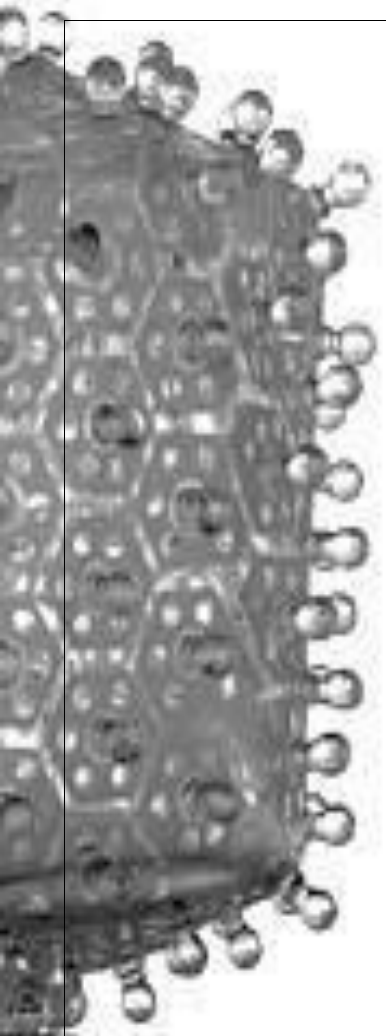


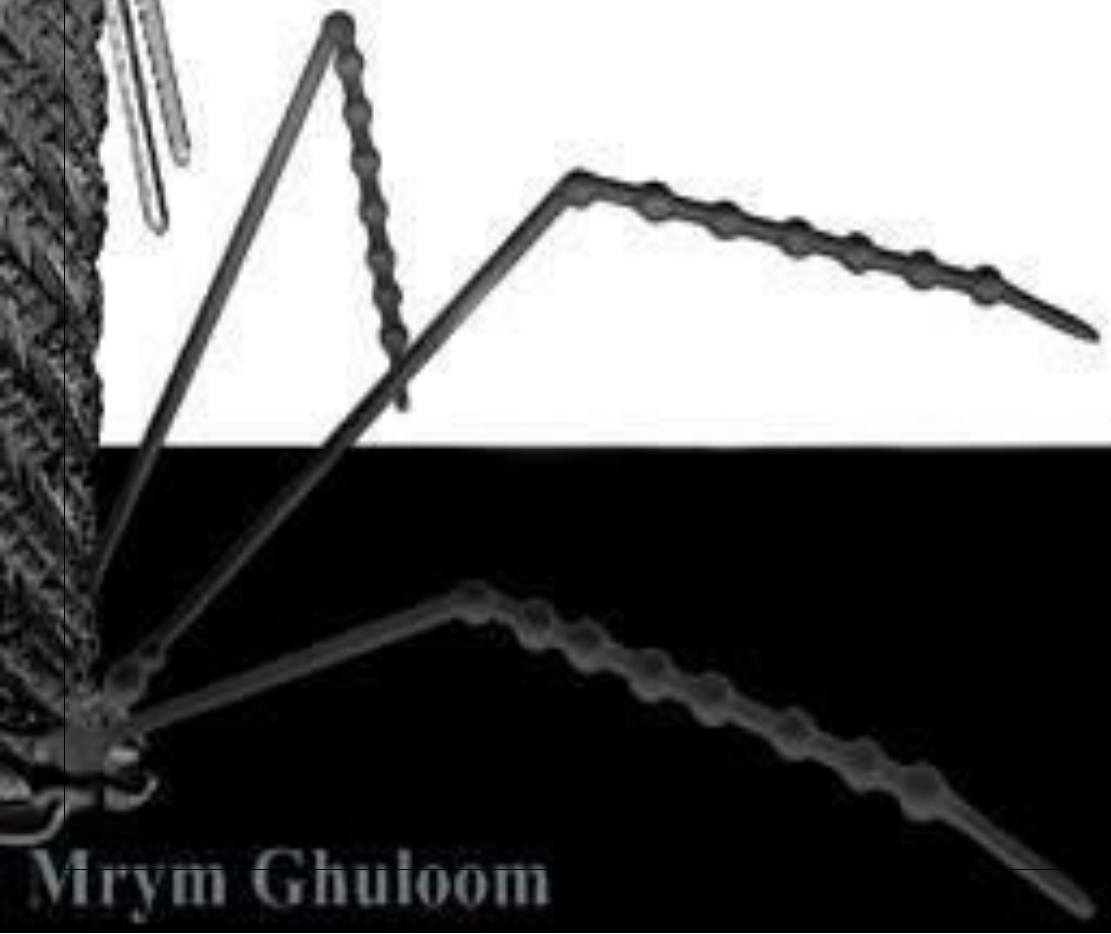
Microbiology

Lecture No: 21 (viro 7)

Dr Name: Hamed

Done by: Mai Ziad

Sheet ■ Slide □



Mrym Ghuloom

Diagnosis of Viral infections-1

Hello☺

This is an easy lecture , yet very important for us as medical students and it's talking about " DIAGNOSIS " we'll take a couple of lectures about this subject and this is the first part ☺ Enjoy it :D

Ps: the doctor said in the lecture that the figures included in our exam are only the ones in our book, and in this lecture one figure is required :D , the other figures are just for clarification .

