Microbiology

Subject :

Lec no : 31

Done By : Tabark Aldaboubi
Dana khalaf

وقل رب زدني علماً

Virological Tests

Virology Lecture 6
Ashraf Khasawneh

Faculty of Medicine
The Hashemite University

Overview

- Clinical virology lab can provide significant benefit to patient care
- Traditionally epidemiologic and academic role
- Current rapid assays impact on therapeutic and public health decisions.
  - Change largely due to molecular methods

* الحاجة هاي الريام للـ Viral diagnosis سبب : now aday many treatment of option

Why Expanding Role for Diagnostic Virology Lab

- Increased pool of immunocompromised
- Increasing antiviral agents

- Results in increasing demand for rapid methods, viral load testing, antiviral susceptibility, genotyping.

Methods in use in virology.

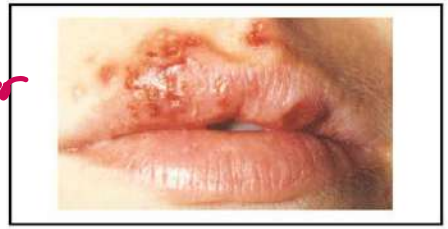
- Detecting Active Infection:
  - Electron Microscopy
  - Viral culture
  - Detection of viral antigens
  - Detection of viral nucleic acid.
  - Histopathology
- Assessing virus-specific immune response
  - Serologic testing

كل هاي ال method of diagnosis -- تكامل مع بعضها حتى تعطينا التشخيص

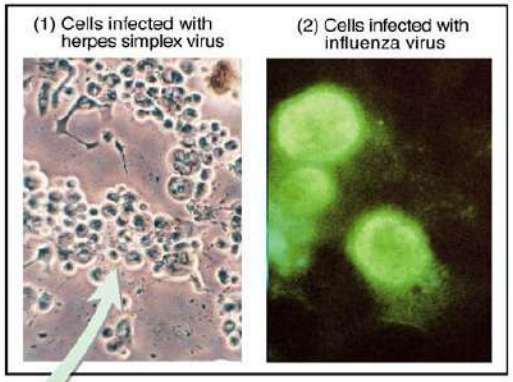
(a) Signs and symptoms: Patient is observed for manifestations of typical virus infections.

Fever blister

Simplex virus ← type 1



(b) Cells taken from patient are examined for evidence of viral infection, such as cytopathic effects *(1)* or virus antigen *(2)*.

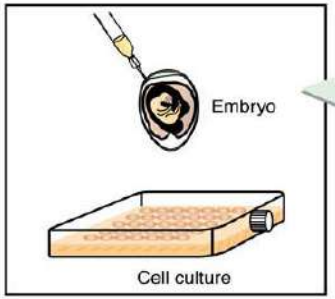


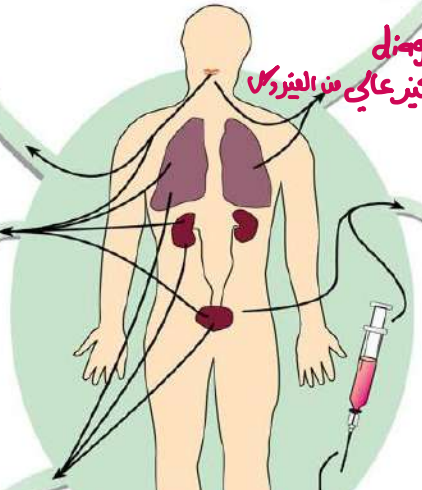
diagnostic tool ك مش كثير بستخدم
لأنو بيحتاج تركيز عالي من الفيروس with in the sample

bacterial culture مش cell culture ←
viruses obligate intracellular parasite لأنو
they need to go inside the cell بالتالي

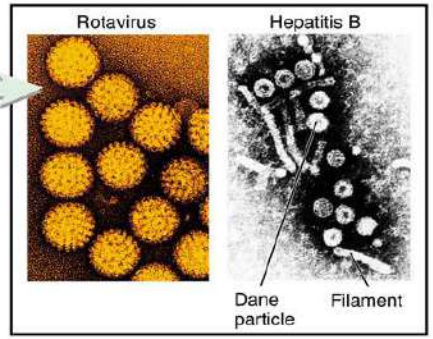
Methods

(f) Culture techniques



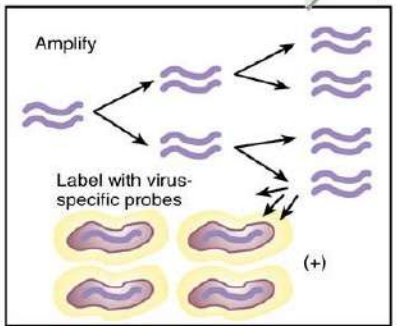


(c) Electron microscope is used to view virus directly.

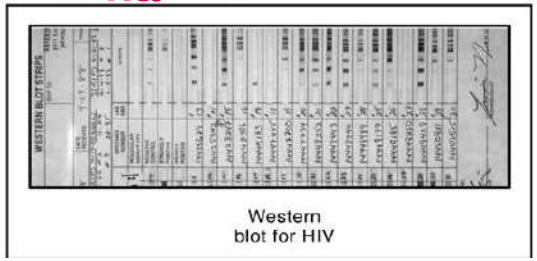


molecular test =

(e) Genetic analysis (PCR)

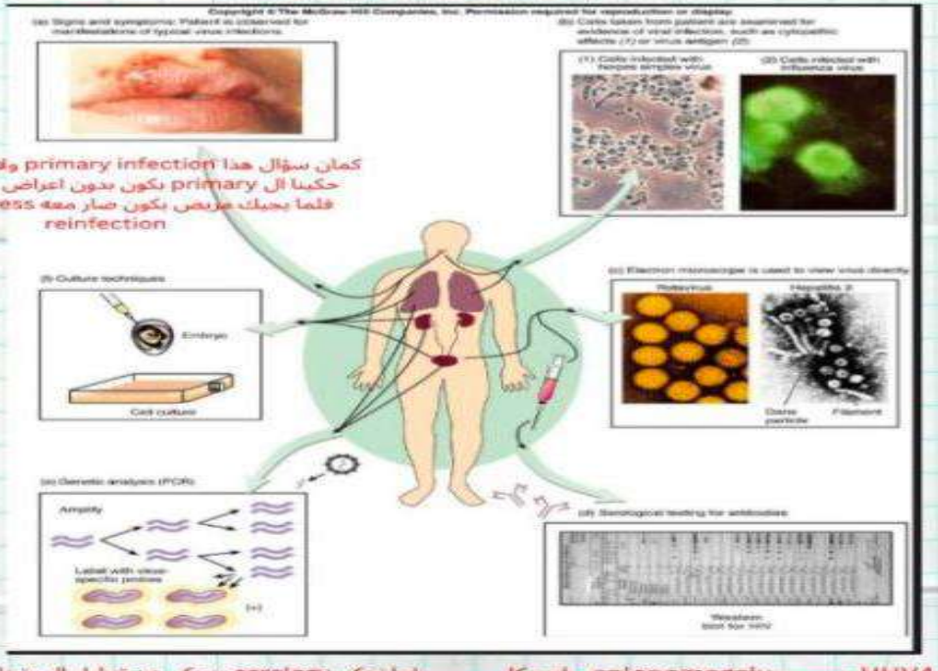


(d) Serological testing for antibodies



Methods

herpes simplex 1
حيكيا اكثر من مرة انه يعمل upper the waist infection بالذات عند الشفايف
و ديروا بالكم تخربطوا بينها و بين ال kissing disease اللي بسببه ال herpes simplex4



كمان سؤال هدا primary infection ولا reinfection ؟
حكينا ال primary يكون بدون اعراض subclinical
فلما بحيك مريض يكون صار معه stress سبب ال reinfection

لما نحكي serology بنحكي عن تحليل البروتينات
لكن لما نحكي PCR بنكون بحكي عن تحليل الجينوم
الوقت مهم لو اخذت العينة بكير او متاخر ما بطلع نتائج

معلومة عالجنب ال HHV4 بسبب splenomegaly و امريكا
بعطوا هذا الفيروس اهتمام لانه عندهم رياضات مش موجودة عن
زي ال NF اللاعب اذا كان عنده splenomegaly و لعب اللعبة
هاي بيصير rupture for spleen

Specimens for Routine Tests

شو علاقة ال fasces و ال throat بال meningitis
عندك فيروسات زي ال pilio بيلش من ال throat و بتنقل لل GI tract تم ال lymph nodes بعدها لل blood و بعدها CNS
وبرضو بتنقل feco oral

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.

قرأهم وبعدها حكا هذول بنطبقوا على كل اشي مش بس الفيروسات

Specimen choice and collection

- Specimen quality limits test quality
- Pathogen detection depends on:
  - Appropriate collection site.
  - Proper timing of specimen collection.
  - Effective and timely processing of sufficient specimen.

* Keep in transport media

Specimens for Routine Tests

!! swap فوجئت عينة من ال meningities كيف لل

Nervous system قبل مايروح replicate in oropharynx ← polio virus قبل

might cause CNS infection ← intervirus * but there intial replecation in oropharynx

we can take a sample from :

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

rota ← localised infection

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.

Specimen storage and transport

- Keep specimens other than blood at 4˚C
- If delay >24hrs, freeze at -70˚C or below.
- Avoid any storage at -20˚C: greater loss in infectivity
- Nonenveloped viruses (adenovirus, enteroviruses) more stable than enveloped (e.g. RSV, VZV, CMV).

