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Current Scenario of Molecular Diagnostics in Indian Healthcare Sector

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Abstract

After successfully accomplishing the Human genome project and opening new avenues for genome based diagnostics and therapy in healthcare sector, development of personalized medicine and advancing molecular diagnostics has been the prime agenda of scientists all-round the globe. Molecular diagnostics has made possible the diagnosis of the previously undetected viral nucleic acids, early access of data to doctors, a deeper understanding of the disease cause, treatment dose and success of the treatment depending upon the case. It has provided an immense scope of novel and more sophisticated biotechnology and biomedical tools to be employed in the sector procreating a new interdisciplinary field. The gene based testing in all fields has flourished in leaps and bounds after the prediction of >5% in 2005. Here we discuss the current scenario, scope and limitations of the Molecular diagnostics in terms of its significance in public health care.

Keywords: Indian Healthcare Sector; Molecular Diagnostic; scope and limitations**.**

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1. Introduction

In the last three decades there has been a revolution in the field of Biomedical Science as there has been a significant shift in clinical practice and healthcare delivery, which is marked by the completion of Human Genome Project in early, 2001. The successful completion of the project has also laid an upfront challenge to correctly extract information from the encoded human genome sequences which can be utilized to create medicines, vaccines and diagnostics tests. Molecular biological methods for the detection and characterisation of microorganisms have revolutionised diagnostic microbiology and are now part of routine specimen processing. The molecular biology has been a tool to research on the human genes and expressed proteins associated in a pathological pathway in health or in disease. However, it is stated that the research focus has been shifting away from analysis of isolated components to understanding the more complex organization (assembly and regulation) in cells, tissues and organs of components in a particular biological order to achieve integration of different functions for homeostatic coordination of body physiology. This biological organization is being compared for normal and pathological state at each level to create a new approach for the detection and modulation of the disease and thereby developing a design consisting ability to predict and to prevent illness. The biological systems which were comprehended as descriptive are being formulated as mechanistic and diagnostics and treatment are based on definitive knowledge of underlying molecular mechanism of disease causation. Therefore it has provided an immense scope of novel and more sophisticated biotechnology and biomedical tools to be employed in the sector procreating a new interdisciplinary field. Today, new diagnostics methods based on genomic and proteomic profiling of the molecular changes associated with disease are being developed in collaboration with diagnostic profiling of the genetic status of how an individual effects, responsiveness to different treatments has come up increasingly important in therapeutic decisions.This gene based testing in all fields has flourished in leaps and bounds after the prediction of >5% in 2005, molecular diagnostics has changed the perspectives of clinical practices and still poses scope for research with the increasing number of complex diseases worldwide [6].

These tests also can detect and quantify the presence of specific viruses, bacteria, or types of cells. Sequencing the entire human genome is a feat that, when it was first accomplished by the Human Genome Project, took an international, governmental research consortium over 10 years and $2.7 billion. (An initial draft of the entire genome sequence was published in 2001, ten years after the project was initiated, and an essentially complete version was published in 2003).

2. Materials and methods

2.1 Molecular genetic-based approaches to treating disease

Once a human disease gene has been characterized, molecular genetic tools can be used to dissect gene function and explore the biological processes involved in the normal and pathogenic states. The resulting information can be used to design novel therapies using conventional drug-based approaches.

Current molecular diagnostic technologies are based on the amplification of specific DNA sequences from extracted nucleic acids, DNA or RNA. Amplification techniques take tiny amounts of nucleic acid material and replicate them many times through enzymatic reactions, some that occur through cycles of heating and cooling. These include methods that involve target amplification (e.g., polymerase chain reaction (PCR), reverse transcriptase–PCR (RT-PCR), strand displacement amplification, transcription amplification), signal amplification (e.g., branched DNA assays, hybrid capture), probe amplification (e.g., ligase chain reaction, cleavase-invader, cycling probes), or postamplification analysis (e.g., sequencing the amplified product or melting curve analysis as is done in real-time PCR).

Nucleic acid-based methods are generally specific and highly sensitive and can be used for all categories of microbes [4, 7]. Amplification methods can identify minute traces of the genetic material of an organism in a specimen, avoiding the need for culture. These techniques are particularly useful for organisms that are difficult to culture or identify using other methods (e.g., viruses, obligate intracellular pathogens), or are present in very low numbers. Results can be provided more rapidly than through most conventional methods, especially culture. However, because amplification methods are so sensitive, false positives from trace contamination of the specimen or equipment can easily occur.

Microarrays or DNA chips are one of the latest methods for rapid infectious disease diagnostics. Microarrays are a recent adaptation of Northern blot technology [8, 17].

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Viruses cause more infectious diarrhoea worldwide than bacteria and other pathogens. The diagnosis of viral diarrhoeal disease has improved with the development of PCR detection. The method of choice for microbiological diagnosis of rotavirus from stool samples is PCR. Norovirus, a calicivirus formerly known as Norwalk virus and responsible for large outbreaks both in the community and health care facilities, can be diagnosed by electron microscopy, enzyme immunoassay and PCR but PCR is the most sensitive and rapid method. PCR is also the most sensitive method for the diagnosis of astroviruses and enteric adenoviruses (serotypes 40 and 41) [5].

