Comprehensive Molecular Diagnostics and Advanced Gene Expression Analysis

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Lecture 40 : Point of Care (POC) diagnostics in Infectious diseases

. Welcome back to the NPTEL lecture series, Comprehensive Molecular Diagnostics and Advanced Gene Expression Analysis. So, we are at the week of the molecular diagnostics in infectious disease. Today we are going to discuss the point of care diagnostics which are available in infectious disease from the perspective of molecular diagnostics. Here we are going to discuss the different detection methods or principles rather which are used in point of care devices and then different platforms which are being used in point of care diagnostics for infectious diseases. So, these are the various types of biomarkers or bio molecules which are exploited when we try to diagnose infectious disease.

So, that can be pathogens nucleic acid like RNA or DNA. Now, majority of the viruses are RNA viruses some of them are DNA as well as different bacterial nucleic acids that can be detected. So, these type of nucleic acids pathogenic nucleic acids they directly reflect the pathogens load during the infection and while going through the treatment procedure these nucleic acid detections can be used in monitoring as well. Then antibodies different anti pathogen antibodies which can be detected in the sera it indicates basically the infectious state and definitely it is easier to detect those and rather than the antigens.

Then again different proteins or antigens can be detected the very well established examples are p 24 antigen in HIV virus. Then capsid antigen or envelope proteins in different pathogens and they help in early detection before the seroconversion before the antibodies appear they are a very important markers biomarkers for infectious disease diagnosis. And it can also be utilized in combination with the antibody diagnosis. Then of course, different types of circulating micro RNA's here you can see a list of micro RNA's pathogenic micro RNA which can detect the presence as well as the monitoring of different pathological states and infection. So, based on that these biomarkers detection their quantification as well as their qualitative presence based on these there are different types of tests which have been utilized in the diagnosis of infectious diseases and those same procedures are incorporated in point of care tests.

Now, why this point of care devices are required and what is this point of care diagnostic devices are providing us. Basically whatever the current conventional diagnostic methods we have to diagnose infectious disease like ELISA like identification of the pathogen in the sample which we detect by staining or by different in different slides. So, those are performed in medical laboratories, laboratories which is not just beside the patient even that can be far far away. Of course, those conventional techniques so, take time needs expertise needs personnel trained personnel who needs to run the test we need expensive machines to conduct those experiments. So, finally, rapid detection is not possible.

So, what we need to decide the treatment protocol to provide a very quick treatment protocol to follow the very quick treatment protocol what we need a very rapid detection technology. So, POC devices or the point of care devices are actually providing diagnosis in the proximity of the patients. So, that is basically a real time diagnosis, rapid diagnosis, accurate and onsite detection at the patients point of need. Definitely WHO has given some fundamental criteria of POC diagnostics like ASSURED and that stands for Affordable, Sensitive, Specific, User friendly, Rapid and Robust, Equipment free and finally, deliverable to the end user. So, these are the different criteria base which should be present in the POC devices.

Now, in the point of care devices there are different methods or different types of reaction principles which are utilized. One of them is electrochemical biosensor. So, basically those are voltammetric or amperometric biosensor where current is generated due to electrolysis means there is electrochemical oxidation and reduction reaction over the working electrode and then the current is measured when there is a potential applied between the indicator electrode and the reference electrode and that signal is obtained from this potential difference and they are measured depending on how quickly the reactant molecule move from the solution to the electrode interface. Now this is the basic principle which is utilized in voltammetric or amperometric biosensor then what is the difference? In voltammetric biosensor the applied potential is increasing gradually at a specific rate whereas, in case of amperometric biosensor the potential remains constant. Now, this electrochemical voltammetric biosensor based molybdenum disulfide nanosheets are utilized for diagnosis of chikungunya virus detection where the DNA is detected based on that.

Similarly, amperometric biosensor has been utilized in micro bacterium tuberculosis diagnosis in sputum. So, what is done basically there is a captured antibody which is coated over the micro tips and when they react with the target bacterial pathogen they form immune complex over those micro tip surface and that causes the potential change or the current changes which has been detected over the sensor. So, this is how the

electrochemical biosensor technology is incorporated in POC devices. Impedimetric biosensor is another type of electrochemical biosensor where the changes of immune sensor which are label free is utilized for detection of dengo NS1 antigen. Similarly, potentiometric biosensor where ion selective electrodes are utilized mostly.

Now, good example is detection of bovine herpes virus 1 detection then there is different types of fluorescence based sensor. Now, the definitely when I am talking about fluorescent based sensor there is fluorophores that fluorophore can be a fluorescent dye can be fluorescent based nano materials. So, very common principle which is used in fluorescent biosensor is FRET that is fluorescence resonance energy transfer. So, there is a transfer of energy from the donor fluorophore to the acceptor fluorophore and that change or transfer of energy is detected in the sensor. Now, that can be a direct fluorescent labeling biosensor fluorescence biosensor can be a direct fluorescence labeled biosensor where the very common example is quantum dot based fluorescence biosensor.

