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LECTURE 16:
VIROLOGICAL TESTS

GENERAL MICROBIOLOGY

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**

عنا دور مهم لل diagnostic tools اللي بنستخدمهم للكشف عن ال biological infection
و هاي الايام و مع التطور صرنا نقدر نطلع نتائج فحوصات الفيروسات بساعات بس
و بناءا ع هاي الفحوصات بنقدر نتخذ قرارات ع مستوى لصحة العامة

Why Expanding Role for Diagnostic Virology Lab

ليش احنا بنهتم بهذال role

اول سبب انه زاد عدد الناس اللي عندهم ضعف مناعة سواء من ال HIV او من autoimmune disease او اللي بوخذوا ادوية immunosuppressive

- **Increased pool of immunocompromised**
- **Increasing antiviral agents**

قبل 40 -50 سنة ما كان عنا هذا ال role لانه حتى لو قدرت توصل للفيروس انت ما عندك antiviral drugs هاي الايام عنا هاي الادوية

- **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**

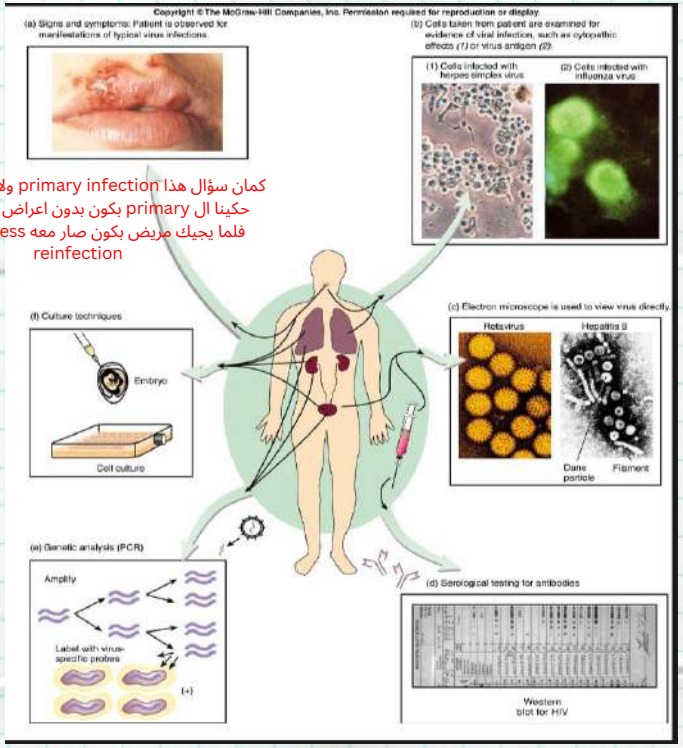
ال electron microscopy
بشوف فيها الفيروس و بحدد اذا هو naked or enveloped

ال viral culture
مشان ازيد عدد الفيروس و بساعدني اشوف خصائصهم
و لما اكشف عال antigens تاعت الفيروس
بسميه serological testing
و بيصير باني اما بكشف ع ال antigens تاعت الفيروس نفسه
او بشوف ال immune response بإني اكشف عال antibody

ال histopathology
و اللي بدور فيها على ال cytopathic effects

Methods

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



كمان سؤال هذا primary infection ولا reinfection ؟
حكينا ال primary بكون بدون اعراض subclinical
فلما يجيك مريض بكون صار معه stress سبب ال reinfection

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

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

Specimens for Routine Tests

شو علاقة ال fasces و ال throat بال meningitis
عندك فيروسات زي ال pilio بيلش من ال throat و بنتقل لل GI tract ثم ال lymph nodes بعدها لل blood و بعدها CNS
وبرضو بنتقل 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.

Specimen storage and transport

- **Keep specimens other than blood at 4℃**
- **If delay >24hrs, freeze at -70℃ or below.**
- **Avoid any storage at -20℃: greater loss in infectivity**

اذا بدنا نحفظ العينة لاكثر من 24 ساعة بنحفظه ع درجة 80- خاصة اذا كان enveloped لانه لو حطيته ع 20- بكون التجميد بطيئ و اللي ما بعطي نتائج حقيقية

- **Nonenveloped viruses (adenovirus, enteroviruses) more stable than enveloped (e.g. RSV, VZV, CMV).**

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**
- **Detect for antibodies using serological or molecular techniques**

BASIC DIAGNOSTIC METHODS

Diagnostic tests can be grouped into 3 categories:

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

direct زي ال electron microscopy
بقدر استفيد بزيادة اذا استخدمت immune electron microscopy
اللي بجيب فيها العينة تاعت المريض و بضيف عليها antibody للفيروس اللي انا شاك انه موجود فلما اشوفها بكون في Fluorescence بلون اما اخضر او احمر بالعينة

Direct Examination

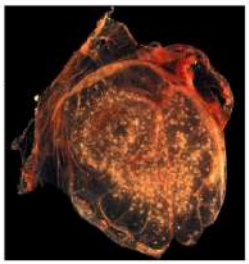
1. Electron Microscopy	morphology of virus particles immune electron microscopy
2. Light Microscopy	histological appearance inclusion bodies
3. Viral Genome Detection	hybridization with specific nucleic acid probes polymerase chain reaction (PCR)

Indirect Examination

ال cytopathic effect حكيناهم
ال hemadsorption بعدين راح نحكي عنها
ال immunofluorescence اللي حكيناه بالصفحة اللي قبل انه بتجيب العينة و بتحطلها antibodies و بتشوف اذا صار florescence

1.Cell Culture	cytopathic effect (CPE) haemadsorption immunofluorescence
2. Eggs	pocks on CAM haemagglutination inclusion bodies
3. Animals	disease or death

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 envelope ال fusion 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

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

Cell Culture

- **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**)

مش كل الفيروسات بتقدر تعمل replication باي نوع خلايا
مثلا ال cell line x بقدر يعمل replication ل 5 انواع
اما cell line y بعمل replication ل نوعين بس
و مشان هيك عنا فيروسات لسا مش عارفين شو ال cell culture المناسب الهم
و معلوماتنا عن ال replication cycle تاعتهم قليلة

Virus Isolation

عنا 3 انواع من ال cell اللي بستخدمهم بال cell culture

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

احسن واحد هو ال primary cells و اللي بجيب فيها خلايا kidney لحيوان و بنحطها ب media تحافظ عليها همه احسن اشي لانهم بحاكوا خلايا الانسان

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

بس ما بتحافظ ع الفيروس الا لاسبوع

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

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

ال hela و ال vero هذول عبارة عن cancerous cell lines
مشان هيك عندهم replication سريع
و هي الاكثر استخداما لانه اكثر وحدة بقدر اعمللها passage

شو يعني passage؟ يعني انا لما اجيب flask و احط عليه الخلايا مشان تنمو راح تنمو عندي بس monolayer يعني ما راح تقدر تنمو فوق بعضها و ال confluency وصلت 90% (confluency يعني قديش السطح اللي تعبى من الصحن بالخلايا)
فانا مضطر اشيل الخلايا و انقل جزء منهم على على flask جديد

<u>Primary cell culture</u> 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.

<u>Continuous cells</u> are the most easy to handle but the range of viruses supported is often limited.

I. Cell Cultures

Growing virus may produce

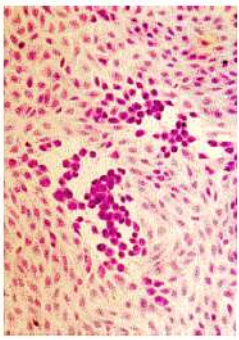
1. Cytopathic Effect (CPE) - such as the ballooning of cells or syncytia formation, may be specific or non-specific.

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.

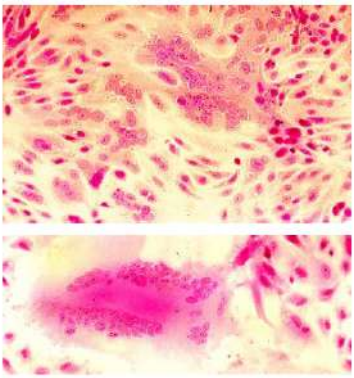
Cytopathic Effect (1)

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 in cell culture caused by RSV (top), and measles virus (bottom).

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

لما تلزق الخلايا ببعض و تعمل giant cell
بنشوفه بال HIV او بال RSV
ال RSV فيروس منتشر بسبب عدوى بمجرى التنفس
بامريكا خافين انه يصير tripledemic
انه يصير انتشار لل covid , flu , RSV

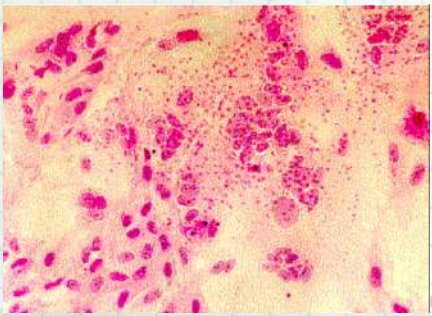
ال RSV خطير ع الاطفال اللي عمرهم سنتين او اقل و بما انه منتشرفهو بتعرضله 90% من الاطفال و اللي بسبب immunity عند ال adults

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.

