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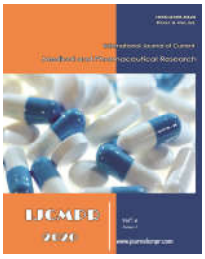

Research Article

PREVAILING AND CROP UP TECHNOLOGIES FOR THE DIAGNOSIS OF MICROBIAL INFECTIONS

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ABSTRACT

There are almost as many ways to look at the world of infectious disease as there are infectious agents. India is a densely populated tropical country with burden of very high. infectious diseases. Cultures and serological assays are usually used for microbial identification in infectious diseases. However culturing and isolation of fastidious pathogens which are not always available can be difficult and may take weeks or months to yield results. On the other hand, culture alone can't distinguish colonization from tissue invasion. Serological tests can be difficult to interpret in the setting of immunosuppression or when only a single sample is available for evaluation.Hence the pivotal role of molecular diagnosis is indispensible in Evidenced-based Laboratory Medicine.The panel-based molecular diagnostics for the rapid detection of pathogens have resulted in thinking about something changes completely in clinical microbiology and clinical practice. These Panel based tests are valid and reliable with rapid turnaround time and the detection of a large number of microorganisms and test interpretation. To observe fastidious organisms like Brucella, in sepsis/ PUO, in traditional blood cultures requires a longer time.CRP/PCT helps in presumptive but not in definitive diagnosis whereas in syndrome panel-based Microarray results can be obtained within an hour. These are fast and sensitive. The film Array blood culture identification panel (Biofire), the range now covers 4 major syndromes like respiratory infections including pneumonia, gastrointestinal infections, Meningitis/Encephalitis, and Septicemia. Sputum cultures are not very sensitive in molecular diagnostics in respiratory diseases and difficult to obtain samples in the pediatric population. Similarly, viral infections cannot be diagnosed using traditional methods. Histological examination, more sensitive, cannot identify the responsible fungi. The Galactomannan for fungal pneumonia needs to be done serially and prone to false-positive results. In order to deal with menace of drug-resistant bacteria, give effective treatment.LIPA (Line probe assay) molecular hybridization tool to diagnose non-tubercular mycobacterium, differentiation within MTB complex and drug susceptibility testing (Identification of resistance genes) India is a hot spot for the emergence of viral diseases. Nipah virus is a zoonotic virus and can be transmitted through contaminated food or directly between people. The molecular diagnosis helped to identify the pathogen. The new molecular test should provide reliable, cost-effective and timely results necessary for diagnosis and management. Test selection should not be based solely on cost. Performance characteristics and impact on patient care and management are critical components of the decision.

INTRODUCTION

Diagnostic assays for detection and monitoring of virus infections have become more and more important and are widely used in routine diagnostics. In particular, the detection of newly emerging infectious diseases is challenging. In addition, travel-associated diseases caused by dengue viruses, chikungunya viruses, influenza viruses, or other viruses draw the attention of physicians. Therapeutic treatment regimens and prevention strategies can also influence the need for diagnostic assays, such as, in the case of the human immunodeficiency virus (HIV) and human papillomavirus (HPV). Moreover, well-known virus infections, such as hepatitis E virus infections, can gain new clinical relevance. (1)

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Immunohistochemistry is especially useful in the identification of microorganisms that are present in low numbers, stain poorly, are fastidious to grow, are non-cultivable, or exhibit an atypical morphology. Finally, it is important to remember that there may be widespread occurrence of common antigens among bacteria and pathogenic fungi and both monoclonal and polyclonal antibodies must be tested for possible cross-reactivity with other organisms.(2)

A primary role for diagnostics is to identify disease and enable management of the individual patient. Nucleic acid–based technologies have enhanced the diagnosis of bacterial and viral infections as the result of increased test sensitivity and rapid turnaround time (3).

A number of potential failures in testing and communicating results to patients were identified, and some specific ideas for improving existing systems emerged (4).