Some definitions I got from wiki : -easy ones -

History : information gained by a physician by asking specific questions, either of the patient or of other people who know the person and can give suitable information ,with the aim of obtaining information useful in formulating a diagnosis and providing medical care to the patient.

Examination:-check up- the process by which a medical professional investigates the body of a patient for signs of disease. It generally follows the taking of the medical history — an account of the symptoms as experienced by the patient. Together with the medical history, the physical examination aids in determining the correct diagnosis and devising the treatment plan. This data then becomes part of the medical record.

Medical sign –from signs and symptoms :P: is an objective[1] indication of some medical fact or characteristic that may be detected by a physician during a physical examination or by a clinical scientist by means of an in vivo examination of a patient.

Where as symptom means : is a departure from normal function or feeling which is noticed by a patient, indicating the presence of disease or abnormality both of them are a part of clinical diagnosis ☺

χ Now let's start χ

First of all what is diagnosis ?

determination of which disease or condition is causing a person's signs and symptoms –wiki blessings :P –

DIAGNOSIS,is devided to 2 parts :, the first part is CLINICAL diagnosis *.* and the second is Investigations *.*

First part:CLINICAL DIAGNOSIS :D >

That is : history and examination.

- Later on you will learn how to do a proper history and examination ☺ * clinical years ♡.♡ *

You have to ask! You have to know what you are dealing with.. is it an infection or not ?

- A proper history and a proper examination can give you an idea about the diagnosis in roughly speaking in 70% of cases.

-example ? : you need to know where is the complaint so you ask the patient..if he/she said it's a respiratory complaint-cough ,fever or shortage of breath then ->the infection is in the respiratory tract – focus on it ☺ ..not urogenital tract for example.

A student told the doctor that what we mentioned above is not always right and that we have something that is called :referred pain is pain perceived at a location other than the site of the painful stimulus. An example is the case of ischemia brought on by a myocardial infarction (heart attack), where pain is often felt in the

neck, shoulders, and back rather than in the chest, the site of the injury

The doctor said that we already said this examination covers 70% of the cases with a right diagnosis ☺ and the left 30% is to be discovered by INVESTIIGATION which is the other part of diagnosis ….:D and that If you have respiratory tract infection then you must have some respiratory tract problems and signs ..you might have some respiratory tract infection symptoms in another disease – cough and fever in heart failure for example- in this case if u r suspicious then go for investigations :D

********** HISTORY AND EXAMINATION are really really important and no matter what a famous doctor you will be "Inshallah" you need to take it in a perfect and detailed way in order not to make any mistake..coz sometimes this mistake might be major and cause the loss of some human people's lives!..**********

In this lecture we are more interested in the **2nd part, which is INVESTIGATION !:D=LAB TESTS !**

Investigations is divided to two parts

Non-microbiology related : like the routine examinations they make when the patient is admitted to the hospital as a baseline for other diseases that are not related to infection