Problems with cell culture

- **Long period (up to 4 weeks) required for result.**

بدك فترات طويلة ببعض الفيروسات

- **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.**

في كثير فيروسات ما بتنمو ب cell culture و اللي بسببعدم فهمنا الكامل الها زي ال hepatitis B , parvovirus , diarrhea virus

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.**

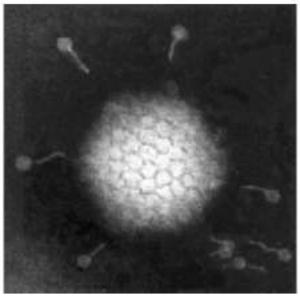
Electron Microscopy

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

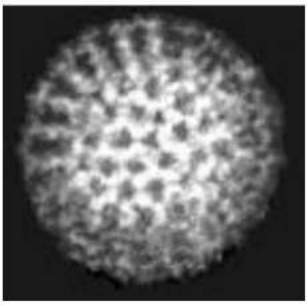
Faeces كلا الفيروسات اللي بتسبب ال gastroenteritis و كلها بتكون naked	Rotavirus, Adenovirus Norwalk like viruses Astrovirus, Calicivirus
Vesicle Fluid زي مثلا ال fluid اللي بطلع من الحب بوخذ منه العينة مثلا ال chicken pox	HSV VZV
Skin scrapings	papillomavirus, orf molluscum contagiosum

ال papillomavirus بسبب زوائد على الجلد و ممكن يسبب بحدوث سرطان فبنوخذ العينة من الجلد

Electronmicrographs



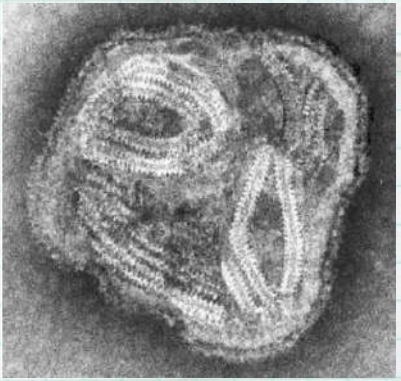
Adenovirus



Rotavirus

بشبه ال wheel مشان هيك سموه rota
rota معناها عجل باللاتيني

Paramyxovirus (Parainfluenza)



عنده helical capsid

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

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

3. ligase chain reaction (LCR),

4. nucleic acid based amplification (NASBA), and

5. branched DNA (bDNA)

بال PCR احنا بنزيد عدد ال genetic material
بالذات ال DNA
طيب بال RNA viruses كيف بعمللها ؟
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 اعمللها amplification
nucleotides
nuclease free water
هذا ال tube بنحطه بال pcr machine

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

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 انا 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 بكون 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 بكون غلط
برضو عنا positive control اللي بقارن فيها النتيجة اللي طلعت معي فيه

Polymerase Chain Reaction

- **PCR allows the in vitro amplification of specific target DNA sequences by a factor of 106 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 94oC), annealing of primers to their complementary sequences (50oC), and primer extension (72oC) 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.**

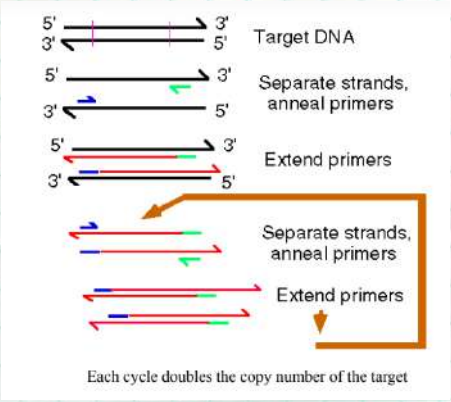
- **Advantages of PCR:**
- **Extremely high sensitivity, may detect down to one viral genome per sample volume**
- **Easy to set up**
- **Fast turnaround time**