The implications of current trends in molecular infectious diseases are moving towards high-throughput, simple, array-type technologies that will provide a wealth of data regarding types of organisms present in a sample and the virulence factors/resistance determinants that influence the severity of disease. As a result of these developments, infectious diseases will be more accurately and effectively treated (5)

The diagnostic procedures for periodic fever syndrome is used in laboratories are highly useful. (6)

Early diagnosis of tuberculosis and drug resistance improves survival and identifying infectious cases.(7)

In a short time, nucleic acid tests have profoundly affected the management of infectious diseases. In contrast to microbial culture methods, molecular methods are rapid, thus allowing early decisions about treatment to be based on data about the pathogens in an individual patient (8).

Detection of Chlamydiae trachomatis is a challenging and important public health issue. Chlamydiae trachomatis is a major cause of genital infection with an estimated one million cases occurring annually among sexually active adolescents and young adults in the US. More than half are asymptomatic(9)

Anorectal infections of men who have sex with men (MSM) should include evaluation of lymphogranuloma venereum (LGV) by identification of genotypes L1, L2 or L3. Detection of CT antigens by enzyme immunoassay (EIAs) or rapid diagnostic tests (RDTs) are unsuitable due to insufficient sensitivity and specificity. (10)

Gonococci is a fastidious organism and is highly susceptible to extreme temperatures and desiccation which can lead to a decreased sensitivity of culture, particularly, when specimen transport is required before culturing (11)

In the past tuberculin skin test (TST) was used to detect host response but the recent development of FDA approved interferon-gamma release assays(IGRASs) has provided an alternative method for detecting immunologic response to M.tuberculosis antigens. IGRA may be used in the evaluation of patients for latent or active TB (12)

In general, the sensitivity of the IGRA is high and comparable to TSTs.IGRA results will come in 24 hrs. Prior ECG vaccination, does not cause a false-positive reaction in IGRA. Amplified nucleic acid techniques (NAAT) are the most sensitive tests for detection of common sexually transmitted infections *Chlamydia trachomatis, Neisseria gonorrhea, T.vaginalis* in urine and urogenital specimens. Culture techniques for detection of these pathogens require special culture, long turnaround time and transport conditions that are often not realized in clinical practice (13)

Since 1980s, immunohistochemistry (IHC) has dramatically transformed the approach to histopathologic diagnosis, specifically in the diagnosis and classification of tumors, and more recently in the diagnosis of infectious diseases in tissue samples (14)

An important role for molecular assays is in the diagnosis of extra-pulmonary MTb infections, such as meningitis, pleuritis or peritonitis, because these infections can be very difficult to diagnose by traditional methods.

The Mycobacterium tuberculosis direct test (MTD) and Amplicor tests have been used on CSF specimens for the diagnosis of MTb meningitis (15)

Molecular diagnostics demonstrated higher sensitivity for pulmonary TB detection in smear-positive specimens. Xpert MTB/RIF, LAMP, LPA, CPA and PCR demonstrated high accuracy overall for pulmonary tuberculosis detection, while SAT-TB had poor performance.(16)

Diagnosis of TB has entered an era of molecular detection that provides faster and more cost-effective methods to diagnose and confirm drug resistance in TB cases, meanwhile, diagnosis by conventional culture systems requires several weeks. New advances in the molecular detection of TB, including the faster and simpler nucleic acid amplification test (NAAT) and whole-genome sequencing (WGS), have resulted in a shorter time for diagnosis and, therefore, faster TB treatments.(17).

Individuals who have cleared the HCV infection will not have detectable RNA, while those with persistent infection will have detectable RNA. Recent data from the CDC have shown that 95% of positive results of screening ELISA that have more than 3.8 can be confirmed as true positives (18)

Molecular tests for an infectious disease should provide reliable, cost-effective and timely results necessary for diagnosis and management. Test selection should not be based solely on cost. Performance characteristics and impact on patient care and management are critical components of the decision. New tests can improve patient care and decrease its cost as in use of genotyping of HIV-1 to detect drug resistance(19)

Until recently, the standard method for the diagnosis of cervical cancer was the detection of abnormal cells by cervical cytology (Pap smear) or Biopsy. The morphological changes associated with HPV infection include atypical squamous cells of undetermined significance (ASCUS), the lowgrade squamous intraepithelial lesion (LIEL) and high-grade squamous intraepithelial lesion (HSIL) (20,21)