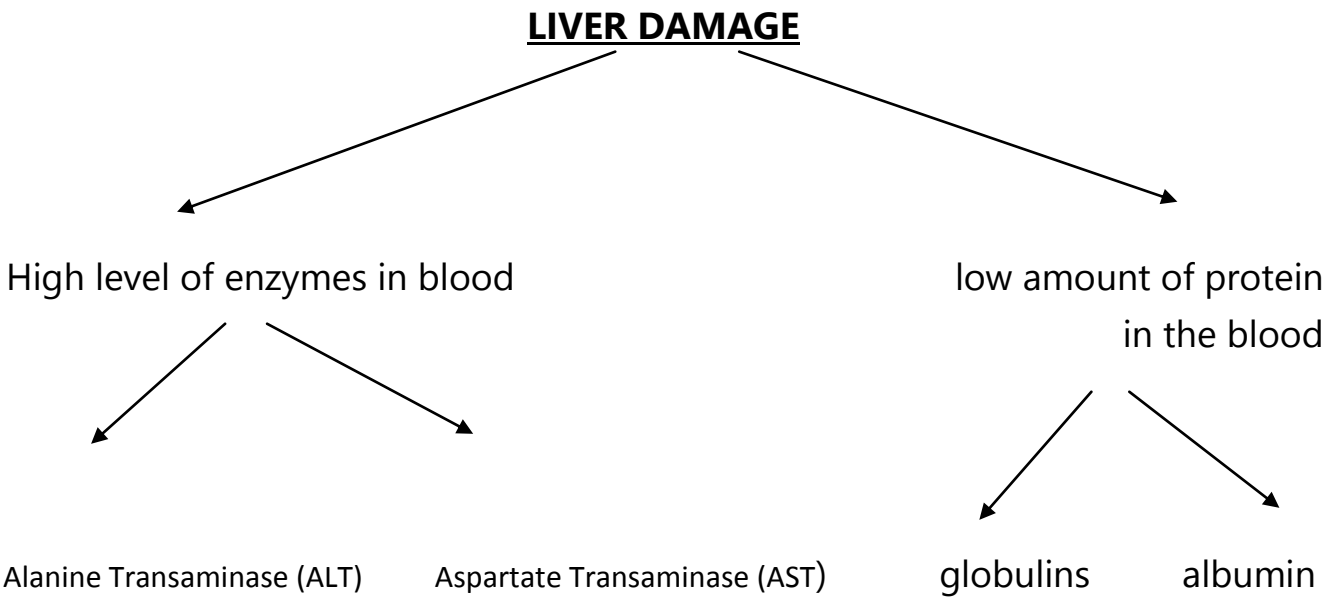
Microbiology related investigation

↓

Virology specific investigations –what matters to us

Some that can matter to us in microbiology examinations : Liver function test and kidney function test.

-liver function test : if there is a decrease in the level of proteins-eg:albumin- and or increase in the level of enzymes-eg:transaminases –damage to liver: elevated enzymes,☺



Note that: if you are thinking of a liver infection ,then you have to consider the →LIVER FUNCTION TEST as virological specific investigation

,but in general if am not considering liver infection→ then it's a routine investigation but it helps me with the dosage of medication, because if the liver is functioning well then it's not like the abnormal functioning liver or defected-☹ SO →when it comes to dealing with medicine doses..you either decrease the dose or change medication and sometimes it might be contraindicated to give some anti-virals so u should stop it..

-**another investigation : CBC complete/full blood count test** (is a blood panel requested by a doctor or other medical professional that gives information about the cells in a patient's blood) is it related to discovering VIRAL infections ? YES!

NOW focus with me..in the CBC… WHAT DO I HAVE ? what does it measure ?

One of the things it measures :WBC -white blood cells number -, if it is increased then you will think about infection! but how will you know if it is not Leukemia? You should take the history and examination and if it appears that this person has a respiratory infection (sign of cough and other signs) and by other investigations then you should exclude leukemia.

***I asked the doctor and he said that leukemia/ the WBC level in blood will be too high ☺ infection would be less than it.. Also in infection the increased WBC's will be differentiated.

how can I Know what causes the infection ? Viral or Bacterial ?!

HIINNTTTT -> blood differential test ;)!

Internet ..what is blood differential test ? – Doctor didn't explain it..

The blood differential test measures the percentage of each type of white blood

↑ Neutrophils →bacterial Infection.

↑ Lymphocytes (Lymphocytosis) → viral Infection.

Ps : in section 1 the doctor didn't say anything about Leukemia : he just said that if viral infection → lymphocytes increase.. if bacterial→ Neutrophils increase ..and that platelets decreases ☺

THOSE ARE THE INVESTIGATIONS NOT DIRECTLY RELATED TO IDENTIFYING THE PATHOGEN… IS IT CLEAR?

****

NOW ..the **investigations that are directly related to identifying the pathogen** and what matters here the viral ones ☺

But before we talk about the investigations..we need to take a proper SAMPLE and send it.

To identify the causative viral agent/pathogen precisely then we have to send the right sample.

For example : if you suspect the patient has CHEST infection it's not logical to take cerebrospinal fluid (CSF) sample …you have to take samples that will help you to isolate the organism.. for example in viral cases we take nasopharyngal swabs or nasopharyngal aspirates or sputum.

If there is a gastroenteritis and diarrhea you have to send a stool sample not a sputum sample..it's LOGIC

- ➔ No results without good quality samples !
- ➔ Underlined words →good quality samples conditions شروط:P

You have to take the **right specimen at the right time** ! for example you can NOT take the specimen after 4 or 5 days from admitting the patient to the hospital and start with medication ! there is like a rule ,,that if there is a fever more than 38.5 before giving any antimicrobial drugs then you have to send a blood sample to the lab (for culture) because if you give an IV antimicrobial then you will destroy the bacteria in the blood and the lab won't be able to isolate the bacteria for you and won't be able to give you the sensitivity and resistance results for this bacteria…☺

And PROPERLY TAKEN: blood culture should be **taken under aseptic techniques** ..you have to sterilize the area and wear gloves.