Now, what it does definitely there is a tagged fluorophore which is targeting the specific antigen or some specific molecule of the specific pathogen and based on that there is changes in the fluorescence. So, this is how Ebola virus glycoprotein the that specific glycoprotein is basically targeted by a specific fluorophore that can be that fluorophore can be tagged over antibody or some other affinity based molecule based on which that Ebola virus glycoprotein is detected and there is fluorescent change. Now, these varieties exploited in signal on fluorescence biosensor as well as signal of fluorescence biosensor. Now, what is signal on fluorescence biosensor? Definitely when the biomolecule is present then there is the then there is the fluorescence. So, when we are getting a positive fluorescence it indicates that the viral antigen or the pathogenic molecule is present.

So, if there is no signal that indicates there is no such antigen. Signal of fluorescent biosensor is just the opposite that there is fluorescence in absence of the targeted molecule or targeted viral antigen. So, this is how signal on and signal of fluorescent biosensor are used. You can see these are the examples of this type of biosensors like influenza H1N1 virus influenza A is detected based on a very good fluorescent probe. Similarly, another antibody functionalized biosensor for infectious disease infectious bronchitis viral detection utilized for signal of fluorescence biosensor.

Then surface enhanced Raman scattering or SARS based biosensors they basically rely on the phenomena of enhanced inelastic light scattering by molecules absorbed on the corrugated metal surface. So, this technique basically utilizes coinage metal nanostructures and it detects single molecules. So, it is highly sensitive multiplexing is possible background noise can be cut off and also these molecules are resistant to photo bleaching. So, this is how based on SARS biosensor different types of molecules can be

detected. Now, what are those type those molecules? Now, the specific bacterial antigen can be targeted via aptamer.

Aptamer which is binding aptamer is the molecule which is binding specifically with some bacterial specific antigen or some pathogenic antigen and those aptamers are magnetic assisted aptamer which are tagged by magnetic nanoparticles. So, those are detected over SARS biosensors. Similarly, rapid SARS based lateral flow immunoassay biosensors are there for detection of E. coli in biological sample. Now, this lateral flow immunoassay again I am going to discuss very soon.

Also this SARS based biosensors are basic can be coupled with PCR procedure and that is utilized in detection of mycoplasma pneumonia DNA. So, this is how different techniques or different principles are utilized in POC diagnostic devices. Again cholerimetric based biosensor where there is changes of color on presence of different targeted analytes. Chemiluminescence based biosensors are also there which is based on the property of chemiluminescence and this property is exploited with DNA hybridization based chemiluminescence biosensor which is a basically multiplex biosensor where multiple simultaneously multiple type of virus can be detected like hepatitis B, C, HIV virus. Then there is magnetic biosensor where the magnetic phenomena of different materials are utilized to detect analytes.

So, that can be a very good example is Z lab that is a magnetic based biosensor for detection of influenza virus these are examples. Similarly magnetic particle spectroscopy or MPS biosensor. So, there is spectroscopic studies over super magnetic iron oxide nanoparticles. So, this is a specialized type of nanoparticles where the spectroscopy is tagged along with the magnetic property. So, there is magnetically saturated regions in sinusoidal magnetic field which finally, produces dynamic magnetic responses and those responses are detected to check on or to diagnose some specific viral or specific pathogenic molecules.

Now I am coming to the different platforms. So, those cholodimetric or chemiluminescence based techniques, then SARS based techniques, electrochemical based techniques these are the principle based on which POC devices are formed, but they are integrated over different types of platforms. So, one such example of POC platform is lab on chip devices or LOC devices. Now LOC or lab on chip by this word which comes in our mind is there is a chip which contains the whole lab. So, the laboratory procedure is basically conducted over a very small chip that is our lab on chip devices.

So, this lab on chip devices how they are fabricated? They are fabricated exploiting the microfluidic principle. So, there are different types of very small microfluidic channels

which are coated over different substrate like glass or polymers. So, a glass content multiple types of microfluidic channels and those channels are very much equipped with the mixing of samples, mixing of reagents, sample separation the whole reaction is conducted over those channels or the channel regions and finally, the detection step is also possible over those channels. So, everything which we do in the lab starting from sample whenever we input the sample in a machine in a laboratory machine. So, there is the input of sample mixing of the reagent then there is the reaction and finally, the detection everything is basically conducted over that small chip.