- **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



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**
- **Presence of IgM**

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

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

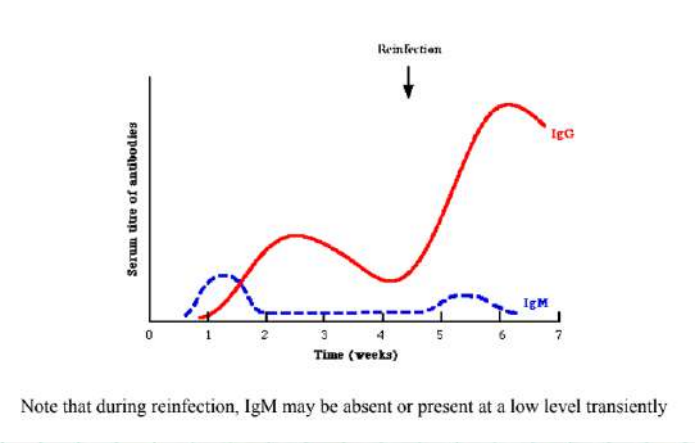
ال seroconversion هي العملية اللي بيصيرعندي فيها antibody منن فيروس معين

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

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**

Typical Serological Profile After Acute Infection



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

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

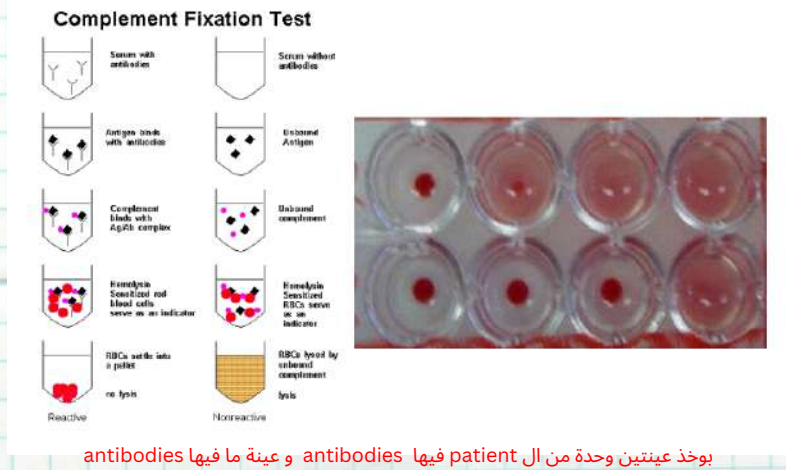
Complement fixation test في شرح تحت الصورة شوفوه بالاول

• 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



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
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**

هذا ال test بنعمله بال microtiter plate و اللي بكون مغى بال antibody او ال antigen

- **Antigen of interest binds if present.**

غالبا بنشتري ال ELISA plate اللي بتكون من الاصل coated by antigen or antibody بدل ما نعمله احنا coating

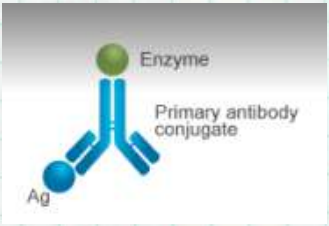
- **Second enzyme-conjugated antibody added**

- **Substrate added and colour generated/read b 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
بعدها بنضيف visible signal اللي راح تعطي لون معين بنقدر نشوفه بال ELISA machine



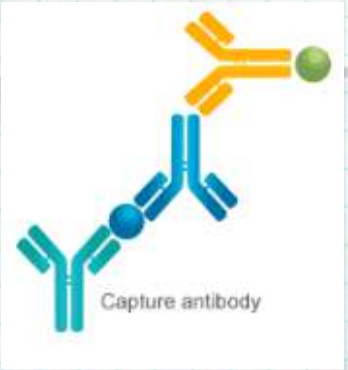
In indirect ELISA, both a primary antibody and a secondary antibody are used. But in this case, the primary antibody is not labeled with an enzyme. Instead, the secondary antibody is labeled with an enzyme. The primary antibody binds to the antigen immobilized to the plate, and then the enzyme-labeled 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

ELISA types

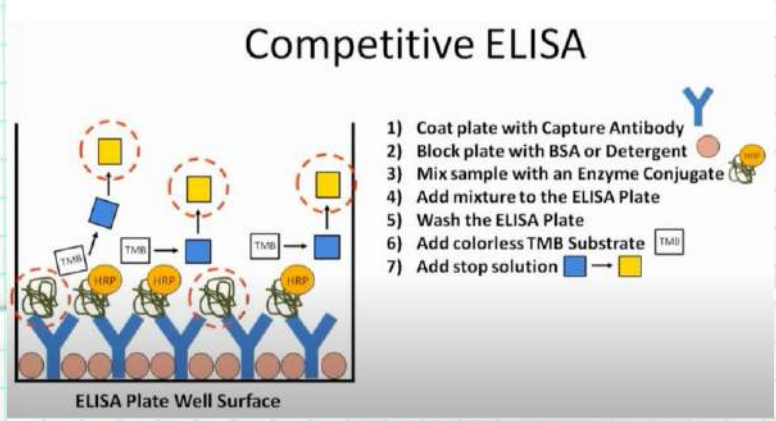
In sandwich ELISA, however, it is the antibody that is immobilized to the plate, and this antibody is called capture antibody. In addition to capture antibody, sandwich ELISA also involves the use of detection antibodies, which generally include the unlabeled primary detection antibody and the enzyme-labeled 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.