A student asked a question : how long will the lab results take to come out?

The doctor said generally in bacterial infections it takes TWO DAYS to three days for identification and determining if the bacteria is sensitive or resistance …

like in E-COLI or klebsiella or stapharues ..depending on how many personnel or the quality of the equipment and efficiency of the lab D:

Some exceptions !: brucella and TB they might take weeks..

In viral infection it is different then bactera.

A student asked another question which the doctor liked; he said it's a bacterial question : What should the doctor do while waiting for the lab results?!

You start as a doctor with emepirical treatment ..AKA BLIND THERABY

It depends on guidelines..there will always be guidelines for choosing antibiotics in every hospital

EG : chest infection → community acquired if he is admitted with the infection (detecting infection within 48 hours of entering the hospital)

→ Hospital acquired after admitting the patient With 2-3 days

You will have guidelines to use ANTIBIOTIC X for community acquired and Y for hospital acquired ☺ why ? because the hospital will be multi resistant organism! It needs different antibiotics

Okay,now the guy who invented those guidelines..why did he chose x and y ?

In each hospital or lab we have YEARLY report saying in chest infections coming from the COMMUNITY must be isolated to type A ,then B then C in terms of frequency – the most common one then the common then the less common ..susceptibility for bacteria A was 100% for DRUG 1 THEN 70% SUSCEPTIBLE for drug 2 then 50% susceptible for drug 3..so I use drug 1 for bacteria A based on my experience and the susceptibility results from last year and according to the international guidelines..so what do I do during this blind theraby ?

I take the sample → send to lab → keep checking the lab results and call then ,in decent places the lab will call you :P → if I isolated bacteria A and the lab results said that its 100 % susceptible to ANTIBIOTIC 1 then GOOD ! but if I've given him antibiotic B and the lab result says its resistant to this drug what should I do ??!!! look at the patient

He is improving ?! then IGNORE lab results! If not improving switch to drug A ☺

- how can I know that he is improving ? shortness of breath decreases fever go away ,WBC getting back to normal levels…meaning:SIGNS are going away ☺

****

***NOW said that we should take GOOD QUALITY samples! samples need to be **stored if they are not transported in the same day; you have to store it in the fridige NOT frozen!**

After we store-in the fridge- it we transport it using VTM - viral transport medium –

VTM contains:

1. tissue culture medium with Anti-fungal and anti-bacterial !

WHY we use anti fungi and anti bacteria ? →to inhibit the growth of fungi and bacteria and to inhibit the contamination.

2.buffering solution to keep the ph at 7.

3 .- Doctor didn't mention this although it is in the slides – protein stabiliser (such as bovine serum albumin) to protect sensitive viruses.

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

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

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

This table is from our test book ☺

Respiratory tract bacteria .like strepto pneumonia .5 to 25% carried as normal flora ..are fine to stay in the respiratory tract but if they go to the blood they become infectious and if the go to brain they might cause meningitis

Hepatitis A can be transmitted fecal-orally while B and C we cna take a sample through serum

Like entero viruses can be transmitted fecal orally and infect the CNS

Unclotted blood – غير متجلط

THERE ARE Specific tubes for such samples that contain anti-coagulant like EDTA and heparin

After studying the conditions for taking good quality samples we should know types of samples and from where we can take them (the table above)

types of sample collection are:

1)**SWABs مسحات:** bacterial swabs are bigger than viral ones, viral ones are thinner with a smaller head.

Must be adequate.

•throat or skin swabs must be taken fairly vigorously.

•transported by viral transport medium

2)**nasopharyngeal aspirates** : enter a tube from the floor of the nose to the throat → you inject 2 ml of normal saline and then you aspirate it back!

-it needs skilled doctor or pediatrician because it can be very uncomfortable for kids..

-its used more commonly For diagnosis upper respiratory tract infections, of young children, e.g. RSV(**Respiratory Syncytial Virus**) and influenza.

3) •**Vesicle fluid for EM**:vesicels of the skin

Collected on the tip of a scalpel blade, spread over an area about

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

4) feces:.

