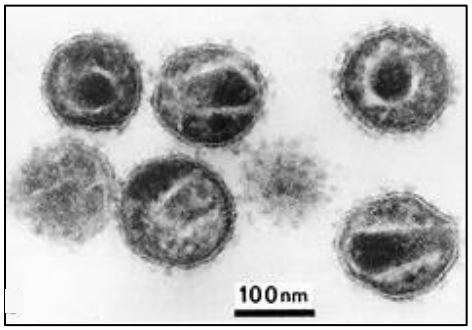
Electron microscopy

- Morphological study of virus

HCV



Retrovirus



- Study of the virus inside cells
- Very high resolution: 1 nm
- Interest of electron microscopy mainly in research

II- Virological diagnosis

4- Indirect diagnosis

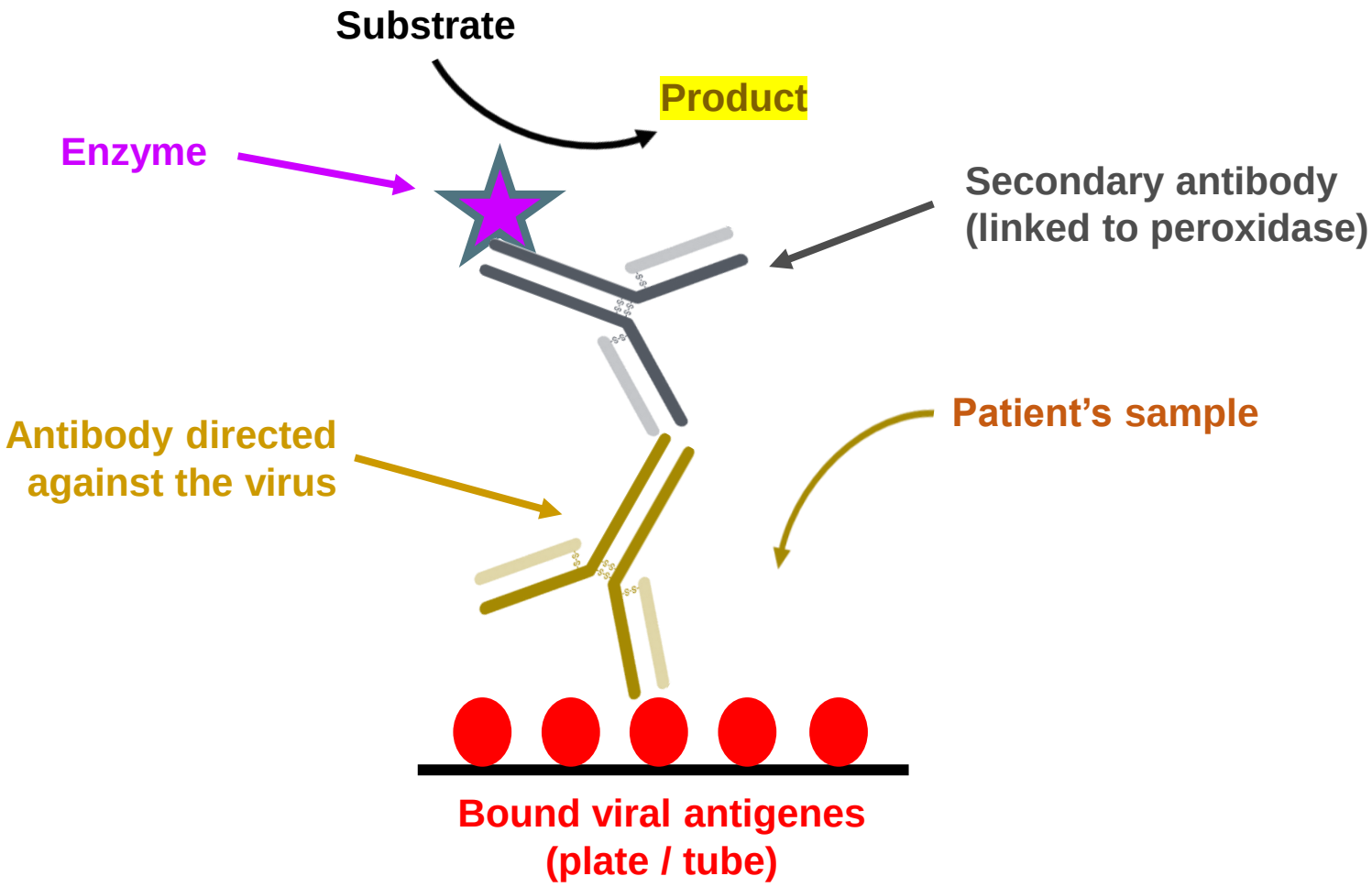
Indirect diagnosis

- Antibody screening
  - serological tests
- Seropositivity