نحكي عن الـ Sample
فيه فرق كبير -20 or -70 اذا كان (+ or -)
بس اذا بدي اعمل assastable infectivity يعني صمود يتكون بتفرق
-20 ⟸ slow freezing جزء بصير له freezing وجزء لا ⟸ loss the envelope ⟸ infectivity على تأثر

Diagnosis of viral diseases

- More difficult than other agents
- Consider overall clinical picture
- Take appropriate sample
  - Infect cell culture- look for characteristic cytopathic effects
  - Screen for parts of the virus → Antigen
  - Detect for antibodies using serological or molecular techniques

* looking at the sign and symptome
* take a sample
* culture ~~> نشوف التغييرات
*

BASIC DIAGNOSTIC METHODS

Diagnostic tests can be grouped into 3 categories:

1. **Direct detection**
2. **Indirect detection（virus isolation)**
3. **Serology**

Direct Examination

See wheel like structure ← Rota بشوف هل الشكل للفيروس مثل الـ

1. **Electron Microscopy** — morphology of virus particles

modified electron microscopy ← immune electron microscopy
which involved targeting the Antigen with Fluorescence Antibodies

2. **Light Microscopy** — histological appearance

cyto pathic effect — inclusion bodies

3. **Viral Genome Detection** — hybridization with specific nucleic acid probes
polymerase chain reaction (PCR)

Indirect Examination

effect بطلع على ال

1.Cell Culture

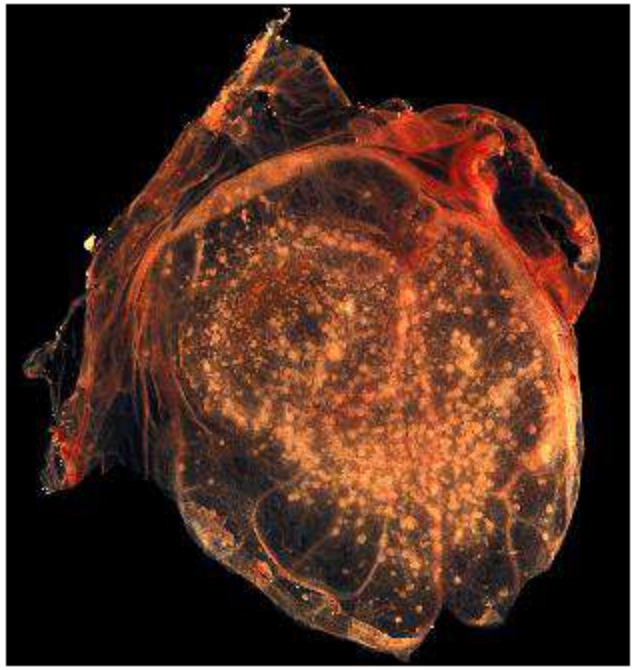
- cytopathic effect (CPE)
- haemadsorption binding
- immunofluorescence ← enveloped virus وحطينا فيه Culture حبينا
  enveloped going to be left as apart of cell membrane
  → Fluorescence antibody بترجع على glycoprotein اذا فيروس infected اذا فيروس الخلايا بتكون

2. Eggs

- pocks on CAM
- haemagglutination ⇐ RBC binding
- inclusion bodies

3. Animals

- disease or death

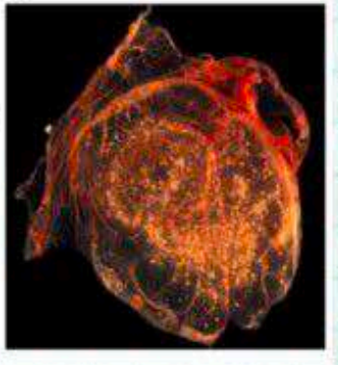


Indirect Examination

ال cytopathic effect حكيناهم
ال hemadsorption بعدين راح نحكي عنها
ال immunofluorescence اللي (CPE) cytopathic effect

1.Cell Culture	haemadsorption
	immunofluorescence
2. Eggs	pocks on CAM
	haemagglutination
	inclusion bodies
3. Animals	disease or death

حكيناه بالصفحة اللي قبل انه بتجيب العينة و بتحطلها antibodies و بتشوف اذا صار florescence



ال influenza من الفيروسات اللي بتنمو بالبيض

طيب ال hemadsorption و ال hemagglutination
اول اشي الثنين بالدم
اللي بيصير اني بجيب فيروس حطيته ع مجموعة cells
بعد 48 ساة انا جبت RBCs و اتطلعت بالمايكروسكوب و لقيت اماكن معينة
ال RBCs بتنجذب لاماكن معينة اكثر من اماكن ثانية هاي هي ال hemadsorption
ليش بيصير هيك
لانه ال enveloped فيروس لما يفوت بال fusion ال envelope بندمج مع ال cell membrane
تبع الخلية و هيك ال spikes راح تكون برضو ع الخلايا من برا فال RBCs بتمسك بال spikes
مشان هيك بس اشوف مكان عليه RBCs مجتمعة و منجذبة اله يعني انه مصاب

طيب ال hemagglutination
هون انا بجيب عينة و انا شاك انه فيها فيروس و بضيف عليها RBCs
بالوضع الطبيعي راح تترسب بس لانه مصاب بالفيروس راح يرتبط كل RBC مع ال glycoprotein اللي
جنبه و اللي بكون شبكة من الفيروسات و ال RBCs مشان هيك ما بترسب

Serology

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

Classical Techniques	Newer Techniques
1. Complement fixation tests (CFT)	1. Radioimmunoassay (RIA)
2. Haemagglutination inhibition tests	2. Enzyme linked immunosorbent assay (EIA)
3. Immunofluorescence techniques (IF)	3. Particle agglutination
4. Neutralization tests	4. Western Blot (WB)
5. Counter-immunoelectrophoresis	5. RIBA, Line immunoassay

Why do yo need cell culture ! progaration of the virus (more copies of the virus)

Cell Culture

certien type of viruses that syant swap not success progarat in the lap.

- Viruses are obligate intracellular organisms – require living cells for virus isolation
- Advantages:
  - Relatively sensitive and specific
  - Can detect many different viruses
  - Provides a viral isolate for further characterization (serotyping, genotyping, susceptibility)

Virus Isolation

Primary is considerd the best , the most expinsive , The least used .

Cell Cultures are most widely used for virus isolation, there are 3 types of cell cultures:

media عدد مرات تغيير الـ

Similiraty لأنها اقرب لـ human cell هاي الاحسن :

1. Primary cells - 1-2 passages (Monkey Kidney)

بتقتل الفرد وتجيب الكلية تاعته وتقلعها Media ثم Flask وبنحطها بال Trushing مشكلتها ← فاقي replication

2. Semi-continuous cells - 20-50 passages (Human embryonic kidney and skin fibroblasts)

continous

3. Continuous cells - Indefinite passages (HeLa, Vero, Hep2, LLC-MK2, MDCK) Tumor cell line

most used in the lap

cell line لأنو عندي بتحفظهم بال liquid Nitrogen

Providing them in the media capble of replecating and produce new cell

Primary cell culture are widely acknowledged as the best cell culture systems available since they support the widest range of viruses. However, they are very expensive and it is often difficult to obtain a reliable supply. **Continuous cells** are the most easy to handle but the range of viruses supported is often limited.

Can survive as Single layer احطهم لل cell ← Flask بتطلع عليه ال

1. Cell Cultures

Growing virus may produce

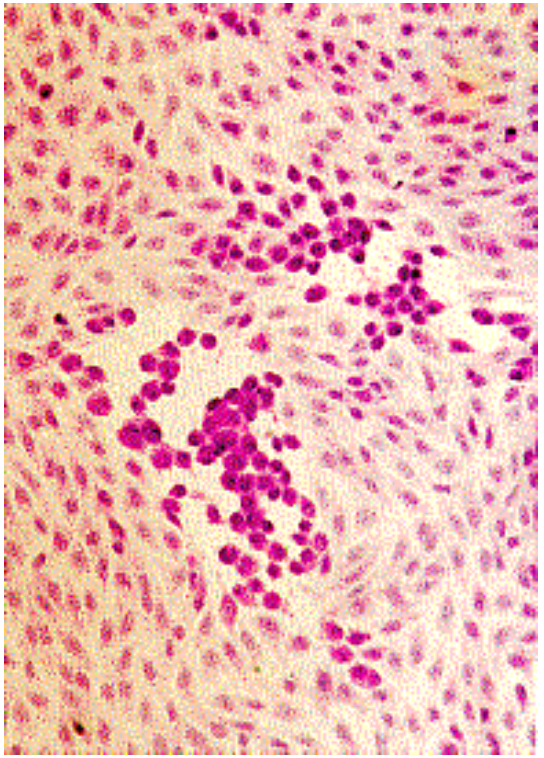
1. Cytopathic Effect (CPE) - such as the ballooning of cells or syncytia formation, may be specific or non-specific.

2. Haemadsorption - cells acquire the ability to stick to mammalian red blood cells.

Confirmation of the identity of the virus may be carried out using neutralization, haemadsorption-inhibition or immunofluorescence tests.

Cytopathic Effect (1)

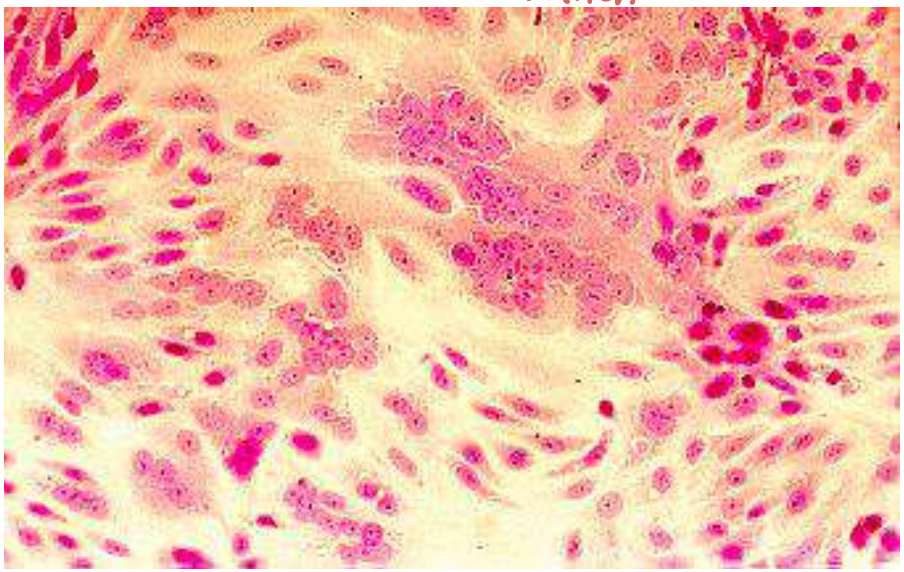
Fig. 1, Cytopathic effects of enterovirus 71 in rhesus monkey kidney cells

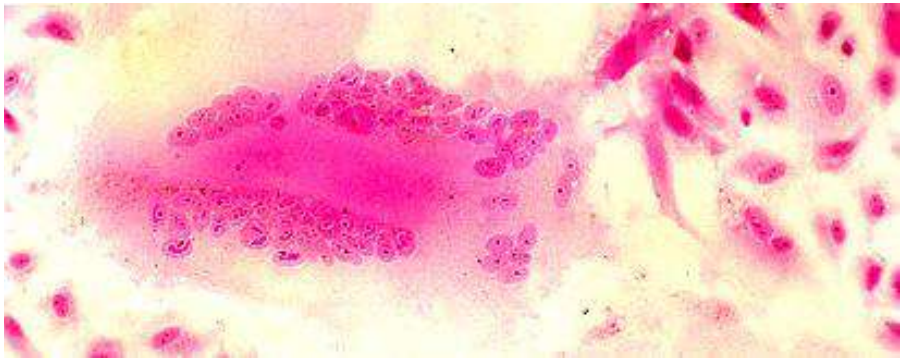


Cytopathic effect of enterovirus 71 and HSV in cell culture: note the ballooning of cells.
(Virology Laboratory, Yale-New Haven Hospital, Linda Stannard, University of Cape Town)

Cytopathic Effect (2)

Syncytium formation بتعطيها ال ← 1 HIV 2 herps virus





Syncytium formation in cell culture caused by[3] RSV (top), and measles virus (bottom).[3]

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

which of the following is associated with syncytium formation !