Current recommendations are to test all women with ASCUS for the presence of high-risk HPV. Those women testing positive for high-risk HPV DNA should be referred coloscopy, while those patients testing negative for HPV DNA can be followed according to routine practice (22)

Chronological record of significant events

ELISAs were first developed in the early The 1970s as a replacement for radioimmunoassay (23)

The 2002 Nobel Prize winner in physiology, Sydney Brenner, has developed a technique whereby a million cDNA sequences can be affixed on separate beads and amplified for 16 to 20 base pairs simultaneously in a flow cell (24)

Recent advances in the field of biological science have sparked new interest in the area of cancer biomarkers. The sequencing of the human genome has provided fundamental structural information about all human genes. The cDNA microarrays and biological mass spectrometry, has allowed thousands of measurements to be performed in short periods of time (25)

Massively parallel genomic sequencing became an accessible laboratory tool from 2005 (26)

Despite considerable research, moving from biomarker discovery to clinical application has presented unique challenges (27)

Since the 1980s, an enormous advancement in molecular technology has dramatically influenced the diagnosis and study of infectious diseases. The application of molecular probes to the study and diagnosis of infectious diseases is a great adjunct to Immunohistochemistry as a diagnostic method and often allows for even more rapid and specific identification of organisms (28,29)

Many viruses, bacteria, and other microorganisms can be localized in tissues by HIS (*in situ* hybridization);these include *Epstein Barr virus,* HPV, *Legionella, Haemophilus influenzae, Polyomaviruses, and Mycobacterium leprae* (30,31,32)

PCR has the advantage of increased sensitivity, minimal tissue requirements, and potential sequencing of the amplified product for specific identification of the microbial genotype (33,34,35)

Literature Gap and Future Research

Modern medicine has become very expensive. It is going to be still more so with new technology invading medical diagnosis and management. It is estimated only a small percentage of the world population has access to modern medicine. In general cultures are considered the gold standard for detection, however testing typically takes place 24-48 hours for completion and longer for fastidious pathogens like anaerobes or slow-growing organisms like mycobacterium. Direct antigen detection assays are widely available and provide quick turnaround time; however, they often have low sensitivity. Molecular methods are becoming common in detecting pathogens due to the high sensitivity and shorter turnaround time than conventional cultures, yet these assays are currently costly.

Research Program for Next Generation World

The molecular revolution is here and is on going.The number of molecular applications for the detection and charectyerization of all types of microorganisms increasing each year, with many commercial venders seeking FDA approval for their applications. Motile parasites of Trichomonas vaginalis, Clue cells in Bacterial vaginosis, Budding yeast cells in Candida infection seen by potassium hydroxide mount. Darkfield microscopy for *Treponema pallidum. N. gonorrhea* in chocolate agar and selective media (like lyses blood agar with specific antibiotics).Identification can be done by colony morphology, staining, oxidize test. Antibiotic testing must be done since antibiotic resistance is reported in gonococci. Candida can be cultured in Sabouraud's dextrose agar Enriched gonococcus agar and enriched Muller Hinton agar can be used for culture of *H. ducreyi.* Tissue culture on Mc Coy, Hep 2 and Hela cell lines for *Chlamydia trachomatis*. Human diploid fibroblast, Vero cell line can be used for herpes viral infection if available. Diamond's Trichomonas medium can be used for *T. vaginalis.*

Nucleic acid testing had a tremendous impact on the practice of infectious diseases. These tests are used in a variety of ways, including diagnosis of pathogens that do not grow using conventional methods or grow very slowly, monitoring response to therapy, assessing the risk of diseases development and determining disease prognosis. In general cultures are considered the gold standard for pathogen detection; however, testing typically takes place 24-48 hrs for completion and longer for pathogens like anaerobes or slow-growing organisms like *Mycobacterium tuberculosis.* Direct antigen detection assays are widely available and provide quick turnaround time than conventional cultures, yet these assays are currently costly. Molecular diagnostics are landmark developments in laboratory science. The molecular diagnostic is used to analyze biological markers in the genome and proteome by applying molecular biology to medical testing..The methods are fully automated as they are rapid comprehensive with easiness with interpret and report. Minimal consumables. Freeze-dried reagents. The main disadvantages are kits are not available outside the USA.