To identify enteroviruses or rotaviruses

•Sent in a dry sterile container

•Better than rectal swabs for virus isolation

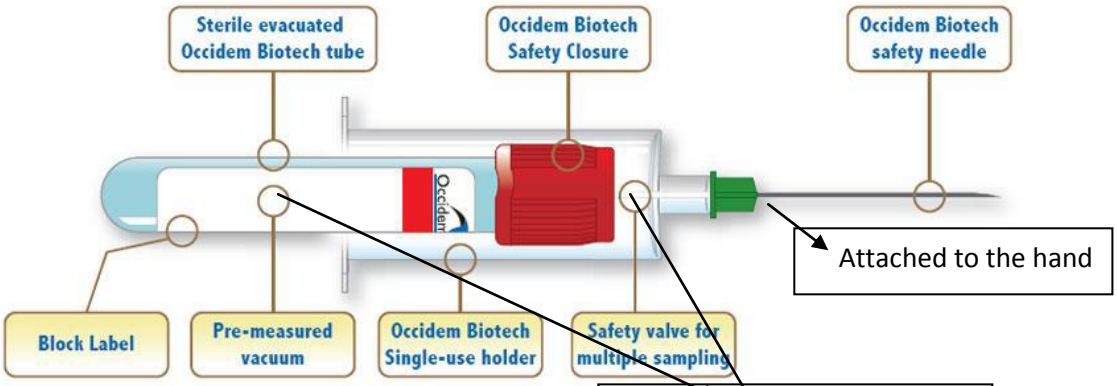
5) clotted blood – 5 to 10 ml –

Take the blood →put it in a tube that doesn't have anti coagulant (heparin or EDTA) you take it via syringe not vacuum(to avoid hemolysis) ..how to take it ?

- You take the blood through syringe then the needle should be removed before expelling the blood to the tube to avoid hemolysis to the RBC's so the sample won't be rejected. – the forceful transfer of blood to the tube might cause membrane rupture of RBC-

Reminder-We use clotted blood for samples but for some cases-like AIDS- we use unclotted blood that has EDTA the anticoagulant

In some cases we use vacuum not syringe so this is how vacuum works ☺



vacuum is easier and more sterilized -

NOW when not having vacuumes we use normal syringes that when using them we take the multiple blood samples by more than one syringe or when the blood comes out we remove the syrigne and leave the needle and put the new tube underneath it so it's not STERiLIZED !

Now after taking the sample when I want to transport it in plastic bags ,I have to write the name of the patient and his age-date of birth! suspected diagnosis, the type of the sample-to know how to deal with it and how much it is precious - and what section of the hospital so the lab can send the results to me!

*********Do not freeze because freezing kills the enveloped viruses ☺

بعرف زهقتو ... انا زهقت اكتر :P:

****

TECHNIQUES IN LAB USED TO IDENTIFY THE VIRUSES :D

They are based on interaction between two complementary things, for example: antigen and antibody ,,or a sequence of DNA with the complementary sequence .

Antibody (positive) and antigen (negative)

1st: Immunofluorescence

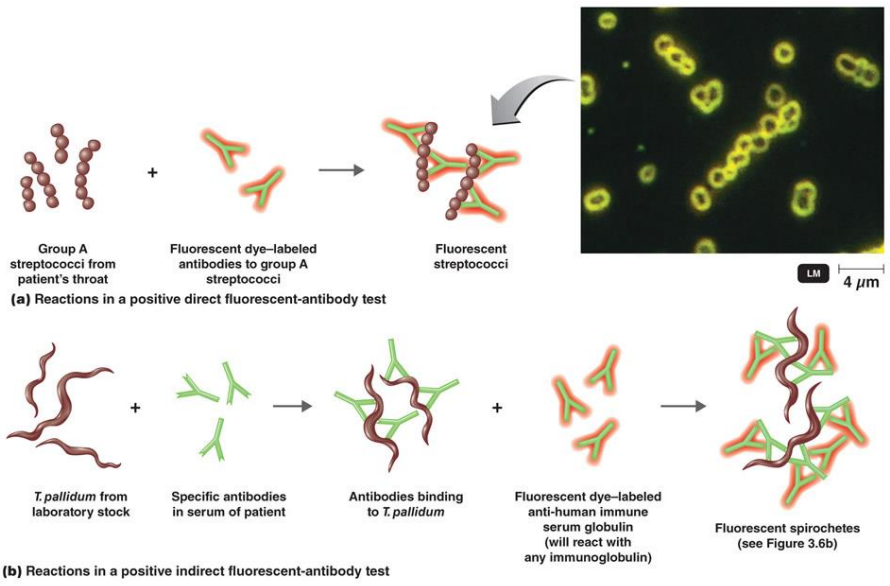
Direct fluorscent antibody method : Detect viral antigen in the clinical sample or from a cell culture to amplify the virus, by reacting the serum with cell culture /sample
This serum will have antibody labeled with substrate
(fluorescein isothiocyanate FITC) .then you have to wash to detect the presence the absence of the antigen..