So, that is our lab on chip and that is completely integrated at an automated in manipulation. So, what are the advantage we are having? Apart from conducting all possible chemical or possible reactions to detect the analyte the device is automated it is a miniaturized version of the lab. So, definitely it is portable multiple type of reactions can be multiplexed over a single chip a chip based method. So, that can so, that is a rapid detection method definitely because that is a chip it is a very small size of chip which contains very small volume channels. So, what you need to input a sample which is of very small volume reagents of very small volume.

So, within that small volume of sample and reagent we can detect what we want to detect and finally, because it is portable on site detection is possible in resource poor regions. So, that is how lab on chip devices are helping in the diagnosis of infectious disease in remote areas where the lab cannot go where different medical laboratories different sophisticated techniques are not available or the expertise are not available. Now, lab on chip devices they are applied in multiple types of infectious disease detection the very common is malaria detection parasitic infection by plasmodium parasites malaria the outbreak occurs in different poor resource poor regions different remote areas where detection is not possible rapidly. So, there this lab on chip devices help in diagnosis. Similar such diagnostic methods are available where plasmodium falciparum gDNA can be detected the infection can be identified in a very fast within minutes within hours.

In fact, and then it is very sensitive you can see the detection limit is very low the pathogen even present in very low concentration can be detected. Then there is immunochromatographic immunochromatographic loc devices where immobilization of captured antibodies are presents antibodies which are directed against LDH of plasmodium falciparum or plasmodium vibax based on that detection of the parasitic infection malaria can be possible and that devices those devices can be incorporated with the smart phone technology. So, over the smart phone you can detect or check the presence of the pathogen. Then bacterial infection as well as viral infection can be diagnosed based on these loc devices mycobacterium tuberculosis and their multiblock resistance strains can be detected. As I told you multiplexing is possible.

So, different species can be detected in a chip genotyping of multidrug resistant tuberculosis can be done from the raw serum raw sputum. So, here for sputum what you need you need to homogenize the sample you need to lysis the cell you need to nucleic isolate the nucleic acid purify and then genotyping is required the whole procedure is possible over the chip. Other bacterial infection like E. coli, Staph aureus, Salmonella enterotica everything is these all bacterial infection detection can be done over loc devices. Viral infectious diseases diagnostics are basically integrated with CRISPR chip.

CRISPR technology we have discussed that CRISPR technology is incorporated in the microfluidic chip and they are multiplexed in such a way that it can detect multiple type of virus. It can be Ebola virus, it can be RT-PCR based detection of HIV, then it can be HPV detection kit HPV genotyping is possible then influenza virus detection that can be possible. So, this is how Lab-on-chip works. It is a multiplex pattern and portable the most important thing is portable and you need a very small amount of sample as well as the reagent. Then coming to the lateral flow devices.

Now, lateral flow devices are known as lateral flow immunochromatographic assay as well. So, basically there is one immune reaction and also there is chromatographic separation procedure. So, in a lateral flow device architecture what we actually have? We have a disk which disk contain different regions like sample pad. So, here you can see this region is our sample pad.

Then we have conjugate pad. Here this grey area this grey area is basically the nitrocellulose membrane and this purple region is the wicking pad. Now how it runs? So, in the sample pad the sample which we suspect that the target infectious disease molecule can be present. So, we give the sample we apply the sample over this sample pad. Now because I told you this is one immunochromatographic assay. So, based on the chromatographic separation procedure capillary flow the samples are adsorbed then after following the capillary flow it is passing towards the conjugate pad along with the nitrocellulose membrane.

Now the nitrocellulose membrane contains multiple types of biomolecule. What are those biomolecule? Over the nitrocellulose membrane in the region of conjugated pad here there can be some specific molecule conjugated with bio labels that can bind with the targeted analyte. Now just think we want to detect a bacteria, bacteria A. Now these bacteria A contains one antigen this is the antigen. Now against these we need some specific biomolecule which has high affinity towards this antigen.

What can be that? That is one antibody the very common one is antibody an antibody which is specifically targeted to the A star antigen. And now these antibody is basically

conjugated with a bio label that can be a bio a bio label which basically gives the color. So, a labeled tag. Now consider the antigen is present in the sample definitely while following the capillary flow it reaches the conjugate pad it definitely we bind with the antigen antibody and it will flow over the nitrocellulose membrane. Now in this region we are having different test pads these are the test pads.

Now the test pads contain capture molecule capture molecule which binds with basically the antigen antibody complex. Antigen antibody complex which contains the bio label along with that we have the capture molecule. Whatever extra sample are passing through will be absorbed by the wicking pad. So, if the test is positive if it contains the analyte in the test line of the positive region it will give a color. So, if you can imagine this is just the kit which we use for pregnancy test which we use for rapid detection of different antigens in our lab.