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



https://www.youtube.com/watch?v=RRbuz3VQ100&ab_channel=openmichigan

هذا الفيديو مشان تتخيلوا اللي بيصير



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

ELISA for HIV antibody

Microplate ELISA for HIV antibody: coloured wells indicate reactivity

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

• **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

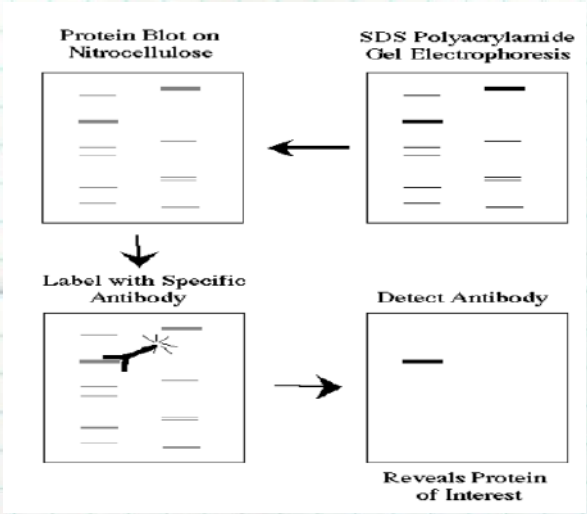
• **The blot is incubated with a generic protein (such as milk proteins) to bind to any remaining sticky places on the nitrocellulose.**

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.**

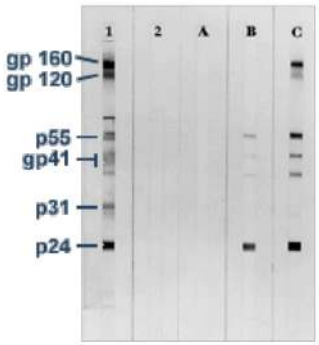
• **The antibody has an enzyme (e.g., alkaline phosphatase or horseradish peroxidase) or dye attached to it which cannot be seen at this time.**

• **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.**



HIV-1 Western Blot

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



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

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)

immunofluorescence

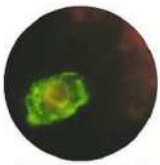
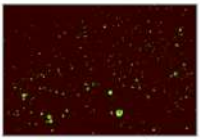


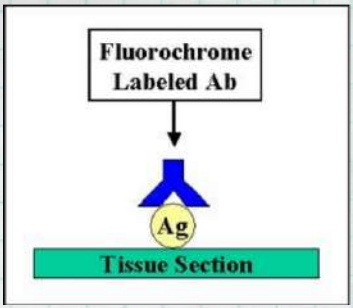
Fig. 3. HSV-infected epithelial cell from skin lesion (DFA)

(Virology Laboratory, Yale-New Haven Hospital)

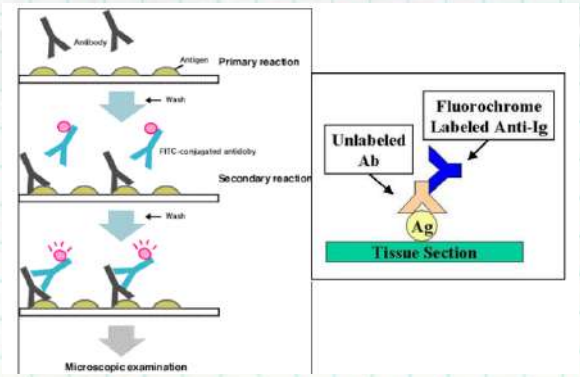


Positive immunofluorescence test for rabies virus antigen. (Source: CDC)

Direct immunofluorescence



Indirect immunofluorescence



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.

هسه بال acute ما بفيد غالبا لانه البيصير المرض
و جسمك لسا ما كون ال Ig

• 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.**

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 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**

CSF antibody titre > _1_ is indicative of meningitis

Serum antibody titre 100

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