Indirect fluorescent antibody method : the dye is attached to a second serum (labeled) , this second serum is usually specific to 1st serum

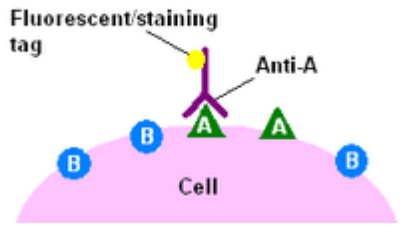
(rabbit and human antibodies).

You will understand it more below..(figures explanation)

This picture is from the internet but very easy !



Written by Mai Ziad ☺



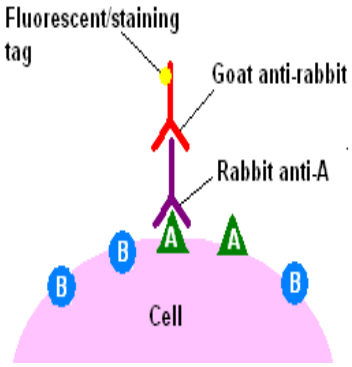
A : antigen (influenza virus for example! lets say it's positive)

We add this serum – known for us and has very specific antibody to the influenza, if it find the Antigen for influenza ,,they bind!

Then we wash: remove the unbound elements then we are left with Antigen and antibody then go to fluorescent microscope we see the FITC with a new lighting color ,means influenza virus is in sample

If there was no influenza virus in sample →

This antibody will not be bound and it will be washed off no lightening color in the sample :D



Indirect method

RABBIT ANTIBODY- negative - bind to ANTIGEN- positive- ! then comes the GOAT anti rabbit- negative- BINDS to rabbit anti body (positive to the goat one)! after that the lightening fluorescence appears :D

inject human immunoglobulins into another species and it will produce anti-human immunoglobulin antibodies

in this picture they injected rabbit Ig INTO GOAT

like the goat antii-rabbit..

<u>Why is there direct and indirect ?! the we get the same final result</u> !

In the direct method many specific sera must be labeled in order to test for a range of viruses(disadvantage of this method)...but in the indirect method (advantage of this method) : we only need one –specific- primary antibody not labeled..and only one labeled (anti-species) serum is needed to test for many viruses....

called a 'sandwich' method, because there are three layers:

- 1. The specimen being tested for a specific virus.

- 2. The specific antiviral serum, prepared in (say) rabbits.
- 3. FITC-labelled antirabbit antibody.

The indirect method is Similar to ELISA but immunoperoxidase instead of FITC is used, which is then reacted with a substrate to give a precipitate visible by ordinary light microscopy ...:D

Now ELISA technique

2nd method of diagnosis ☺

It's an abbreviation for **Enzyme-linked immunosorbent assay and radioimmunoassay** -,it's a quantitative method and relies on the positive ,negative (for control)

*commercially available (capture antigens on bread)

*how does it work ? مبدأ العمل:P

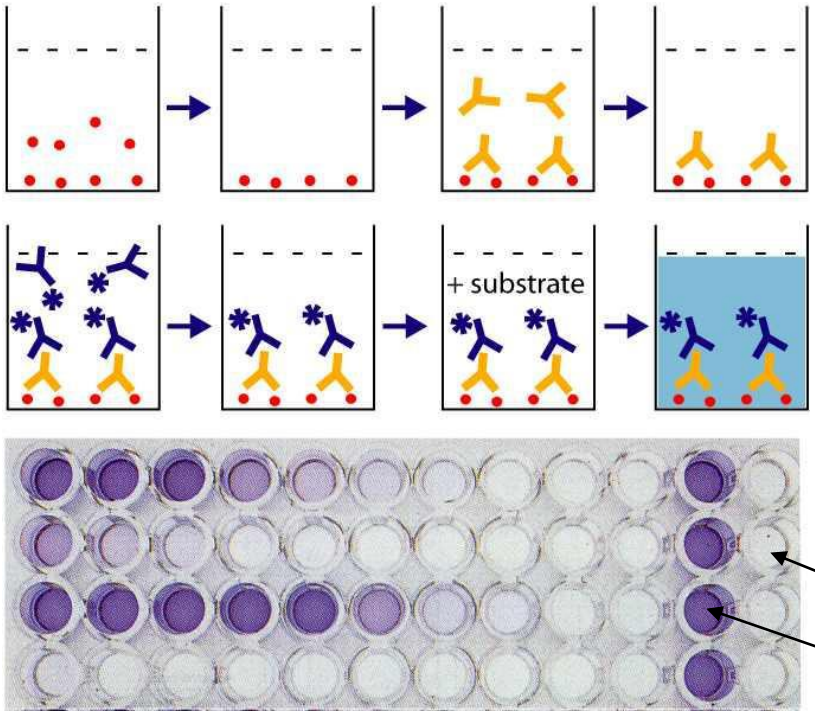
We have this multiwell plate (96 wells) this picture (below the purple wells in it) represents a part of the plate ☺what happens ?!

