Comprehensive Molecular Diagnostics and Advanced Gene Expression Analysis

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Lecture 36 : Syndromic Panels and Multiplex Assay : Molecular identification of Microorganism

Namaskar. Welcome back. We are starting a new week where we are going to discuss molecular diagnostics in infectious disease. So, this is another important region of comprehensive molecular diagnostics and advanced gene expression analysis. Very first class for this week is syndromic panel and multiplex assay molecular identification of microorganism which is for the infectious disease panel. So, these are the very basic things which we are going to cover the very basic concept of syndromic panel, what are the different advantages, then the different tests, then what are the available different syndromic panels and finally, their limitations.

So, before going to the advantages of syndromic panels, why this syndromic panels are used or what is actually this syndromic panels. So, the infectious disease appears in pool of symptoms. Some syndromes are basically based on the upper respiratory tract infection where running nose, then sore throat, fever, pain in the ears or throats or cough. So, this is a symptomatic pool or syndrome which indicate towards upper respiratory tract infection.

Similarly, lower respiratory tract infection has separate syndromes, separate pool of symptoms, GI symptoms gastrointestinal infection, it comes with another pool of symptoms starting from loose stool or blood in stool, abdominal pain, fever. So, based on this type of common symptoms the approach is to suspect a pool of microbiome. Now what can be the pathogens, what are the pathogens which can be causing these type of symptoms. So, what we do we can check suppose this type of suppose this upper respiratory tract infection can be caused by 10 pathogens. So, how they can be identified? Individually we can check each 10 organisms one by one, but that will take time.

So, what happens we can prepare a mode where simultaneously a rapid detection of all these 10 organisms can be done. So, that is our syndromic panel where based on the syndrome or based on the pool of symptom or rapid syndromic panel based multiplex

assay is designed in such a way that multiple identifying marker of different pathogens are put over those panels or multiplexed. So, how that can help? Of course, that is one comprehensive approach. So, it is going to the comprehensive panel or syndromic panel of multiplex assay is basically highlighting the comprehensive approach towards all the causative organisms or as much as possible the causative organisms can be included. Also it is streamlining the testing process.

So, basically the physicians they are waiting till the actual report which indicating the causative organism and for that time period what needs to be done empirical microbial therapy. That is a general microbial approach antimicrobial approach or antimicrobial therapy is targeted a broad spectrum therapy is given which can cover all positive organism till the exact report or exact causative organism is identified, but what that is causing that is going to raise antimicrobial resistance in the population. So, the streamline testing process not only help in avoidance of the antimicrobial resistance emergence it is also saving time in diagnosis and treatment. Then the syndromic panels comes with improved sensitivity and specificity. Now, in compared to the traditional culture based methods for identification these techniques or these panels are helpful in identifying slow growing or fastidious bacteria or difficult to culture pathogens.

Again these are very simple in testing basically numerous pathogens simultaneously can be checked which is basically reducing the need of multiple testing and simplifying the interpretation. So, these are the advantages of syndromic panels. What are the tests available? So, these are the different test based on which the multiplex assays are prepared. So, the very common one the commonest one is multiplex PCR. Here the multiple target sequences are basically multiplexed and simultaneously amplified in a single reaction tube.

So, in a single reaction the one causative organism or multiple causative organisms can be identified by multiplex PCR. Nucleic acid hybridization test you know that is the hybridization probe based technique based on which the target organism can be identified. Then there is bead based assay. Now, this bead based assay one such example is Luminex technology. So, these are the color coded microspheres which are coated with specific probes that can capture the target nucleic acid and can identify the pathogens.

Then there is immunological assays antigen antibody based techniques ELISA or lateral flow immunoassays that can also be utilized for identification of target pathogen. Also mass spectrometry is available there from the clinical sample directly the proteins of the microorganism can be identified and based on which the microorganisms can also be identified and finally, sequencing. So, next generation sequencing where a comprehensive pathogen detection can be done using the sequencing of the genome. So,

these are the different techniques or principles based on with the complex or sorry the complete multiplex assay is formed. Now, the syndromic panels are basically divided based on the different types of symptom.