So, this is the lateral flow devices. Now the lateral flow devices can be of sandwich type can be of competitive type. The sandwich type lateral flow is basically the target analyte bound with the antigen, antigen with antibody tagged complex. They are captured over the test zone by the capturing molecule and which finally, gives rise to the visible test control lines. Now these are possible for those molecule which are larger in size basically which contains multiple types of binding sites or epitopes. So, here the target concentration or target concentration means the analyte concentration is basically proportional to the test lines signals intensity.

Competitive lateral flow device is for those molecule which are smaller in size the analytes which are smaller in size and that contains fewer binding sites. So, how it is done here is competitive displacement or competitive competition for binding of those captured antibody. So, what will happen the target analyte when it is bound with the antigen sorry antibody it will reach the test region. Now in the test region there is already antibody which is bound with the captured molecules. These antigen antibody complex they will displace the previously bound antibody which previously used to be giving the signal.

So, what will happen initially before even applying the analyte there was a signal which will be absent in presence of the target. Again so, what will happen those antibodies so, here we have the antibodies and here we have the test line. Now these antibodies are bio labeled and here there is the capture molecule. So, when there is no analyte present no virus present so, definitely there is no viral antigen present. So, if you give the sample simply those antibodies will go reach the test region will bind with the captured antibody and give the positive result.

Whereas, if there is sample they will bind with this antibodies and will replace the

bound antibody which will give rise to negative signal. So, if there is analyte there will be no line in the signal test signal region. Again the target concentration is basically inversely proportional to the test line signals intensity and this sandwich lateral flow device as well as the competitive lateral flow device those both can be utilized as qualitative and quantitative detection for the targets. Lateral flow devices they are utilized for detection of parasites, malarial malaria biomarker detection, then bacterial detection for mycobacterium tuberculosis, then virus detection can be done E. coli, salmonella, enterate IDs, HIV, HBV DNA different influenza viruses they can be detected using this lateral flow devices.

Then there is miniaturized form of PCR devices basically in case of conventional PCR what is the problem there is a slow heating or cooling temperature control region. So, to maintain the reactions temperature we need to control the set in such a time taking way that it takes around 1 to 2 hours to complete the whole run. So, to recover that the very common strategy is to increase the heat transfer rate by using better heaters as well as if we reduce the sample volume we need this temperature control a bit in lesser quantity and that is what is utilized in ultrafast NAT or nucleic acid test. So, shrinking the sample volume as well as using some better heaters to control the temperature is what utilized over the miniaturized PCR devices and those are applied over the microfluidic and MEMS platforms. So, these miniaturized PCR devices are utilized for differential identification of malarial parasite, falciparum and vivax parasite, then brucem allyl detection is possible, bacterial detection, multiplex cassettes are utilized for detection of entero hemorrhagic E.

coli. Then chip based two NAT devices which are utilized very much it is in present in diagnostic utilization for detection of pulmonary and extra pulmonary tuberculosis, then viral detection for SARS-CoV virus, then different influenza virus they are possible. So, this is how miniaturized PCR devices are used in infectious disease diagnostics. Then coming to isothermal nucleic acid amplification devices. Now isothermal nucleic acid is basically amplification of nucleic acid which is which is conducted in constant temperature condition. Remember in thermo cycler in PCR we need to raise the temperature, then we need to decrease the temperature that specific temperature control is not needed.

So, what we do not need is the temperature control as well as the thermo cycler. There are multiple techniques for isothermal nucleic acid amplification techniques which we will definitely discussed in next week session. So, using this isothermal nucleic acid amplification devices parasite detection possible over the microfluidic platform where trypanosoma bruce DNA detection is possible. Bacterial detection nucleic acid extraction and amplification is possible, then E. coli detection is possible fluorescence paper based lamp device for mycobacterium tuberculosis g DNA detection is possible.

Viral virus detection is also possible for HPV DNA, then Zika virus different influenza virus and that can be incorporated with smart phone based multiple control devices where this isothermal nucleic acid amplification devices are incorporated for signal generation over the digital platform. So, this is how different POC devices are used in diagnosis of infectious diseases. Coming to the summary then requirement for POC devices in infectious disease is basically coming from the need of initiate a timely treatment for which we need a rapid diagnosis in resource limited regions. So, we need a portable rapid detection platform that is provided by the POC devices. Different molecular techniques are utilized the very common molecular techniques are PCR and isothermal nucleic acid amplification tests.

POC device platforms can be microfluidic devices can be lateral flow devices different cartridge based systems and if you remember which you see in day to day life COVID 19 antigen test, influenza rapid detection, different other parasitic detection they can be possible utilizing this POC devices. So, these are my references these are very good references where different types of POC platforms are well described. So, you can go through the references. Thank you all and see you in the next week class. .