هيبقى ممكن السؤال يجي :

الدكتورة بتجب هاي الاسئلة المباشرة بحطها بكيس وبشرح الحالة

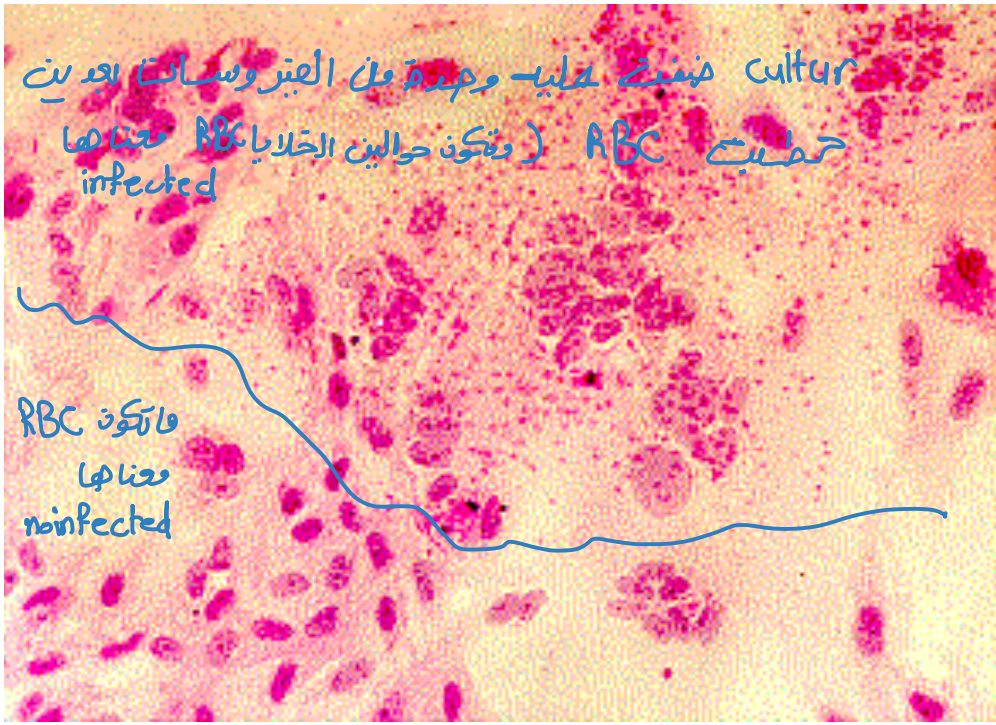
وبجيبن سبأل السؤال

Haemadsorption

Orthomyxoviruses (influenza) and some paramyxoviruses (parainfluenza, measles, mumps)

Insert viral glycoproteins (haemaglutinin) into host cell membrane.

Promotes attachment of RBC of certain species (e.g guinea pig) to cell membrane.



* Syncytial formation caused by mumps virus and haemadsorption of erythrocytes onto the surface of the cell sheet.

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

Problems with cell culture

- Long period (up to 4 weeks) required for result.
- Often very poor sensitivity, sensitivity depends on a large extent on the condition of the specimen.
- Susceptible to bacterial contamination.
- Susceptible to toxic substances which may be present in the specimen.
- Many viruses will not grow in cell culture e.g., Hepatitis B, Diarrheal viruses, parvovirus, papillomavirus.

Viruses Isolated by Cell Culture

Viruses readily isolated by cell culture	Less frequently isolated viruses
Herpes Simplex	Varicella-Zoster
Cytomegalovirus	Measles
Adenoviruses	Rubella
Polioviruses	Rhinoviruses
Coxsackie B viruses	Coxsackie A viruses
Echoviruses	
Influenza	
Parainfluenza	
Mumps	
Respiratory Syncytial Virus	

Egg culture

Eggs are used mainly for the isolation of influenza viruses. Ten to 12 day-old chick embryos are used.

- Routes of Inoculation

Viruses can be cultivated in embryonated hen's egg at different stages of development by the following routes:

1. Amniotic
2. Yolk sac
3. Allantoic
4. Chorioallantoic membrane

Direct Detection of Virus or Viral Antigen: Electron Microscopy

- Quick
- Looks for many viruses
- Useful if unknown pathogen
- Less prone to cross contamination vs molecular.
- Expensive equipment, need expertise to read
- Not well suited to screening large numbers of samples.
- Low sensitivity – need 10^5-10^8 viral particles/ml to detect.

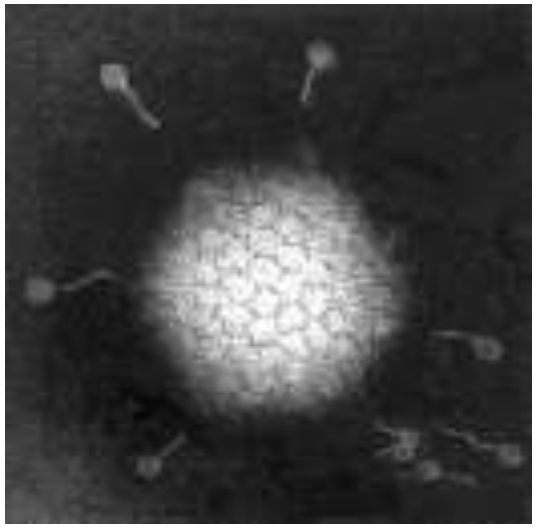
نوخذ عينة و نطلع عليها بالـ electrone microscope بنقدر
نعرف هل هو envelop or not و Type of capsid

Electron Microscopy

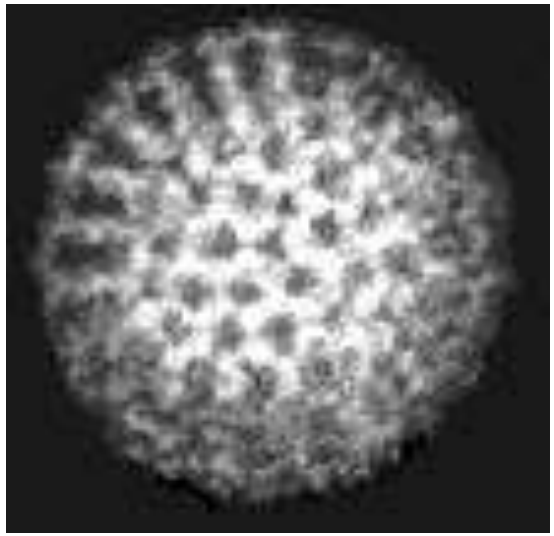
10^6 virus particles per ml required for visualization, □ 50,000 - 60,000 magnification normally used. Viruses may be detected in the following specimens.

Faeces gastroenteritis	Rotavirus, Adenovirus
	Norwalk like viruses
	Astrovirus, Calicivirus
Vesicle Fluid	HSV
	VZV
Skin scrapings	papillomavirus, orf
	molluscum contagiosum

Electronmicrographs



Adenovirus

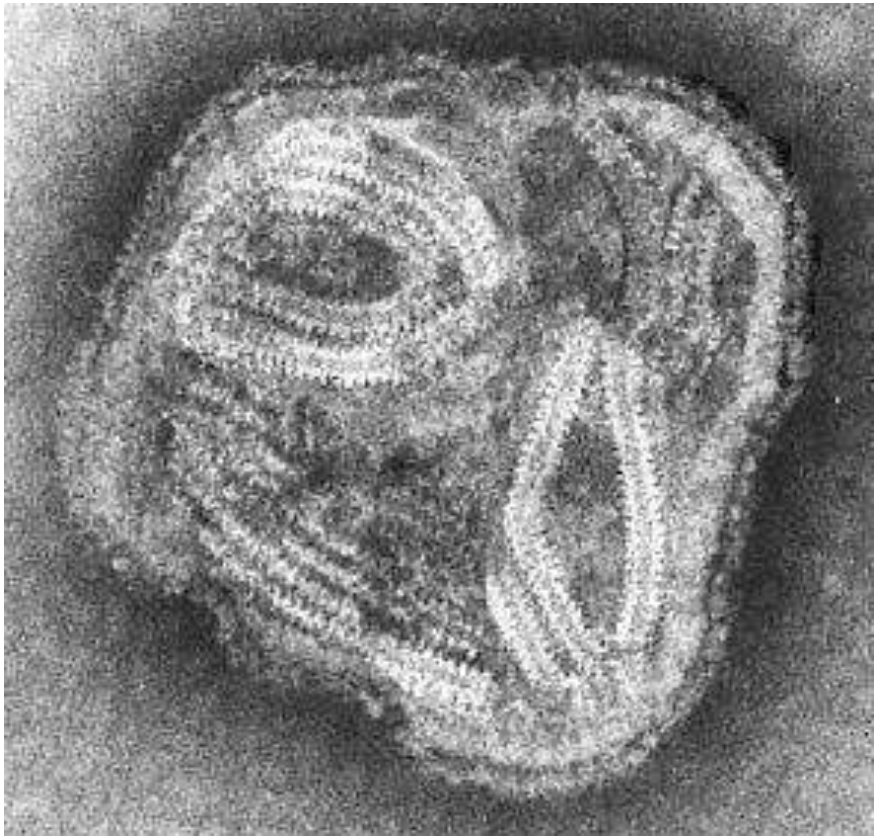


Rotavirus

Naked الجدتين
وبشوف الcapsid

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

Paramyxovirus (Parainfluenza)



helical capsid - enveloped

Problems with Electron Microscopy

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

Light Microscopy

Replicating virus often produce histological changes in infected cells. These changes may be characteristic or non-specific.