So, one such symptom is septicemia where blood stream pathogens are need to be detected as early as possible. Remember septicemia is one very treated condition where the mortality is very high and if delayed if the pathogens are the positive pathogens is properly not identified in that case death rates are very high. So, for that what is required the syndromic testing from the positive blood culture broth are required which rapidly identifies the pathogens or it can also discriminate the common contaminants. Contaminants which are basically the colonization or normal colonizing bacteria which colonizing microbes which can be separated from the actual causative pathogens. It also can detect the antimicrobial resistance gene and within 18 to 24 hours it can give report, but again it has some limitation.

So, in the panel in the multiplex panel remember that is the problem with multiplex panels those come as a pool of pathogens which are previously plated over it or the target markers are previously plated over it. But if there is some off target pathogens which is not whose marker is not included in the multiplex panel this syndromic panel are not able to diagnose those pathogens. Susceptibility information is not a broad one there is limited information regarding that potential false positive results can also be obtained of course, it is one expensive method and the reports of antimicrobial resistant information are coming from variable sources and there is showing variable data. So, at the end traditional testing are still required for getting the complete information. So, even if the syndromic panel is helpful these are the limitations.

For the upper respiratory panels here are some examples of syndromic panels applied bio code respiratory pathogen panel it consists their target common target organisms like body tiller parthesis, chlamydia pneumonia, mycoplasma pneumonia viral panel including influenza A A1H1, 3, 1 2009, influenza B, para influenza virus, human metapneumovirus, rhinovirus, enterovirus. So, this is the panel. So, if either of these organism is positive for causing this upper respiratory tract syndromes it can be identified rapidly, but problem is for off target pathogens. Again Luminex Verigin respiratory pathogen flex test here you can see these are the panels panel of organism the markers are included here. Coming to lower respiratory panel one such lower respiratory panel is BioFire film array pneumonia plus panel.

So, remember I am giving examples of such panel, but multiple FDA cleared panel are available in the market. So, here you can see there is a pool of bacteria virus as well as some resistance gene which are included here. So, methicillin resistance gene, ESBL, CTXM, carbapenemase can be identified KPC. These are the resistance gene which are

incorporated in this lower respiratory panel BioFire's panel and the resistance gene can be identified as well. Then there is GI panel.

So, here you can see applied BioCore GI pathogen panel GPP panel which can give reports within 4 hours and it contains bacterial species, some viral species and also parasites are included here in this GI panel. So, what are the limitations of GI panel? So, some targets of some pathogenic targets which are included they are of questionable significance sometimes. Then positive for multiple targets can the reports can be and why because you know our GI tract is the colonization colonizing area for different virus or other organisms and that can be present in asymptomatic people as well. So, the problem is to identify the exact pathogenic organism which is causing the trouble. Also false positive results can be obtained from low incidence targets like Vibrio cholerae or Entomiba histolyticia.

Then Clostridioid is difficile and this organism has highest rate of colonization in GI tract around 5 to 10 percent in adult and more than 48 to more than 50 percent in children. So, whether this Clostridium difficile is causing the infection or that is only the colonizing bacteria that is hard to identify from this panels. So, here are the advantage that it is rapidly identifying and is helping in starting the exact and appropriate antimicrobial agent. But the problem is that if the specific causative organism is not in the panel that cannot be identified and also it cannot differentiate between the colonization and the pathogenic organism. So, coming to the summary syndromic panel is basically helping in faster detection, it helps in avoidance of antimicrobial resistance emergence.

These are the techniques like multiplex PCR, nucleic acid hybridization, bead based assay, ELISA and lateral flow, immunoassay, mass spectrometry, sequencing these are helping to form the different multiplex based detection in syndromic panels. These are the references. Thank you and see you in the next class.