We have the antigen its bound to the well ! you add the sample that contain the antibody ...so ? the antibody binds to the antigen!-positive and negative – then you add a secondary LABELED and is specific to the primary antibody and so it will bind to the primary antibody ...

Ps: after each step u wash! To remove the unbound things so after we add the antibody we wash , then after we add the second antibody we wash و هكذاا:P

How do you detect the presence of the antigen ?! make sure ?? by adding a substrate ☺ the secondary labeled antibody has a label (peroxidase label) if you add the substrate to it, then it will catalyze a reaction leading to production of a chromogeneic substrate.

Chromogeneic substrate : A substrate that changes color when modified by a specific enzyme



Originally the wells are clear .which contains all the reactions, and contents; it contains the antigen, antibody 1 and antibody 2..

When you add the substrate the colour will change to purple :D

Why did it change !? because the secondary antibody (labeled)

It exists and It catalyzes the reaction, if it exists means it's bound to the primary antibody and the later one is bound to the antigen so the antigen is there and you can quantify the results

Always use a positive and negative control

To know that this result is POSITIVE we compare it with the positive control ☺

Written by Mai Ziad ☺

From the book ☺ -the figure is from the book and the doctor had to explain it ☺

Antibody 1 antigen antibody 2 (labeled)

Antibody bind antigen then the secondary bind the antigen ! its labeled! If I detect a change in colour then the antigen is there☺

Something that might confuse you ,how can the secondary antibody bind the antigen same antigen bound to antibody 1 ?

The presence of different epitops (epitops : zy l ba9meh ll antigen :P

or same epitope on different places on the antigen

This is for antigen detection.

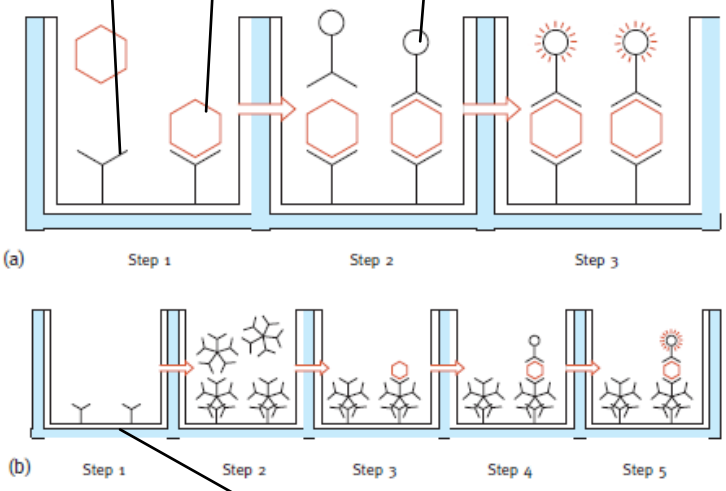


Fig. 36.2 (a) Direct identification of antigen by captures and ELISA. Step 1: Addition of specimen containing antigen that combines with the specific 'capture' antibody on a plastic surface. Step 2: Addition of enzyme-labelled specific antibody. Step 3: Substrate is added, reacts with bound enzyme, and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

In this example this is a capture antibody; it will capture the igM antibody, it is specific; it will be negative and the igM will be positive.

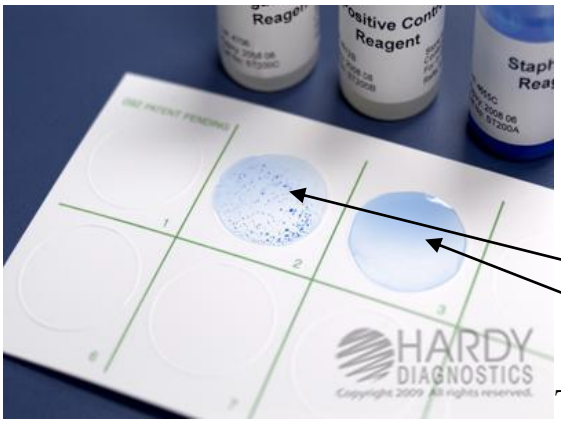
what's on the plastic surface is antibody to igM-capture it- (tho igM is antibody but when we inject it the body will develop antibodies against it and consider it as antigen) .. so antibody for igM then we add a patient sample with igM and those igM are captured by the anti igM then antigen is added →igM is positive and the antigen is negative so they bind→ then we add a secondary antibody(labeled)and →then we add substrate so it will change colour and we will realize that there is an igM ☺
By the way this is for antibody detection.

