Proceedings of National Conference
held on 6th -7th January 2012 on
'Biotechnology in Diagnostics'

Organized by

Department of Biotechnology and Microbiology,
Vidya Prasarak Mandal's
B. N. Bandodkar College of Science,
NAAC reaccredited 'A' grade
Best College Award, University of Mumbai

Edited By

Mrs. Jayashree Pawar
Assist. Prof. Dept of Biotechnology and Microbiology,
B. N. Bandodkar College of Science, Thane

Advisory Panel:

Dr. Siddhan Subramanium

Dr. Sudhakar Agarkar

Dr. P. M. Kelkar

Dr. V. V. Bedekar

Dr. Meghana Joshi

Dr. Kalpita Mulye

Convenor:

Dr. (Mrs.) M. K. Pejevar

Program Committee

Teaching Staff	**Non-teaching Staff**
Dr. (Mrs.) M. K. Pejevar	Ms. Priyaka Manore
Dr. Vijaya Srinivasan	Mrs. Vasudha Shetye
Dr. Meghana Joshi	Mr. Sachin Adivarekar
Dr. Kalpita Mulye	Mr. Sunil Babar
Mrs. Jayashree Pawar	Mr. Jitendra Khairnar
Mrs. Nilambai Daripkar	
Ms. Rajitha Radhakrishnan	
Ms. Sonal Mathias	
Dr. Ashwini Pande	

From the Convenors desk

Biotechnology and Microbiology departments are comparatively younger departments of our college. In comparison with other colleges of University of Mumbai we were late to enter in the field. But with the inception of this department, we started with many innovative methods for students' interaction.

We run a movie club in which short movies are shown to the students which help in understanding the subject better. Students also get acquainted with the working environment of laboratories.

Students are given small research projects, due to which the students get acquainted with the research methodologies and get training in the laboratory techniques.

We run a journal club where the students have to go through the research journals including journals like Lancet and have to discuss the research papers published in the journals. This not only gives a deep insight, but also introduces the research aptitude in students.

With these practices we also thought of conducting the National Conference on a very important topic 'Biotechnology in Diagnostics'. Keeping in mind the field of human health which is dependent on new Biotechnological tools, the conference will be helpful for the students to get a chance of interaction with the experts in the field. This will help students to choose their path in the research career if they wish to.

I am very happy as a convener of the conference and principal of the college to hand over these proceedings in your hands.

Dr. (Mrs.) M. K. Pejavar
Principal,
B. N. Bandodkar College of Science, Thane

Messege From The Department In-Charge

Our Biotechnology and Microbiology departments are fairly new and I am glad to be a part of it. The chairman of Vidya Prasarak Mandal and the Principal encouraged me to put my ideas into practice such as journal club, movie club, guest seminars and I hope the students will benefit by it in the coming years.

This is the first national-level conference organized by our department and we hope to engage in many more such initiatives that will bring together academicians, scientists, students and industry professionals at our VPM campus.

The diagnostic market in India is one of the most profitable markets in the world. The market will continue to grow in the coming years with increasing disease prevalence and patient awareness. We have players innovating in diagnostics ranging from point of care diagnostics to basic research supporting diagnostics.

I hope this conference exposes the students to the field of diagnostic research and creates enough interest for them to pursue it to later on commercialize diagnostic technology.

Dr. Meghana Joshi
In- Charge,
Department of Biotechnology and Microbiology.
B. N. Bandodkar College of Science, Thane

Editor's Note

This publication contains the Conference Proceedings of the two-day conference on 'Biotechnology in Diagnostics' held at B. N. Bandodkar college of Science, Thane, on January 6-7, 2012.

On behalf of VPM's B. N. Bandodkar College of Science, I take this opportunity to welcome all the Honourable speakers and participants for this conference on 'Biotechnology in Diagnostics'

Over the years, B. N. Bandodkar college of Science, Thane, has been known to pioneer efforts to provide innovative programs in newer domains of Biotechnology. This conference is another effort to inculcate research aptitude in the students.

The theme of Conference is 'Biotechnology in Diagnostics'. Many eminent personalities from all over India, working on different aspects of diagnostics from Academia, Industry and Research Institutions, will be presenting recent breakthroughs in diagnostics. The conference aims to open a dialogue amongst these eminent personalities and the participants.

One of the academic objective of the conference is to give the students, an insight of the research field. Two preparatory workshops have been conducted as a part of the conference on 24th September and 26th November 2011 at B. N. Bandodkar College of Science, Thane. We have tried to impart the basic technical information about the subject to the students, which will widen their understanding of today's conference theme.

This conference also includes poster presentations by undergraduate and post graduate students. We aim to give them a platform to interact with the scientific community and prepare them for the competitive world which they will be facing in near future. We got an overwhelming response from students for poster presentations.

I request all of you to actively participate in this valuable discussion. I am sure that at the end of the second day we will have ample vital information as output of this conference for academic, industrial and research reference.

