

Molecular approaches to the diagnosis of infectious diseases

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Abstract- Molecular diagnostics, a combination of laboratory medicine and molecular genetics, has come a long way in understanding the prognosis of disease and effect of therapy to cure it. It is also used to detect and quantify specific microorganisms causing disease. PCR is an important technique used to specifically amplify specific DNA and RNA in molecular diagnostics. Fluorescent in situ Hybridization, Micro array, Capillary electrophoresis and multiple next generation sequencing help in amplifying and mapping out the target proteins and genes, expression of a large number of genes and in prognosis and targeted therapy. Molecular diagnostics also predicts a persons predisposition to different types of cancers. Nanotechnology would help molecular diagnostics to be made cheaper and available to general public in near future.

Index Terms- Disease, Molecular diagnostics, PCR amplification, Therapy

1 INTRODUCTION

“Molecular diagnostics” is defined as a class of diagnostic tests that assess a person’s health at a molecular level, detecting and measuring specific gene sequences in nucleic acids or the proteins they express. Since such tests can identify genotypic changes, they provide information on a person’s predisposition to disease as well as treatment options and likely outcomes. In the field of medical microbiology these tests are used to detect and quantify specific micro organisms.

Molecular diagnostics is a combination of laboratory medicine and molecular genetics and has now become an integral part of our health care system. These tests range from genetic tests which influence cancer treatment to tests which detect genotypes of micro organisms which helps to provide the appropriate antibiotic to the patient. Molecular diagnostic tests help to predict the likelihood of recurrence of cancer, or, to detect the carriers of a mutated disease causing gene, or to determine the microbial strain of a bacterium or virus in an infected patient. Molecular diagnostics are responsible for the discovery of biomarkers and hence in the development of specific treatment targeting these biomarkers. Companion diagnostics, is an new field of molecular diagnostics which identifies whether a specific treatment is likely to be effective for an individual patient [1].

Molecular diagnosis influences various stages of medical care—prevention, detection, diagnosis, and treatment. Molecular methods may be preferred to conventional microbiologic testing in detecting and identifying infectious agents for which routine culture and microscopy may not be adequate and are most appropriate for infectious agents which are fastidious, and difficult to cultivate and identify.

A number of techniques are employed in modern diagnostics to detect and quantify specific DNA or RNA sequences, as well as proteins. One of the most fundamental of those techniques, the polymerase chain reaction (PCR), is used to amplify specific sequences of DNA or RNA. Nucleic acid amplification is used to amplify specific targets which may be present in low concentrations; amplification-based methods such as PCR are perhaps the most widely used molecular diagnostic technique in both research and clinical laboratories. PCR not only detects the presence of the target DNA segment, but also quantifies the amount of target DNA that was originally present in the sample. Quantitative PCR is helpful for understanding the prognosis of disease in a patient and the effect of therapy [2].

Other tests used are in situ hybridization which uses a labeled DNA or RNA strand that hybridizes with the target, complementary sequence and quantifies the target sequence in the sample. FisH stands for Fluorescent in situ Hybridization and it uses a fluorescent probe for identification of target sequence. Microarrays measure nucleic acids and also the expression of a large number of genes. Mass spectrometry is a technology that determines the molecular mass of a charged particle by measuring its mass-to-charge (m/z) ratio. It is used to find and analyze protein based biomarkers, identify and type micro organisms etc. Sequencing is used to map out the sequence of the nucleotides that comprise a strand of DNA. This can be done via capillary electrophoresis or through multiple next generation sequencing (NGS) methods.

Certain molecular diagnostic tests influences therapeutic decision-making and an example is K-ras testing. K-ras is a protein, encoded by the Kras gene that plays a critical role in cell division and differentiation. K-ras mutations produces an abnormal, overactive K-ras protein which are detected in certain cancers such as pancreatic, colorectal, and lung cancers. A patient with a Kras mutation is unlikely to respond to certain therapies in colorectal and lung cancer and disease progression is faster in such patients. Hence Kras mutation

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testing is typically performed for patients with metastatic colorectal cancers.

The high costs of molecular diagnostic tests are a matter of concern and prudent use of these techniques is necessary. New technologies also will bring greater efficiency to medical diagnosis. There has been a great deal of excitement about the promise of nanotechnology to support the development of the molecular diagnostics field. Companies have commercialized technology based on gold nanoparticles that allow increased sensitivity and specificity for molecular diagnostics assays. Nanotechnology also enables multiplexing of assays and innovative ways to sequence DNA. The success of molecular diagnostics depends on the commercialization of rapid, user-friendly, inexpensive and high-quality tests. The new age physician has to necessarily understand the application of molecular diagnostics because medical therapy is becoming increasingly dependent on molecular tests for treatment decisions[3].

References:

[1] F Sidoti , M Bergallo , C Costa , R Cavallo. Alternative molecular tests for virological diagnosis. *Mol. Biotechnol.* 53(3):352-62, 2013. doi: 10.1007/s12033-012-9533-8.

[2] D.P Bora , G Venkatesan , V. Bhanuprakash, V. Balamurugan, M.Prabhu, M.S Siva Sankar, R. Yogisharadhya TaqMan real-time PCR assay based on DNA polymerase gene for rapid detection of Orf infection. *J. Virol Methods.* 178(1-2):249-522011.

[3] S. Belák, P. Thorén, N. LeBlanc, G. Viljoen. "Advances in viral disease **diagnostic** and **molecular** epidemiological technologies". *Expert Rev Mol Diagn.* May 9(4):367-81.-2009.