Viral inclusion bodies are basically collections of replicating virus particles either in the nucleus or cytoplasm. Examples of inclusion bodies include

1. the **negri bodies** found in rabies infection
2. **cytomegalic inclusion bodies** found in CMV infection

DNA virus (cytoplasmic و intranuclear)
استثناء

Although not sensitive or specific, histology nevertheless serves as a useful adjunct in the diagnosis of certain viral infections.

Molecular Methods

- Methods based on the detection of viral genome are also commonly known as molecular methods. It is often said that molecular methods is the future direction of viral diagnosis.
- However in practice, although the use of these methods is indeed increasing, the role played by molecular methods in a routine diagnostic virus laboratory is still small compared to conventional methods.

- Classical molecular techniques include

1. dot-blot and Southern-blot which depend on the use of specific DNA/RNA probes for hybridization.
2. the polymerase chain reaction (PCR) and RT-PCR which depend on the use of specific primers Amplification of DNA
3. ligase chain reaction (LCR),
4. nucleic acid based amplification (NASBA), and
5. branched DNA (bDNA)

* DNA seprate the band according the size (base bairs)
* DNA leder → Molecular wheight مقسم حسب
* DNA horizontal
* protein seperat according Molecular wheight → (UV) كيف بدي اشوفهم بعدين !!
ببينوا على شكل Fat

2. the polymerase chain reaction (PCR) and RT-PCR which depend on the use of specific primers

بال PCR احنا بنزيد عدد ال genetic material
بالذات ال DNA
طيب بال RNA viruses كيف بعملها ؟
بعمل reverse transcriptase
و بحولها ل DNA
طيب كيف بعمل ال PCR
بنجيب tube حجمه 0.2ml
فلنفرض بدي احط فيه 50microl
شو بكون فيه ال tube ؟
primers
DNA pol
ال genetic material اللي بدي اعملها amplification
nucleotides
nuclease free water
هذا ال tube بنحطه بال pcr machine

3. ligase chain reaction (LCR),

4. nucleic acid based amplification (NASBA), and

5. branched DNA (bDNA)

هسه عنا 3 خطوات الاولى ال denturation و اللي برفع الحرارة ل 90 و بنفصل السلسلتين عن بعض
بعدها ال annealing اللي بنزل فيها الحرارة ل 60 تقريبا مشان ترتبط ال primers
بعدها ال extension اللي بنزيد الحرارة فيها ل 70 و بيصير ال DNA بنسى
هذا الحكي بنعاد 30-35 مرة بكل cycle كل DNA بتضاعف

13

Nucleic Acid Detection

- Short length of viral genome makes them ideal candidate for nucleic-acid based diagnosis
- PCR → horizontal – gel – UV light
  - conventional PCR – agarose gel detection of product
  - Real-time PCR- products detected using probes or intercalating dyes within the reaction.

Nucleic Acid Detection

- **Short length of viral genome makes them ideal candidate for nucleic-acid based diagnosis**
- **PCR**

عنا نوعين من ال PCR
conventional and real -time

في شرط انه ال primer يكون specific يعني مثلا اذا عارف لو استخدت هذا ال primer اني قاعد بكشف ع HIV

- **conventional PCR – agarose gel detection of product**
- **Real-time PCR- products detected using probes or intercalating dyes within the reaction.**

بال conventional بنمشيه على agarose gel مادة جلاتينية
بعدين بمشي فيه electrophoresis اللي راح يفصل قطع ال DNA بناءا على طولها
و التقطيع فيه يكون horizontal
ال real time بتكون مشبوكة بشاشة بتعطيك و مع كل cycle بتعطي قراءة و بتعمل s shape curve

في عنا negative control لازم دايما يكون negative لانه لو اعطانا positive كل الشغل يكون غلط
برضو عنا positive control اللي بقارن فيها النتيجة اللي طلعت معي فيه

Polymerase Chain Reaction

Polymerase Chain Reaction

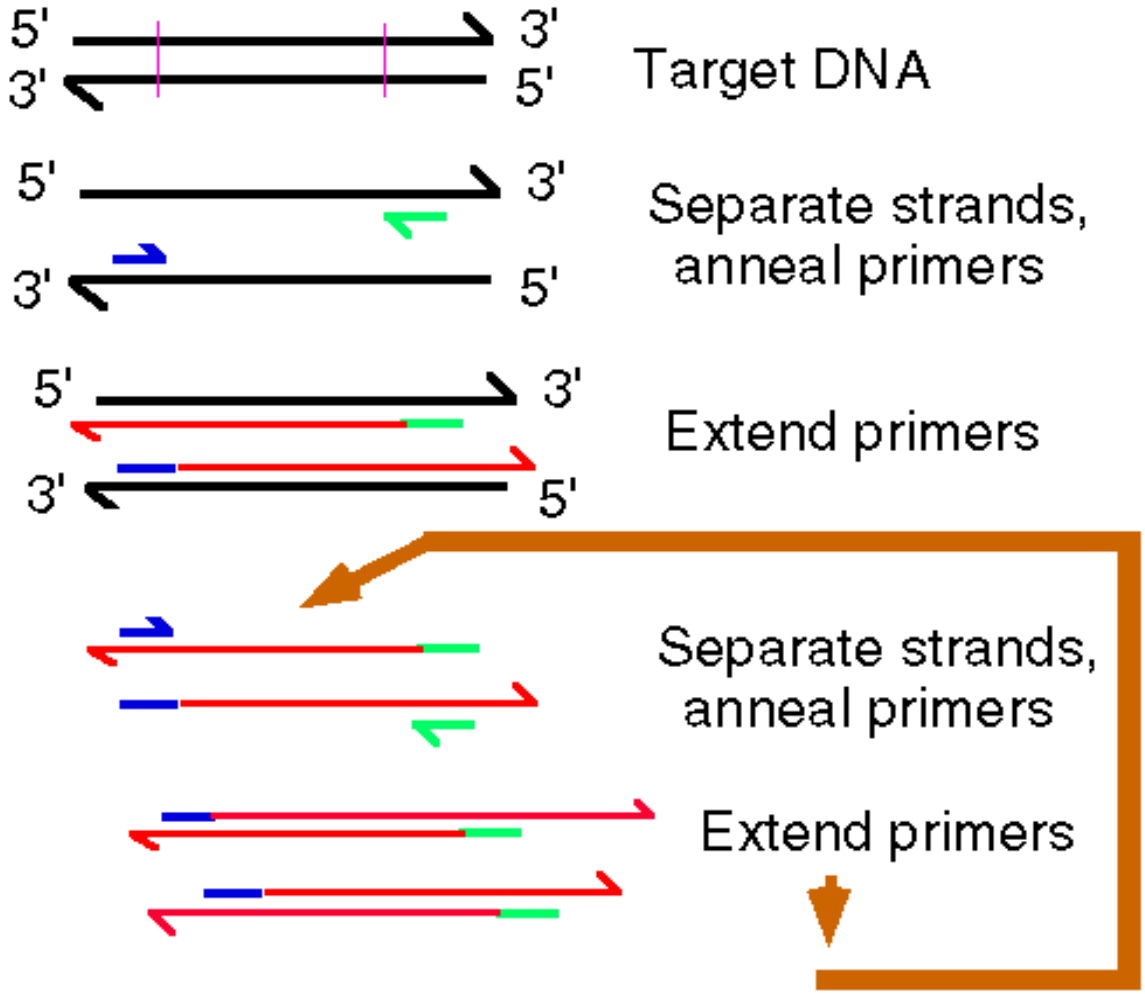
- PCR allows the in vitro amplification of specific target DNA sequences by a factor of 10^6 and is thus an extremely sensitive technique.
- It is based on an enzymatic reaction involving the use of synthetic oligonucleotides flanking the target nucleic sequence of interest.
- These oligonucleotides act as primers for the thermostable Taq polymerase. Repeated cycles (usually 25 to 40) of denaturation of the template DNA (at 94^{o}C), annealing of primers to their complementary sequences (50^{o}C), and primer extension (72^{o}C) result in the exponential production of the specific target fragment.
- Further sensitivity and specificity may be obtained by the nested PCR.
- Detection and identification of the PCR product is usually carried out by agarose gel electrophoresis, hybridization with a specific oligonucleotide probe, restriction enzyme analysis, or DNA sequencing.

Polymerase Chain Reaction

اقرأوهم لحالكم ...

- Advantages of PCR:
  - Extremely high sensitivity, may detect down to one viral genome per sample volume
  - Easy to set up
  - Fast turnaround time
- Disadvantages of PCR
  - Extremely liable to contamination
  - High degree of operator skill required
  - Not easy to set up a quantitative assay.
  - A positive result may be difficult to interpret, especially with latent viruses such as CMV, where any seropositive person will have virus present in their blood irrespective whether they have disease or not.
- These problems are being addressed by the arrival of commercial closed systems such as the Roche Cobas Amplicor which requires minimum handling. The use of synthetic internal competitive targets in these commercial assays has facilitated the accurate quantification of results. However, these assays are very expensive.

Schematic of PCR



Each cycle doubles the copy number of the target

جهاز PCR قديم ...