Timeline of scientific discoveries

Molecular genetic testing is a diagnostic discipline in the clinical laboratory. Molecular diagnostics can also be referred to as biotechnology. Since the complete human genome sequence becomes available in 2003, molecular genetic testing has been expanded extensively. Even with high standardized methods, these tests are susceptible to laboratory errors as any other laboratory procedure. Molecular diagnostics has advanced in precision, accuracy, speed, detection, and cost. Applications range from detection of infectious diseases to cellular and tissue antigens. Molecular diagnostic testing using nucleic acid-based assays provides rapid and accurate diagnosis and identification of infectious diseases that previously involved a long waiting period for pathogen identification. Past challenges such as contamination, have become less of a problem because of the use of new techniques **(36)**

Nucleic acids are the critical molecules of life. Nucleic acid analysis includes electrophoresis hybridization assays, and amplification techniques, sequencing and polymorphism detection. Complete assays or diagnostic tests often combine several of these techniques, such as amplification, electrophoresis, and hybridization. Nucleic acid hybridization is a fundamental concept in nucleic acid biochemistry. It is defined as the interaction between the single stranded nucleic acid molecules to form a duplex (double stranded) molecules based on the complementary base pairing of their respective sequences.(37)

When hybridization reaction is used to analyze the nucleic acid content of an unknown sample, the process is known as hybridization assay and the specificity of hybridization reaction is described as probe**)** The polymerase chain reaction(PCR) is an in vitro method that amplifies low levels of specific DNA sequences.PCR analysis can lead to the detection of gene mutations, identification of viral DNA associated with specific cancers, and detection of genetic mutations. In addition the advanced labs also doing probe hybridization assays, Southern blot techniques(Eg Sickle cell anaemia and Hemophilia A) Northern blot for specific mRNA. Proteis are separated by electrophoretically, transferred to membranes and identified through labeled antibodies. It is used to detect antibodies to specific epitopes of an antigen subspecies and confirm the specificity of antibodies detected by ELISA screening.

Micro arrays (DNA Chips) are the products of bonding or synthesis of specific DNA probes on a stationary support. These chips are used to examine gene activith and identify genetic mutation in malignancies, test for genetic disease and detect virally resistant mutations. In situ hybridization is a specialized type of solid support assay involves taking morphologically intact tissue,cells or chromosomes afixed to a glass microscope slide through the hybridization process. Inaddion, polymorphism detection assays are also available in advanced laboratories. 99% of human genome identical in all individuals, there are still millions of regions of DNA that vary between individuals. Besides PCR, many other methods for amplification have been developed. Transcription mediated amplification (TMA) is modeled after the replication of retro viruses. Ligase chain reaction (LCR), Strand displacement amplification (SDA) and loop mediated amplifications (LAMP),etc are also currently available. Whole genome, transcriptome or exome amplification, branched chain signal amplification, signal probe amplification techniques, Rolling chain amplification methods are also used in molecular diagnostics**(38)**

The use of genetic typing of microbes to demonstrate relatedness among microorganisms continues to provide useful information regarding the transmission of infectious diseases.

Current altercation.

The diagnosis of fungal diseases is now shifting to sensitive assays for the direct detection of fungal antigens in clinical specimens and in biologic specimens and in biologic fluids, often leading to same day results.

Most of the advances in diagnostic clinical Parasitology over the past five years involve the introduction of new serologic techniques for the detection of antibodies and antigens in blood and other fluid secretions. Enzyme immunoassays (EIAs) and indirect and direct hem agglutination inhibition (IHA) assays, indirect and direct fluorescent assays and complement fixation constitutes the spectrum of procedures used in longer clinical and reference laboratories.