2.2 Molecular Diagnostics used for Ebola Test

Previous year the most contagious Ebola Virus Disease, created a devastating situation of epidemic in the West African region. In order to assist the affected communities the national and international disease control centres

with aid workers worked tirelessly to control the epidemic situation. Ebola, which is a single stranded RNA virus, has five known strains, four of which are pathogenic to humans. Zaire Ebola Virus (EBOV) strain was responsible for the recent outbreak which previously caused 78% of high mortality rate.([1Gire Sk 2014)] According to a recent report of the World Health Organization, approximately 2,600 deaths resulted due to the viral infection for the last several months. Therefore to combat this swelling up of epidemic, clinicians are initiating the utilization of medical diagnostics to rapidly identify the Ebola strains thereby accelerating the quarantine (isolation of infected patients) and subsequent treatment. The current detection methods involve conventional processes including viral culture, electron microscopy (TEM), and antibody-based detection such as immunohistochemistry and ELISA[20].Moreover, the Centres for Disease Control and Prevention are enquiring further the use of probe-based RT-qPCR assays which is a molecular diagnostic test for acute infections. This RT-qPCR detection was previously used for the outbreaks as well as the current crisis as a reliable means to identify the potentially virus-infected individuals.This diagnostic test which is commercially known as "The LightMix^{R} Ebola Zaire test developed by Roche Diagnostics Division has received emergency permission from FDA (US-Food and Drug Administration) for the use of Ebola Test, aiding in the detection of virus and starting the patient's treatment as early as possible.

3. Results

3.1 Limitations of Molecular Methods

Despite significant advantages of molecular diagnostics it cannot yet replace conventional methods for a range of infectious diseases since many common tests performed in the clinical microbiology laboratory are rapid and inexpensive. Advances in conventional technologies have resulted in many rapid antigen tests requiring only minutes for results and the modern automated culture systems allow relatively rapid identification and susceptibility testing. Unlike bacterial culture, which can detect a large number of cultivable bacteria without initially knowing the specific organism responsible, all PCR tests except broad-range PCR can only detect the organism whose DNA is complementary to the primers used. Therefore to cover a similar breadth of possible organisms would require the introduction of inexpensive and simple microarray technologies [19] that are not yet available.

3.2 False Positive and False Negative Results

Another problem restricting the application of molecular techniques to routine diagnosis is that of false positive and false negative results. To avoid false positive results due to laboratory contamination relatively large laboratory areas are required for physical separation of reagent preparation, specimen preparation and product detection areas together with a high level of staff training and skill. Amplicon laboratory contamination can be reduced by ultraviolet light irradiation of reagents and chemical inactivation of surface contamination with sodium hypochlorite. Amplicons can be destroyed by the use of dUTP to replace dTTP for amplification then uracil *N*-glycosylase (UNG) treatment of preassembled starting reactions to destroy the dUTP-containing amplicons. Intersample contamination can be reduced by the use of disposable equipment and cotton filter tips, and using disposable personal protective equipment such as caps, gowns and gloves. Even with scrupulous

technique problems can be encountered, especially with broad-range PCR due to the presence of foreign DNA in the PCR reagents. It is therefore crucial that appropriate negative controls are included in every PCR run to detect any contamination. The advent of real-time PCR has reduced this risk due to single tube PCR reaction and detection systems.

Poor primer design can also lead to erroneously positive results. Primers may be poorly designed such that incidental amplification of microorganisms other than those sought occurs. Also primers are designed based on the known sequences available through international databases but organisms or sequences yet to be discovered can subsequently reduce the specificity of the PCR [12]

In addition,Nucleic acid based techniques depend on enzymatic activity, false-negatives also occur when a sample contains contaminants that inhibit enzyme activity [10].

4. Conclusions

4.1 Scope of molecular techniques in Future

Technologies will continue to evolve, allowing faster, more sensitive and less expensive methods for pathogen surveillance and discovery. Although multiplex PCR is relatively mature, microarray technology is still in its infancy; near-term modifications already in development include microfluidic sample processing and direct measurement of conductance changes associated with hybridization. We have only touched the surface of proteomics and host response profiling. It is conceivable that biomarkers will be found that are specific for classes of infectious agents and/or provide insights that can guide clinical management. In chronic diseases the most substantive advances are likely to come not from technical improvements, but from investments in prospective serial sample collections and an appreciation that many diseases reflect intersections of genes and environment in a temporal context. Rapid development in genetic based molecular diagnostics has made wide application of tools, from basic research to detection of abnormalities in human health, possible. However, the industry faces various challenges in implementing genetic based molecular diagnostic at clinical stage

Methods for cloning nucleic acids of microbial pathogens directly from clinical specimens offer new opportunities to investigate microbial associations in chronic diseases [14]. Microarray and gene chip assays, first published in 1991, have the advantages of miniaturization and automated construction using industrial robots together with sensitivity and rapid reading of large amounts of detailed genetic information.

Commercially, initial efforts focused on applications of microarrays for use in detecting drug resistance and mycobacterial identification but the biotechnology companies are now assessing the market for molecular diagnosis using microarrays in the infectious diseases laboratories. For example, Affymetrix produce GeneChip microarrays for pathogen identification, virulence factor identification, pathogen response to drugs, and vaccine development. However, these companies will need to compete with the multiplex real-time PCR kits becoming commercially available such as those produced by Prodesse for the detection of the common respiratory pathogens.

Over the past decade, the application of molecular pathogen discovery methods resulted in identification of novel agents associated with both acute and chronic diseases, including Borna disease virus, hepatitis C virus, Sin Nombre virus, HHV-6, HHV-8, *Bartonella henselae*, *Tropheryma whippelii*, West Nile virus, and SARS coronavirus [1, 2 ,3,11,13,15,16, 18].

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