Serology

Criteria for diagnosing Primary Infection

- 4 fold or more increase in titre of IgG or total antibody between acute and convalescent sera 50 → 250
- Presence of IgM
- Seroconversion - is the development of detectable specific antibodies to microorganisms in the blood serum as a result of infection or immunization. vaccine او يكون ماخد infected by the virus ممكن المريض يكون
- 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 100-170
- Absence or slight increase in IgM

Serology

ال primary لما تنصاب اول مرة بالفيروس و حكينا بعد ما تصاب اول Ig بطلع هو ال IgM

Criteria for diagnosing Primary Infection

- **4 fold or more increase in titre of IgG or total antibody between acute and convalescent sera**

هسه اذا قسنا ال IgG للمريض بعد 3 ايام من ال infection طلع معنا 30 و بعدها قسناهم بعد اسبوع طلعوا 150 يعني صاروا 5 اضعاف هذا بعتبره primary infection لو اقل من 4 اضعاف بكون reinfection

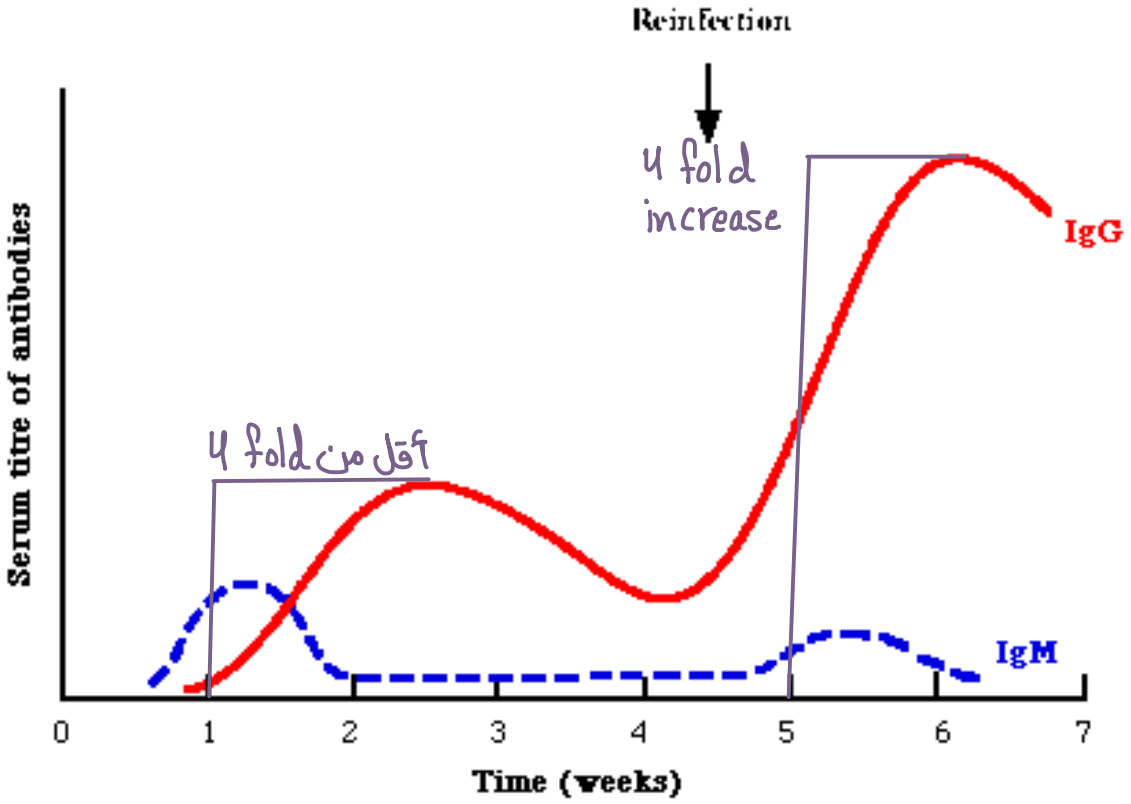
- **Presence of IgM**
- **Seroconversion - is the development of detectable specific antibodies to microorganisms in the blood serum as a result of infection or immunization.**

ال seroconversion هي العملية اللي بيصيرعندي فيها antibody منن فيروس معين

- **A single high titre of IgG (or total antibody) - very unreliable**

Typical Serological Profile After Acute Infection

innate immunity ال complement نوع من ال
complement ال هذا بشغل antibodies and antigens تبعه Triger ال
و بروح بعمل membrane attack complex يعني بعمل ثقب بال membrane و بقتل الفروس



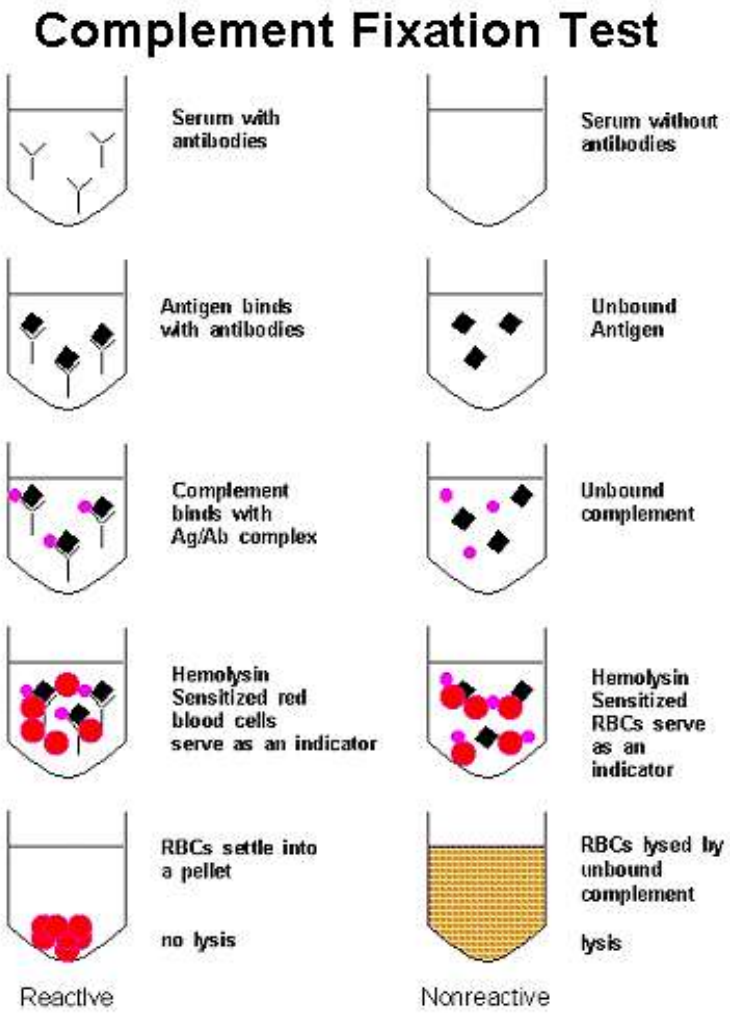
Note that during reinfection, IgM may be absent or present at a low level transiently

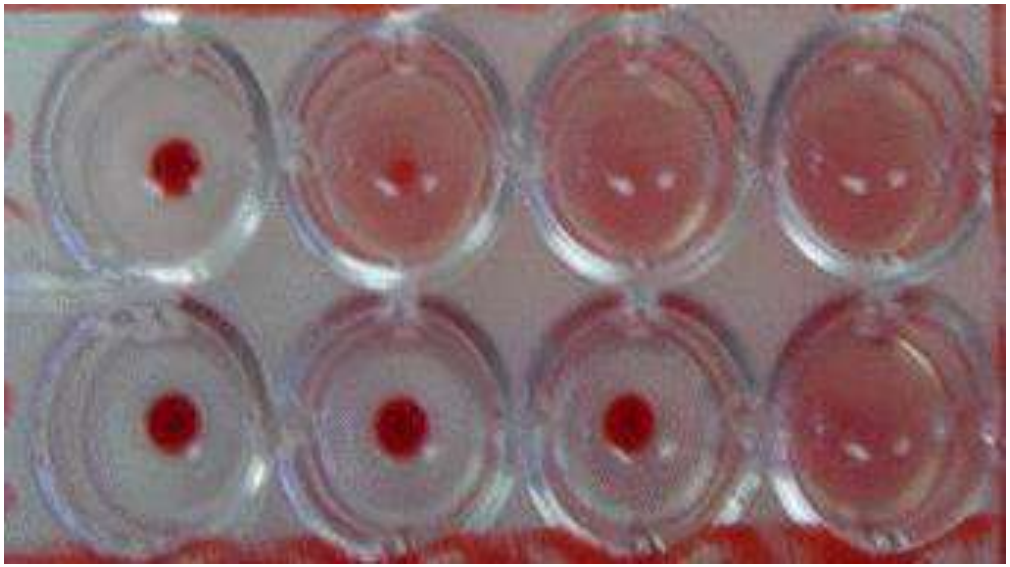
Complement fixation test

شوفو سلايد ٤٥ لتفهمو الفكرة

- The **complement fixation test** is an ***immunological medical*** test looking for evidence of infection. It tests for the presence of either specific ***antibody*** or specific ***antigen*** in a patient's serum. It uses sheep ***red blood cells*** (sRBC), anti-sRBC antibody and complement, plus specific antigen (if looking for antibody in serum) or specific antibody (if looking for antigen in serum).
- If either the antibody or antigen is present in the patient's serum, then the complement is completely utilized, so the sRBCs are not lysed. But if the antibody (or antigen) is not present, then the complement is not used up, so it binds anti-sRBC antibody, and the sRBCs are lysed.
- The ***Wassermann test*** is one form of complement fixation test.