Application of molecular biology into diagnostic Parasitology or to use Persing's sound bite from Trenches to Benches is an exciting evolution coming from with in the research laboratories

It has been shown that biopsy specimens with anti-*T.pallidum* polyclonal antibody is more sensitive and specific than silver staining methods,with sensitivities ranging from 71% to 94%**((39)**

Identification of M.tuberculosis is routinely achieved by acid-fast bacilli(ASB) staining, culture of biopsy specimens,or both.Neverthless AFB staining has low sensitivity, and it is not specific because it does not differentiate mycobacterial species**(40)**

Molecular methods have had a significant impact on the diagnosis of viral infections because of their superior sensitivity and rapid turnaround time compared with conventional diagnostic methods.

Molecular diagnostics can also be referred to as biotechnology. Last two decades has witnessed phenomenal advances in the field of medicine following revolutionary changes taking place in the application of technology and as a consequence, Biochemistry and Microbiology have evolved as the most important branch of evidence based medicine. Even with high standardized methods, these tests are susceptible to laboratory errors as any other laboratory procedure. Molecular diagnostics has advanced in precision, accuracy, speed, detection, and cost. (41)

Efforts to detect disease early can always be accompanied by unintended harms. New diagnostic tests: more harm than good,**(42)**

Immunohistochemical methods have the advantage of providing rapid and specific identification of several fungi, that frequently results in cross-reactivity with polyclonal antibodies and even with some monoclonal antibodies.

Therefore assessing crossreactivity using a panel of fungi is important in the evaluation of immunohistochemical methods **(43)**

Microarray assays save time while cutting costs in biomedical diagnostic application and research. Working with smaller volumes reduces reagent consumption, improved reaction kinetics. Over the last several years there has been a remarkable decrease in error rates. Array technology provides a unique and powerful approach to screen a sample for a dozen to thousands of genes. Current array fabrication allows for diverse platforms with better detection and reduced cost for high-density applications. A false-negative result may be caused by inhibition of or decreased efficiency of amplification, and proper controls. Insufficient sample, inappropriate specimen type inappropriate timings of sample collection and degradation of nucleic acid during transport and handling are other sources of false-negative results.

Recent advances in diagnostic technology

Biomarkers are used ubiquitously as indicators of biological health. The development of genomic and proteomic multiplex technologies have enormously amplified biomarker discovery and application to diagnostic and therapeutic decisions in clinical practice. New technologies are now available that simultaneously identify a wide spectrum of biomarkers and save time and costs. Multiplexed assays can be coupled to other disease specific indicators (i.e., cytokines, single nucleotide polymorphisms) in order to get more powerful information. However, there is an urgent need for validation/standardization of the new assays before they are adopted into clinical diagnostics. It is worthy to note a new assay, T cell interferon gamma release (TIGRAs), which has

recently been introduced in the diagnosis of latent tuberculosis infection (44)

Discovery of Bio markers

Bio marker discovery is a medical term describing the process by which bio markers are discovered. Many commonly used blood tests in medicine are bio markers. There is interest in bio marker discovery on the part of the pharmaceutical industry; blood-test or other bio markers could serve as intermediate markers of disease in clinical trials, and as possible drug targets.

Bio markers and Molecular Diagnostics

Biomarkers provide a dynamic and powerful approach to understanding the spectrum of neurological disease with applications in observational and analytic epidemiology, randomized clinical trials, screening and diagnosis and prognosis. Defined as alterations in the constituents of tissues or body fluids, these markers offer the means for homogeneous classification of a disease and risk factors, and the can extend our base information about the underlying pathogenesis of disease. Bio markers can also reflect the entire spectrum of disease from the earliest manifestations to the terminal stages (45)

Furthermore, the implementation of these advanced molecular diagnostics tools for discovery, quantification and validation of molecular biomarkers will be presented. Since 2004 the history of this conference series was primarily focused on quantitative PCR (qPCR) related techniques and applications, but since 2010 the focus was broadened to digital PCR (dPCR), next generation sequencing (NGS) and the underlying complex data analysis applying bioinformatical tools. This history is nicely summarized by the BDQ editors in one of the previous editorials 'qPCR, dPCR, NGS – A journey'(46)

Today, biomarkers have immense scientific and potential clinical value in the diagnostic testing pipeline. They span the broad diagnostic sector from the genome to the phenome over various '-ome' levels and have been used since the earliest days of the application of molecular biology. A biomarker signature is capable of revealing specific biological traits or measurable physiological changes, according to a disease status, physiological or pathological condition, or after drug application(47)