Now lets move to another diagnosis method

3rd: Latex agglutination tests :

same thing antigen and antibody (positive and negative) BUT it's easier, more rapid, commercially available and not complicated ☺ doesn't need a trained staff or complicated equipments ..

We use it to diagnose some infections like the rotavirus infection in the gastrointestinal tract HOWEVER Its liable to prozone (you will know what this means now) effects, giving false negative results at low dilutions of serum



This one is the negative and the second one → positive ,.. let's say you have a sample (stool sample for example) ,,the antigen (rota virus)was in the stool sample, when you added the antibodies there was an interaction and this interactions appeared as precipitation(clumps), it means the positive and negative met and we have these dots as a result of reaction ,but the negative result →it will stay clear without any clumps or dots because there is no antigen to react with the antibody ☺

Prozon effect: false negative result because of the high concentration of antibodies..meaning you have a sample with a lot of antibodies and regardless of antigens ,,so the epitope might be covered by the high concentration of the antibodies! → and no antibody will bind to the epitope of the antigen ! because of high competition..and another cause that there might be an interfering antibody for another disease and it will cover the eptiope يحجب الرؤيه

How to deal with this???! dilute the sample of antibodies !:)

4th :Electron microscope :

We use Phosphotungstic acid stain (PTA) that negatively stain the sample

-NEGATIVE staining means : the background will be black and the virus particles will be white .. because it will not be penetrated by this stain! And you have to have a million copy of the virus to be able to detect it in the electron microscope

-Its useful to diagnose some viruses according to the morphology like: HSV(herpes simplex virus) and VZV (Varicella zoster virus) (but it's difficult to distinguish between those two viruses by morphology itself) and we use it to diagnose SARS coronavirus.

So we finished detection of antigen and antibody now detection of VIRAL GENOME !

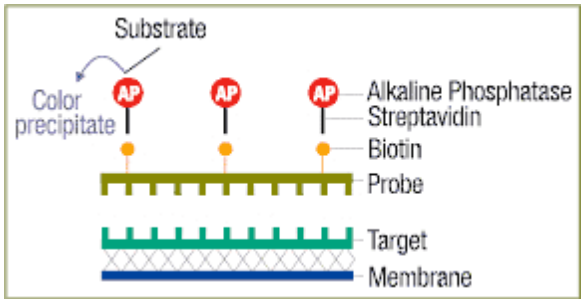
بهدله و تهديد انو يصعب الاسئله ازا بنحكي اكتر :p

5th: Detection of viral genome by nucleic acid hybridization :

-------Dot blot hybridization:

By using PROPES (labeled)

And we call it like that because we use nitrocellulose paper to test some samples that might contain genetic material for a virus



For example this sequence is your target ,it's a part of a virus sequence..you add a complementary sequence that is labeled (here we are talking about sequence and anti-sequence (sense and anti-sense) instead of antigen and antibody), if there is ACTG then you expect the prope to be TGAC, you mix it with the sample if the target is there then the prope will bind to it and it will be detected by the microscope – There is something called hybridization in situ

that is similar to dot-plot hybridization but you do the test immediately on the clinical sample.

SUMMARY OF THE PRECEDURE : (From Internet)

1)extract and purify DNA or RNA from different sources .

2)apply directly as DOTS on nitrocelluolose paper.

3)if DNA then use alkali to denature it and form single strands

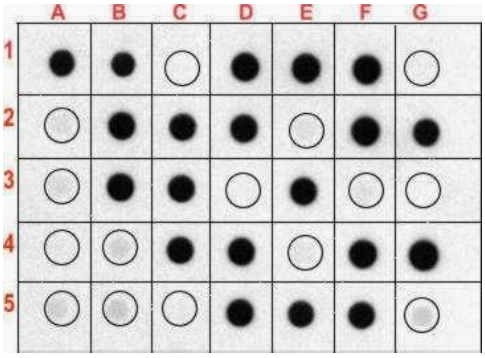
4)immobilize by baking at 70-80 c for 2-3 hours

5)add labeled probe for hybridization to take place

6)wash off unbound probe

7)autoradiograph

Black dots represents samples where target dna is present or probe has bound☺



PROBE : a **hybridization probe** is a fragment of DNA or RNA of variable length (usually 100-1000 bases long) which is used in DNA or RNA samples to detect the presence of nucleotide sequences (the DNA target) that are complementary to the sequence in the probe...

-===== Hybridization *in situ is similar, except that it is used directly in* tissue sections

!!!! كلها صف حكيAND FINALLLLY WE ARE DONE WITH THIS LECTURE!

. اااا المملة جدا عالمحاضره سامحونا قوGOOOOD LUCK EVERYONE :D