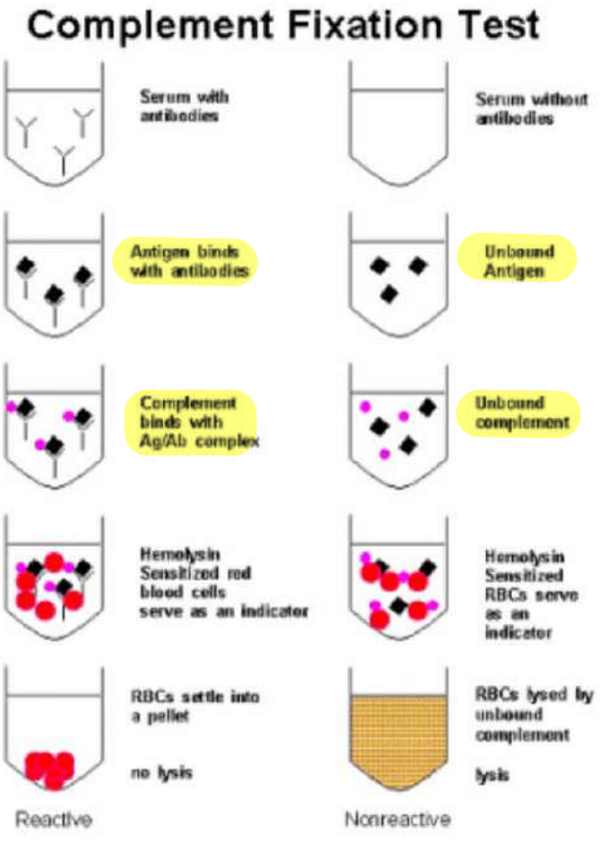
Complement fixation test



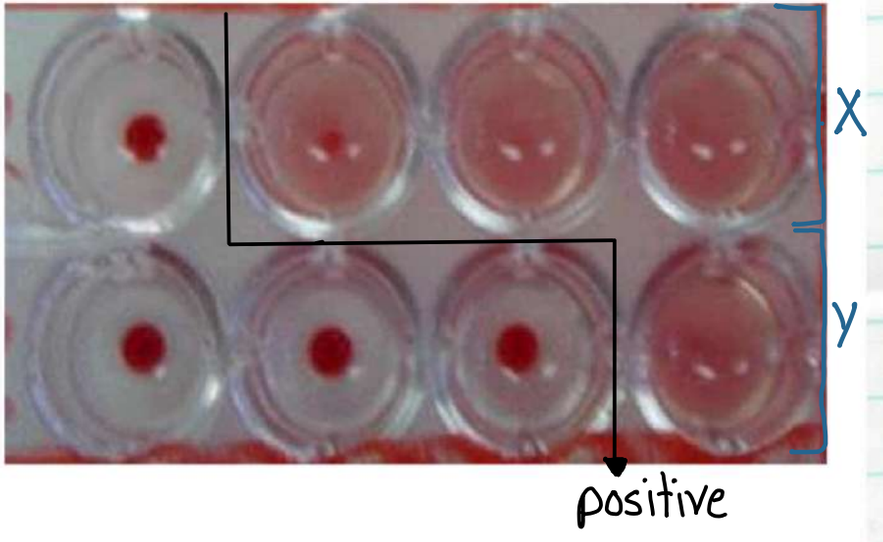


Complement fixation test

ارجعو لشرح الدكتور بعد ساعة من بداية المحاضرة ، لانو حكى شغلات مو عارفة اكتبها



باخد من العينة الاولى ١٠ مايكرو وبحطها عالتانية بعدها بعمللها maxing
وبرجع باخد ١٠ مايكرو وبحطها عالتالت ،، هيك بعمل serial dilutions

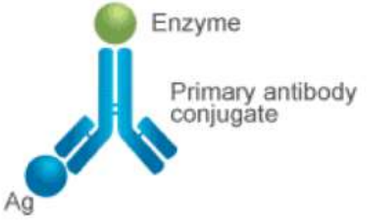


بوخذ عينتين وحدة من ال patient فيها antibodies و عينة ما فيها antibodies
بضيف antigen بكمية معروفة هسه راح يكون عندي antigen-antibody complex فعينة
و العينة الثانية ما بتكون لانه في antibodies ← antibodies ما في
بضيف ال complement اللي راح يكسر ال complex و يخلص منه
اما لو ما في ال complex فال complement ما حيعمل اشي
اول هسه بال test بكون عندي RBCs
اذا ما تحللت يعني positive يعني في antibodies
اذا تحللت يعني فش antibodies لانه ال comp راح يرتبط بال RBCs يحللها

ELISA

- Surface of solid phase (microtitre plate) coated with antibody
- Antigen of interest binds if present.
- Second enzyme-conjugated antibody added
- Substrate added and colour generated/read by spectrophotometer.

ELISA types



In direct ELISA, only an enzyme-labeled primary antibody is used, meaning that secondary antibodies are not needed. The enzyme-labeled primary antibody "directly" binds to the target (antigen) that is immobilized to the plate (solid surface). Next, the enzyme linked to the primary antibody reacts with its substrate to produce a visible signal that can be measured. In this way, the antigen of interest is detected.

عنا 4 انواع لل elisa

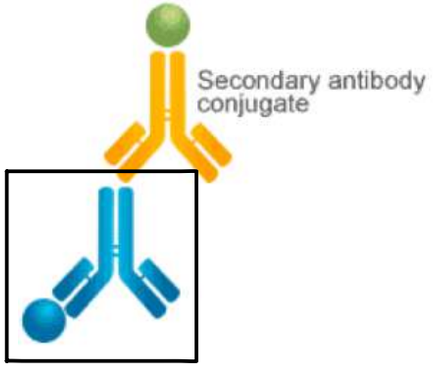
اول واحد ال direct اللي بوخذ فيها ال plate بتكون فيه coated by antigen

و بعدها بنضيف ال antibody اللي بكون معه enzyme

ال antibody برتبط مع ال antigen

visible signal اللي راح تعطي لون معين بنقدر نشوفه بال ELISA machine

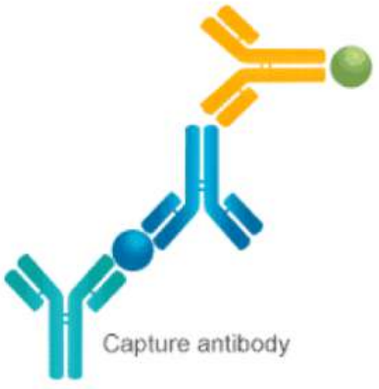
بعدها بنضيف



antigen
تبع المريض

In indirect ELISA, both a primary antibody and a secondary antibody are used. But in this case, the primary antibody is not labeled with an enzyme. Instead, the secondary antibody is labeled with an enzyme.
The primary antibody binds to the antigen immobilized to the plate, and then the enzyme-labeled secondary antibody binds to the primary antibody.
Finally, the enzyme linked to the secondary antibody reacts with its substrate to produce a visible signal that can be measured.

عندك ثاني نوع ال indirect
ال plate بروض coated by antigen و برضو primary antibody
و secondary antibody
و برضو بضيف مادة بتتفاعل مع ال enzyme و بتعطيك اللون



In sandwich ELISA, however, it is the antibody that is immobilized to the plate, and this antibody is called capture antibody. In addition to capture antibody, sandwich ELISA also involves the use of detection antibodies, which generally include the unlabeled primary detection antibody and the enzyme-labeled secondary detection antibody.
Firstly, the antigen of interest binds to the capture antibody immobilized to the plate. Secondly, the primary detection antibody binds to the antigen. Thirdly, the secondary detection antibody binds to the primary detection antibody, and then the enzyme reacts with its substrate to produce a visible signal that can be measured.

47

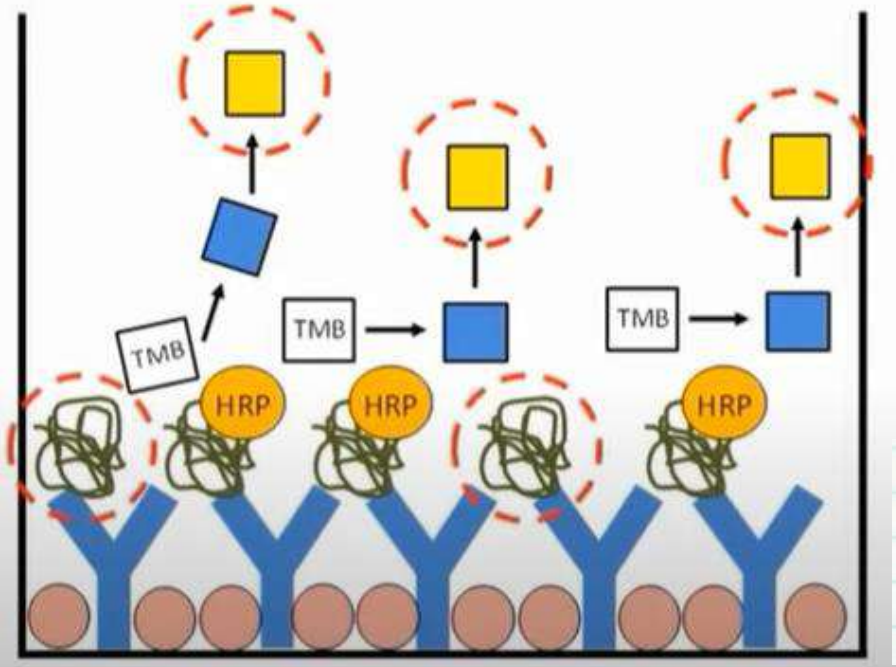
زيادة. ←

اخر نوع الsandwish
عنا ال plate عليه antibody بدل الantigen
بعدها بنضيف antigen و بعدها primary بعدها secondary
و بعدها اللون

الدكتور حكى مابدو تفاصيل وماشرحها

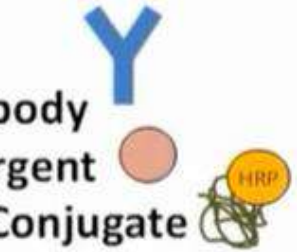
Competitive ELISA

الدكتور حكى مابدو تفاصيل وماشرحها



ELISA Plate Well Surface

1) Coat plate with Capture Antibody
2) Block plate with BSA or Detergent
3) Mix sample with an Enzyme Conjugate
4) Add mixture to the ELISA Plate
5) Wash the ELISA Plate
6) Add colorless TMB Substrate
7) Add stop solution

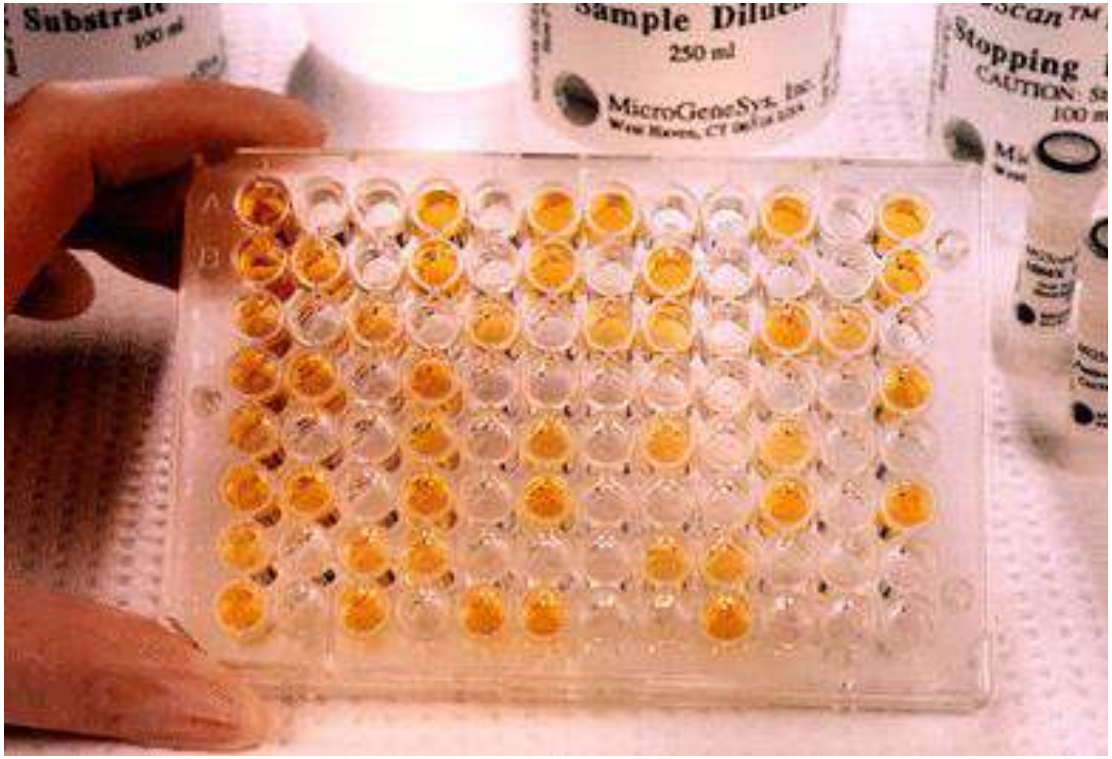


النوع الرابع هو الcompetitive
ال plate بكون coated by antibodies
و بضيف بروتين مشان اعمل block لل plate
ال sample بخلط معه enzyme و بيصيروا يتنافسوا
و طبعا اللي بكون اكثر هو اللي راح يوخذ اماكن اكثر
اذا كان ال enzyme conjugate اللون طبيعي يكون اغمق من لما يكون ال proteins sample اكثر
فيعني كل ما زاد غمق اللون زاد ال antibodies