As novel gene-based diagnostics proliferate, they will be increasingly important to drug development, approval and later in clinical practice. There are numerous promising singular biomarkers or more complex multiple biomarker signatures available, the most important of which are currently used for assessing drug development, patient stratification or measuring the efficacy of treatment in therapeutic medicine. Clearly there is a translation problem to transfer the results from molecular diagnostics research to drug development and finally clinical practice(48,49)

The first goal in the biomarker development pipeline is the generation of reliable biological data from applied diagnostic techniques and applications. Therefore, an international consortium of scientists, working in various fields of nucleic acids molecular diagnostics established working guidelines for qPCR and dPCR (50,51)

Challenges

Some of the challenges may be different in interpreting other microbiological tests such as culture and serology. Assessment of the accuracy of molecular methods is challenging when, as often happens, the methods have lower detection limits than the established methods **(52)**

Molecular assays available for detecting influenza virus infection include rapid molecular assays, Reverse Transcription-Polymerase Chain Reaction (RT-PCR), and other nucleic acid amplification tests. These tests can detect influenza viral RNA or nucleic acids in respiratory specimens with high sensitivity and high specificity.(53)

False-positive results can also occur as the result of carryover contamination of amplified products. This is not a problem with signal amplification methods, but can be of significant concern for target amplification methods, such as PCR, nucleic acid sequenced based amplification (NABA) transcription-mediated amplification(TMA) and strand displacement assay(SDA)Cross-contamination of clinical specimens with target DNA during specimen collection,transport and processing can occur with any method. Strict attention to good laboratory practices is needed to minimize the risk of cross-contamination (54)

"Our technical capabilities are exceeding our ability to apply them effectively and economically to human problems".Dr Raymond Barltt. (Known for developing Barlett's criteria for sputum Gram staining. Molecular diagnostic techniques are very sensitive and the interpretation might get tough when more sensitivity comes at the price of specificity.

An opinion arrived at through a process of reasoning

Diagnosing diseases and disorders requires highly developed skill on the part of the physician or other medical professional .Usually the diagnosis calls for systematic use of standard equipment with sop's quality control regularly with diagnostic kits and reagents.

Quality systems are the mainstay of clinical laboratory management. The comprehensive laboratory testing process must be continually monitored and evaluated to ensure reliable test results and set the foundation for quality improvement. While such efforts have resulted in significant improvements in many of the processes, errors still occur. In order to implement corrections and improve the testing process, the laboratory technician must identify the various sources of errors.

Last two decades has witnessed phenomenal advances in the field of medicine following revolutionary changes taking place in the application of technology and as a consequence, Biochemistry and Microbiology have evolved as the most important branch of evidence based medicine.

The quality of any laboratory test result is dependent on many variables, It begins with skill, and knowledge when preparing the patient and specimen are essential to the provision of the highest quality standards for testing and services. The patient must first be properly prepared so that the best possible specimen can be collected. Next, the actual collection of the specimen must be completed. Then, the specimen should be properly processed, packaged and transported to the laboratory in a timely manner and under environmental conditions that will not compromise the integrity of the specimen. After all of these activities take place, a quality analysis can be performed.

Molecular diagnostics has advanced in precision, accuracy, speed, detection, and cost. Applications range from detection of infectious diseases to cellular and tissue antigens. Molecular diagnostic testing using nucleic acid-based assays provides rapid and accurate diagnosis and identification of infectious diseases that previously involved a long waiting period for pathogen identification. Past challenges, such as contamination, have become less of a problem because of the use of these new techniques. Developing countries are expanding their use of molecular diagnostics in HIV diagnosis and viral load testing. In cancer detection and multidrug testing is still considered an immature industry. A clinical laboratory is a place of translation of these insights into effective health care.

A shortened version of a larger work

The implications of current trends in molecular infectious diseases are moving towards high-throughput, simple, array-type technologies that will provide a wealth of data regarding types of organisms present in a sample and the virulence factors/resistance determinants that influence the severity of disease. As a result of these developments, infectious diseases will be more accurately and effectively treated.(55)

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