- Seroconversion
- IgM and IgG detection
- IgG avidity assay

  CMV, Rubella virus

ELISA (Enzyme-linked immunosorbent assay)

☞ Indirect ELISA

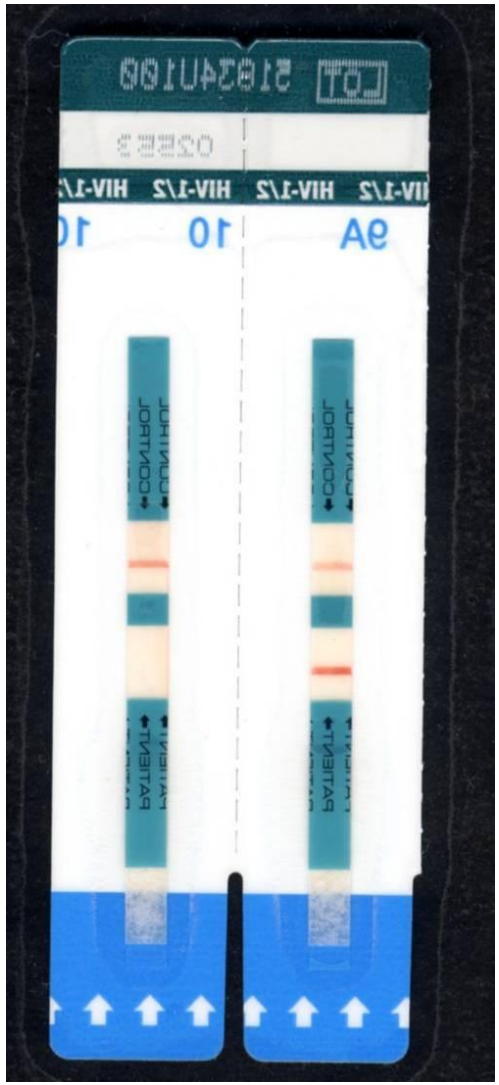


Rapid Diagnostic Test (RDT) (1)

- Unit test
- Visual reading
- Rapid
- Detection of anti-HIV-1 and anti-HIV-2 antibodies
  - 2 tests detect p24 antigen and anti-HIV-1 and anti-HIV-2 antibodies

Rapid Diagnostic Test (RDT) (2)