زيادة ←

ELISA for HIV antibody

كل ما زاد ال depth لل yellow colour this has a higher type



Microplate ELISA for HIV antibody: coloured wells indicate reactivity

Western Blot

- Western blots allow investigators to determine the molecular weight of a protein and to measure relative amounts of the protein present in different samples.

Western Blot

- Proteins are separated by gel electrophoresis, usually SDS-PAGE.Vertical gel
- The proteins are transferred to a sheet of special blotting paper called nitrocellulose.
- The proteins retain the same pattern of separation they had on the gel.

Western Blot

- The blot is incubated with a generic protein (such as milk proteins) to bind to any remaining sticky places on the nitrocellulose.
- An antibody is then added to the solution which is able to bind to its specific protein.
- The antibody has an enzyme (e.g., alkaline phosphatase or horseradish peroxidase) or dye attached to it which cannot be seen at this time.

Western Blot

- The location of the antibody is revealed by incubating it with a colorless substrate that the attached enzyme converts to a colored product that can be seen and photographed.

another serological method

Western Blot

- **Western blots allow investigators to determine the molecular weight of a protein and to measure relative amounts of the protein present in different samples.**

احنا هون بنحكي عن البروتينات
الفكرة انك بتدرس بروتين في بيئة فيها بروتينات ثانية
البروتين اللي بندور عليه هو ال antigen

لحتى تفهموا

- **Proteins are separated by gel electrophoresis, usually SDS-PAGE.**

هون ال gel electrophoresis بكون vertical
فوق حكينا بال DNA بكون horizontal

- **The proteins are transferred to a sheet of special blotting paper called nitrocellulose.**

- **The proteins retain the same pattern of separation they had on the gel.**

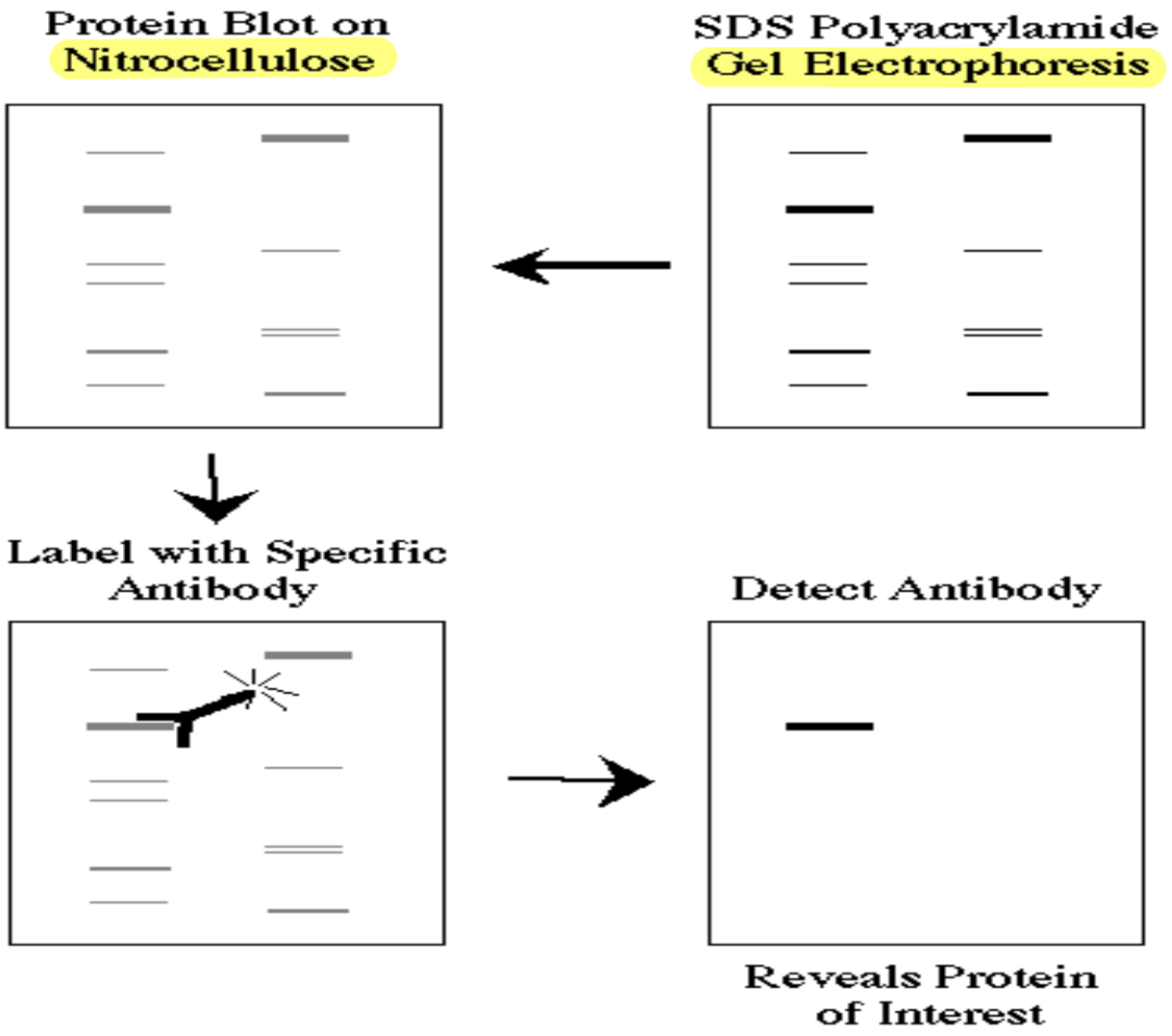
بيصير في تقسيم للبروتين تبعا لل weight
ال gel هون thin فهو عرضة انه يتكسر
مشان هيك بحط مقابله nitrocellulose membrane
مشان انقل ترتيب البروتينات عليه

- **The blot is incubated with a generic protein (such as milk proteins) to bind to any remaining sticky places on the nitrocellulose.**

هسه حتى بعد ما نقلتها لسا انا مش شايف البروتين
بعدين بضيف primary antibody و بعدها ال secondary antibody
بعدها بتجيب ال nitrocellulose و ممكن يعطيك اكثر من نوع antibody

- **An antibody is then added to the solution which is able to bind to its specific protein.**

- **The antibody has an enzyme (e.g., alkaline phosphatase or horseradish peroxidase) or dye attached to it which cannot be seen at this time.**



مهم تسمعوا شرح الدكتور.

Western Blot

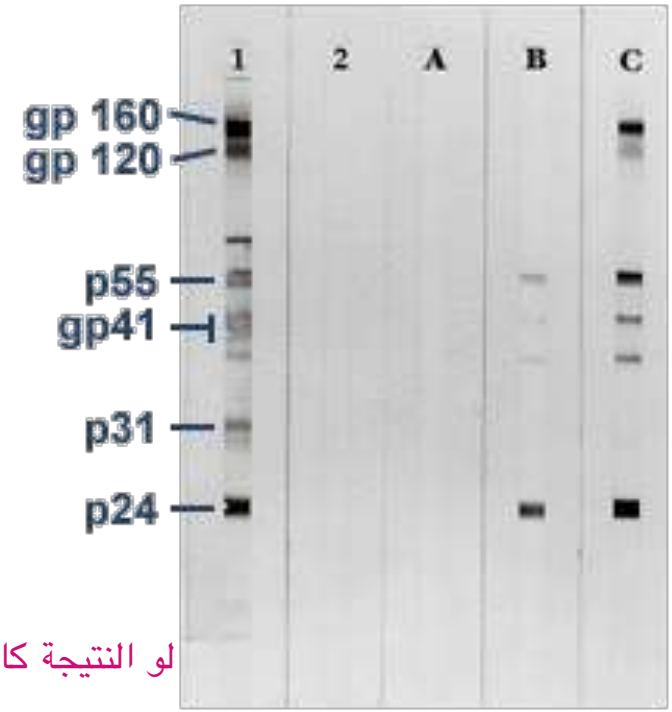
نردع نحكي انه ال positive control ما اعطاك positive او
ال negative ما اعطاك negative يعني شغلك غلط

حالة B حتى لو بروتين خفيف الواحد مش طبيعي يكون عنده بروتين للHIV بجسمه
و اللي 90% بكون positive
لكن في فحصين للHIV ال screening و ال confirmatory
الدكتور طلب نقرأ عنهم

HIV-1 Western Blot

- Lane1: Positive Control
- Lane 2: Negative Control
- Sample A: Negative
- Sample B: Indeterminate
- Sample C: Positive