Negative Positive



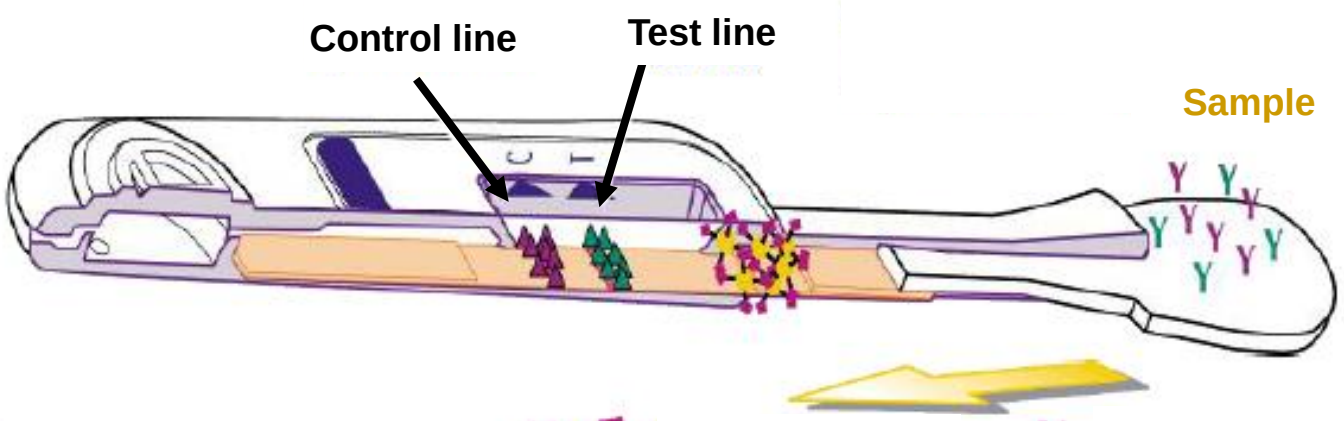
Positive control band →

HIV band →

Deposit area →

Direction of migration

Principle of immunochromatography for HIV



Antibodies against human antibodies

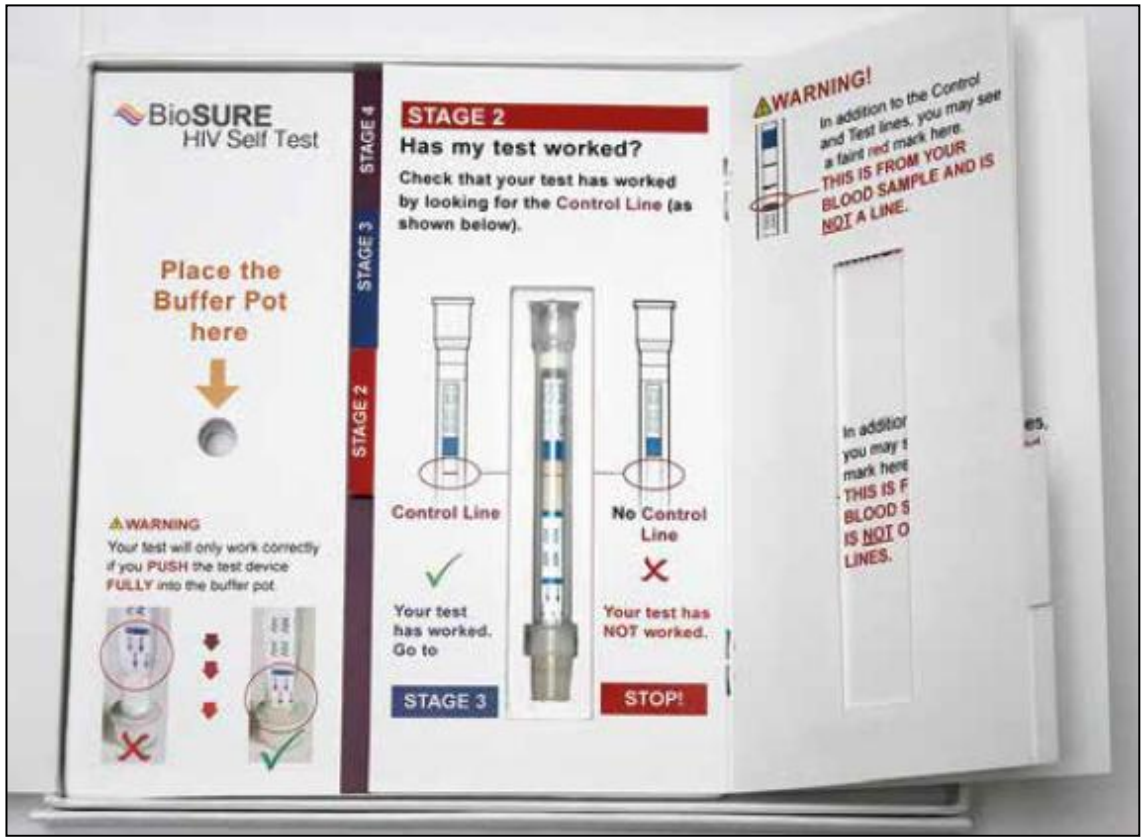
HIV-1/2 antigens

Colloidal gold conjugated with protein A

Non-HIV human antibodies

Anti-HIV-1 and anti-HIV-2 antibodies

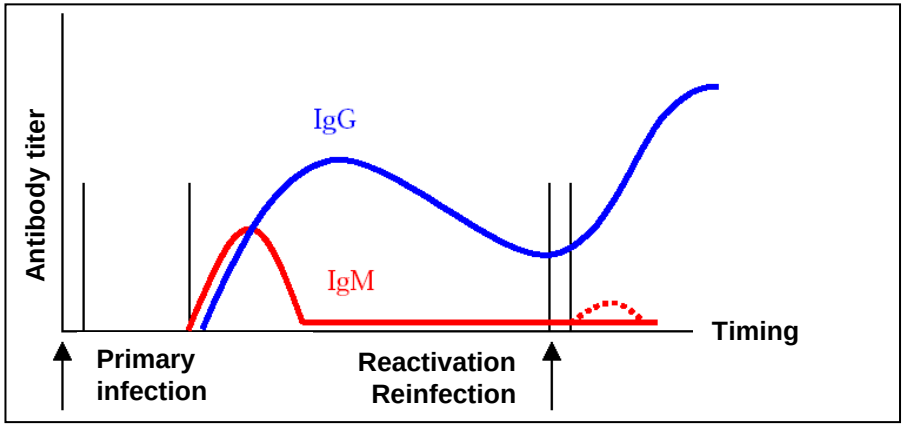
HIV self-tests

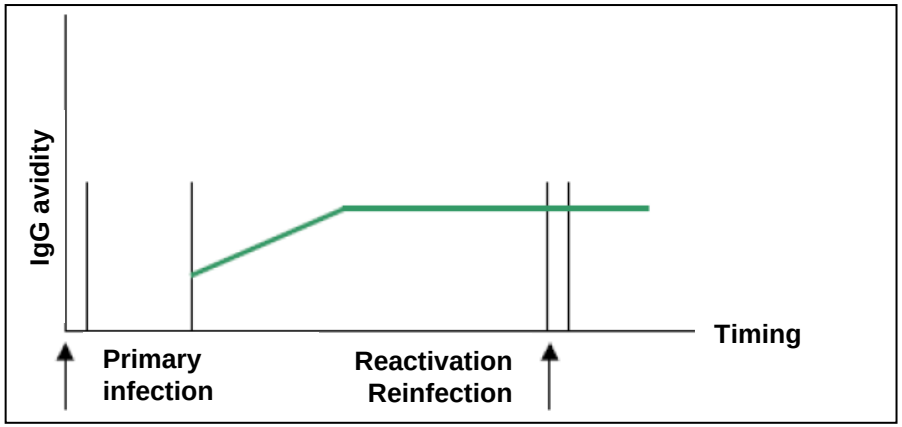


[Laboratoire Mylan]

IgG avidity assay

☞ For CMV





Serology (1)

- Indications
  - Evidence of more or less recent contact with a virus
    - Seroconversion of specific antibodies
      - requires 2 samples at 15 days interval
    - Presence of specific IgM
    - Significant increase in specific IgG
    - Measurement of antibody avidity

Serology (2)

- Advantages
  - Good sensitivity and specificity
  - Automation
  - Time to result
- Limitations
  - Reduced sensitivity in certain categories of patients
    - infants and immunocompromised
  - Sometimes difficult interpretation
    - cross-reactivity within the same family of viruses, presence of IgM during reactivation (CMV)

III- Context of the diagnosis

How and when to use these diagnostic methods?