For different patients

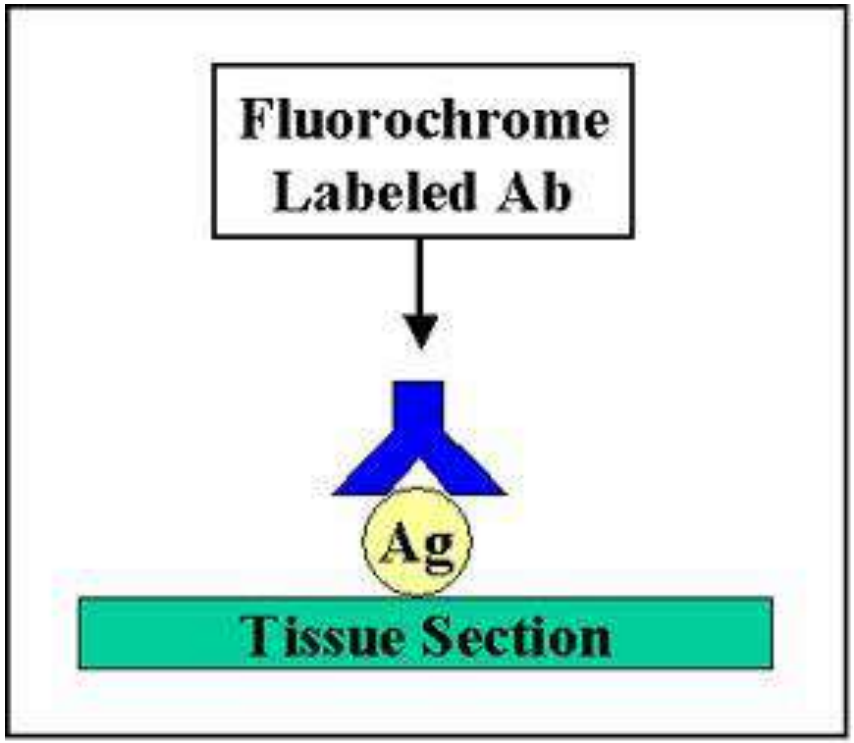


لو النتيجة كانت positive لازم أتأكد منها

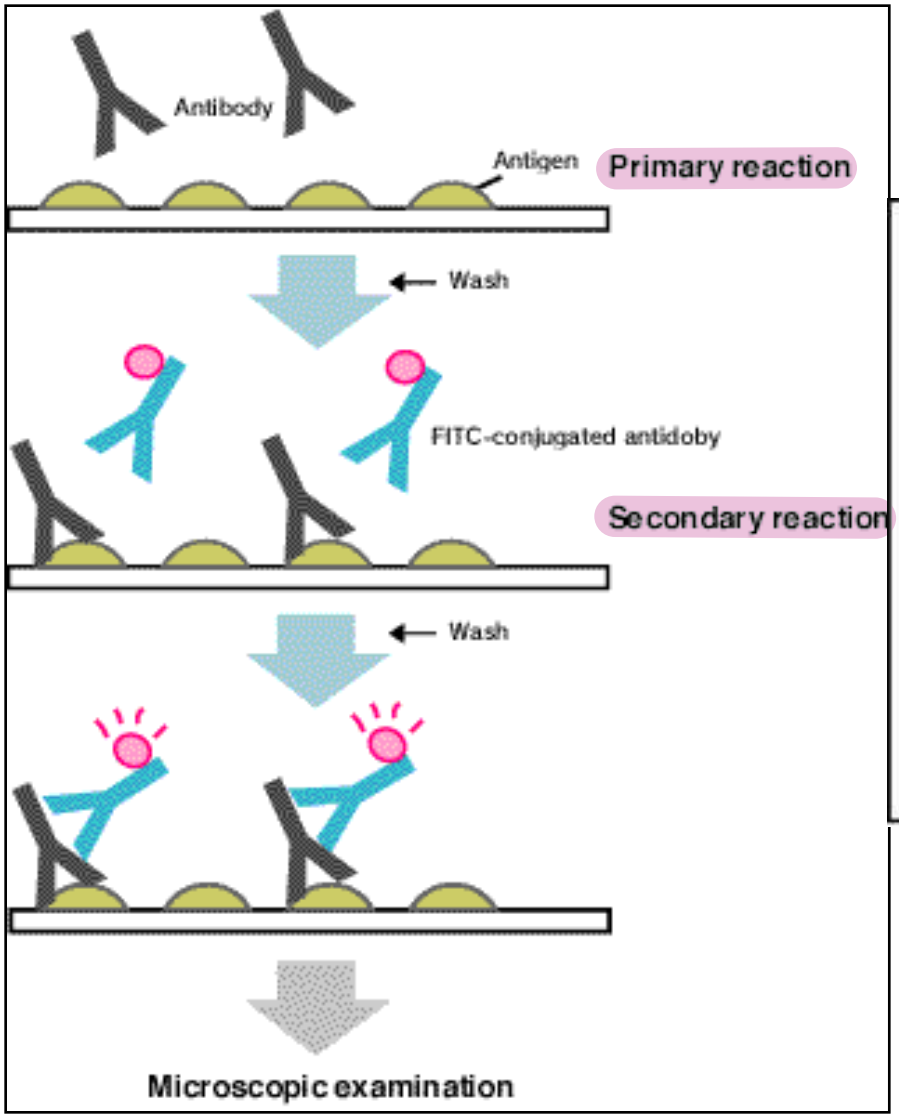
Rapid Diagnosis Based on the Detection of Viral Antigens

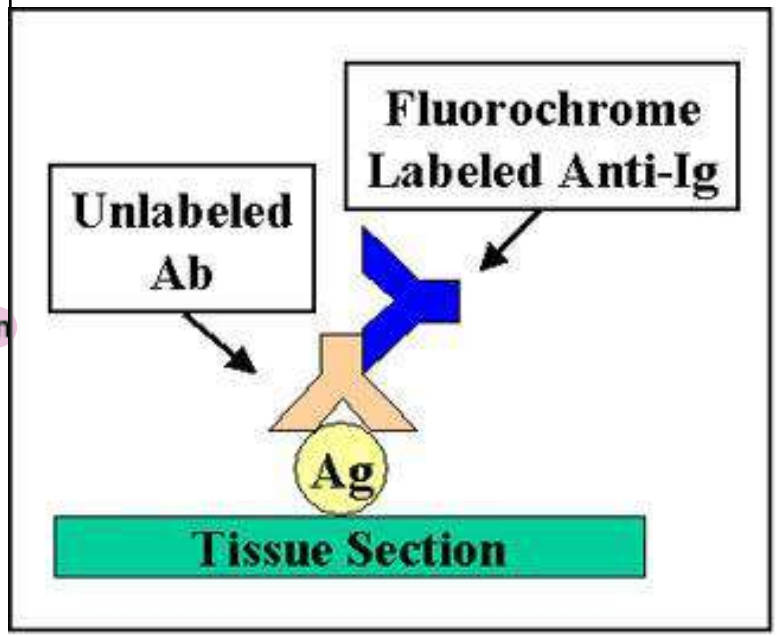
Nasopharyngeal Aspirate	RSV Influenza A and B Parainfluenza Adenovirus
Faeces	Rotaviruses Adenoviruses Astrovirus
Skin	HSV VZV
Blood	CMV (pp65 antigenaemia test)

Direct immunofluorescence



Indirect immunofluorescence





Immunofluorescence

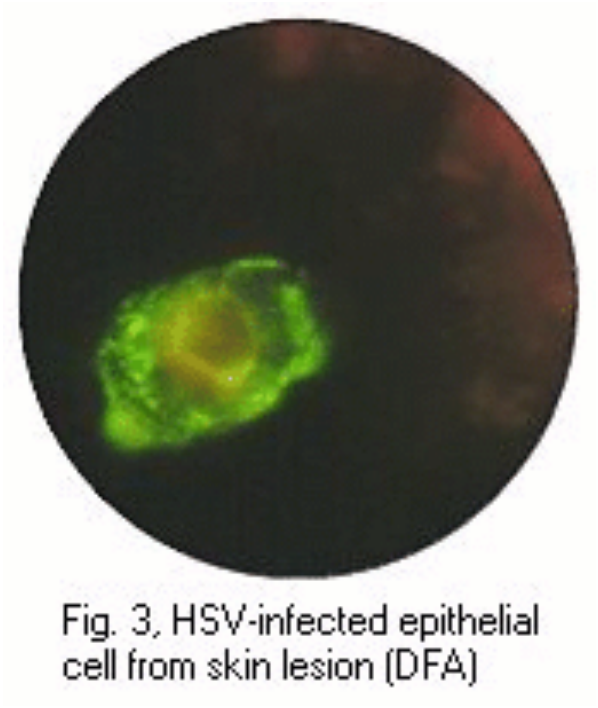
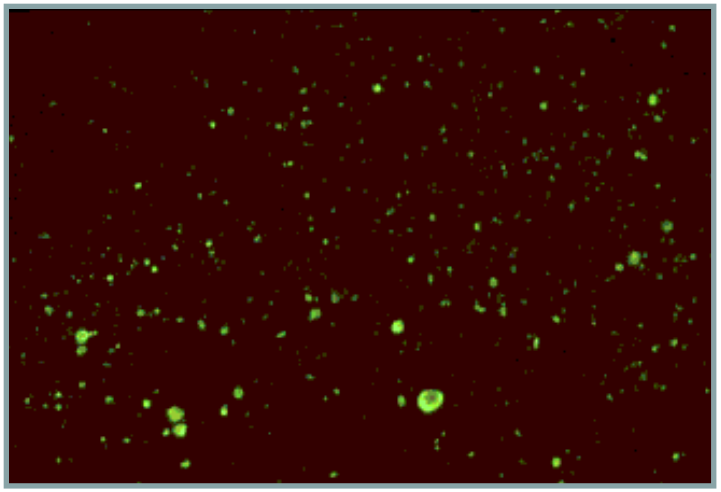


Fig. 3, HSV-infected epithelial cell from skin lesion (DFA)

(Virology Laboratory, Yale-New Haven Hospital)



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

اقرأوهم لحالكم 😊

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.

Usefulness of Serological Results

- How useful a serological result is depends on the individual virus.
- For example, for viruses such as rubella, 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.

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.**

cross reactivity معناها انه ال antibodies اللي عملها الجسم لل HSV
ممكن تشبك مع ال VZV لانهم من نفس العيلة و ممكن يعطي نتيجة كاذبة طبعا
الموضوع نادر

- **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**

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

- Diagnosis depends on the presence of an intact blood-brain barrier

"مايزيدني طمأنينة أن الله يعلم وأنا لا أعلم، يعلم بأن فوضى أيامي ليست فوضى بل مرتبة بشكل دقيق لا أدركه، يرى الرؤية الغير واضحة لي بشكل واضح جدًا، إن الأمور كلها أمام الله تتجلى بشكلها الحقيقي دون أن يخفى عليه شيء.. أليس الله بقادرٍ عليها؟"