- Characterization of a virus
- Confirmation of an acute infection
- Evidence of the viral origin of an infection
- Search for immunity through ancient infection or vaccination
- Detection of a persistent infection with or without symptoms
- Monitoring of the progress of a viral infection with or without treatment
- Monitoring of the effectiveness of treatment (viral load)
- Blood, organ and tissue donations
- Epidemiological studies

Viral load

- (RT)-PCR measurement / molecular hybridization
  - by quantitative methods
- Viral load
  - = virus concentration in the blood
- Follow-up of infection
- Monitoring of treatment effectiveness
- Introduction of treatment
  - = preemptive treatment

IV- Presentation of the OTU 01

Content of practicals

- Theory about diagnostic methods
- Virus isolation: HSV
  - Preparation of the cells
  - Inoculation of the different samples
  - Observation of CPE 1 week later
- ELISA: HBV diagnosis
  - Ag HBs, anti-HBs and anti-HBc
- Real-time PCR: CMV
  - Taqman technology
- Diagnosis of toxoplasmosis
- Tutorials about bacteria diagnosis and clinical cases

Infectious risk management

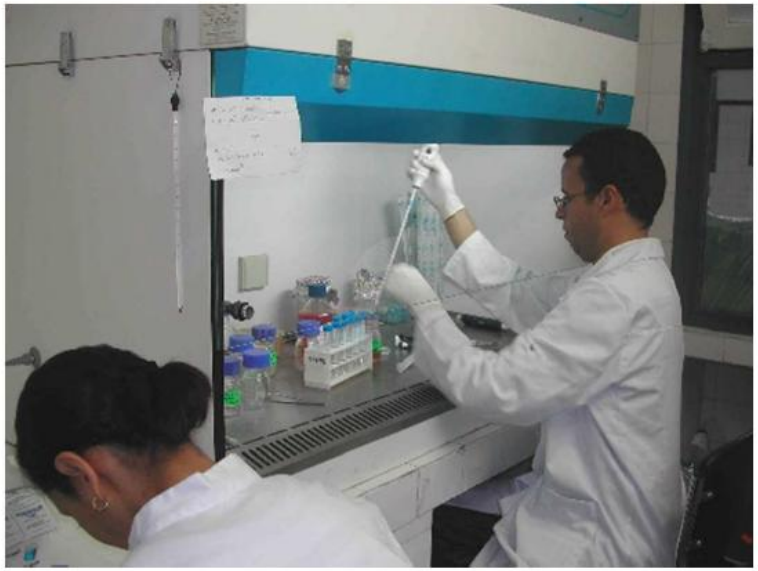
- Potentially pathogenic samples
- Handling with gloves and a lab coat

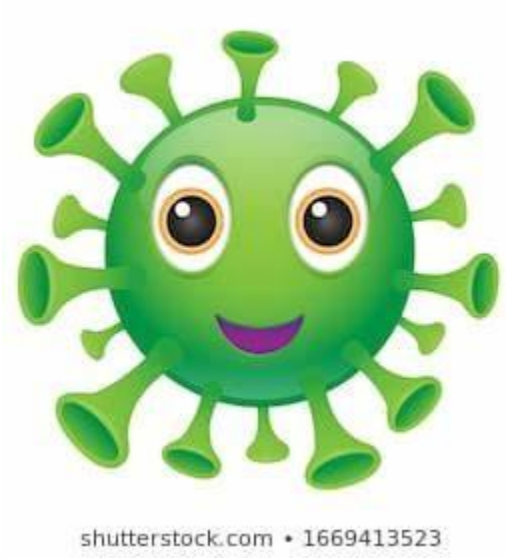
+

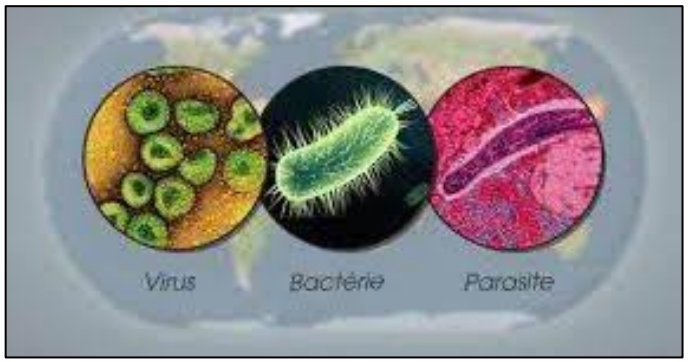
= bad idea

- Waste management
  - yellow bin for biological waste

Biological safety hoods







Enjoy this OTU 01!!!!!!