I am grateful to Vidya Prasarak Mandal, Our Principal Dr. M. K. Pejavar and our Department in-charge, Dr. Meghana Joshi for giving me this opportunity to work on the conference proceedings. Last, but not the least, I would like to thank my Departmental collegues, and the non- teaching staff for their immense support.

Mrs. Jayashree Pawar
Assistant Prof.,
Department of Biotechnology and Microbiology.
B. N. Bandodkar College of Science, Thane

Table of Contents

SECTION I
INAUGURAL SESSION

Molecular medicine in the "Omics" era

Hon'ble Dr. Bharat B. Chattoo

Founder Vice Chancellor of the Shri Mata Vaishno Devi University, Jammu.
Director, Genome Research Centre
Department of Microbiology and Biotechnology Centre
M. S. University of Baroda, Vadodara – 390002 (Gujarat), India

Recent advances in genomics have made it possible to analyse various aspects of genome structure and dynamics in much greater depth. It is now possible to simultaneously monitor the expression of a large number of genes and to study the interaction among various proteins that are critical to many cellular processes, using various tools involving judicious use of genetic systems and advances in analytical methods. Information now available from various genome sequencing projects is leading towards an increasingly improved understanding of the genome architecture of various organisms and is providing new insights into the evolutionary relationships among various species. The documentation of genetic polymorphisms in humans is leading towards a better understanding of how different genetic differences might influence predisposition to disease and response to various drugs. This opens up new avenues for molecular diagnostics and identification of novel targets for drug development on the one hand and towards development of personalized medicine on the other. Some of these tools are also making it possible to study the vast majority of microorganisms that have not been possible so far to cultivate in culture. This is providing new insights for the study of the microbiome and its implications for human health. One of the major challenges that the availability of huge amounts of data from various "Omics" projects poses is to develop innovative methods of analysis to handle large data sets and to design experiments so that the biological significance of various processes can be studied experimentally. Understanding of how the instructions from the genome and epigenome are modulated in various cellular processes within a cell also promises to be an exciting area of future work.

Monoclonal Antibodies in Cancer Diagnosis and Therapy

Prof. S. V. Chiplunkar

Principle investigation, Advanced centre for treatment, research, and education in cancer, Tata Memorial Centre, Kharghar, Navi Mumbai - 410210 India

The potential for using antibodies as 'magic bullets' against cancer has been alluring investigators since Kohler and Milstein described the making of monoclonal antibodies. These are invaluable tools in research, medicine, diagnosis and treatment of diseases. The monoclonal antibody market has experienced explosive growth since the process for creating monoclonal antibodies was introduced. More than fifteen therapeutic monoclonal antibodies have been approved, several of which have reached blockbuster status. Numerous diagnostic monoclonal antibodies have also been approved, making this one of the fastest growing fields in biotechnology. Today, the industry is working as furiously as ever to perfect the design and production of monoclonal antibodies for therapeutic, diagnostic, and other purposes. The recent clinical and commercial success of anticancer antibodies such as rituximab and trastuzumab has created great interest in antibody-based diagnostic and therapeutics for hematopoietic malignant neoplasms and solid tumors. Radioimaging also known as radioimmunoscintigraphy uses radionuclide labeled antibody or antibody fragments to diagnose tumors in cancer patients in an antigen dependant manner. Several monoclonal antibody based ELISA kits are available in the market for diagnosis of colon, breast, and prostate cancers. Immunophenotyping of leukemias and lymphomas using monoclonal antibodies are now in routine practice for diagnosis and prognosis of the disease.

In cancer therapy, the purpose of antibody administration is to induce the direct or indirect destruction of cancer cells, either by specifically targeting the tumor or the vasculature that nourishes the tumor. Traditionally monoclonal antibodies have been produced as native molecules in murine hybridoma lines. The development of genetic engineering has been central to the clinical use of antibodies. This technology has allowed the conversion of existing mouse monoclonal antibodies into mouse-human chimerized antibody and humanized reagents where only the antibody complementarity determining regions (CDR) are of murine origin. More recently, the production of fully human monoclonal antibody has been made using phage display technology or transgenic mice. The 'humanized' antibodies have facilitated improvement in the therapeutic potential of monoclonal antibodies as these are less immunogenic, more effective, and do not evoke a human anti-mouse antibody response (HAMA).Therapeutic antibodies have become a major strategy in clinical oncology owing to their ability to bind specifically to primary and metastatic cancer cells with high affinity and create antitumor effects by complement-mediated cytolysis and antibody-dependent, cell-mediated cytotoxicity (naked antibodies) or by the focused delivery of radiation or cellular toxins (conjugated antibodies).

SECTION II
INVITED TALKS

Copy Number Variations as new tools for molecular diagnostics

Dr. Anu Ghosh

Low Level Radiation Studies Section, Radiation Biology & Health Sciences Division, Bio-Medical Group, Bhabha Atomic Research Centre, Mumbai – 400085
Email: anugh@barc.gov.in

Copy Number Variations (CNVs) are now recognized to be a highly prevalent form of genetic variation in humans. These can be microscopic or submicroscopic and result when the number of copies of a particular DNA segment shows difference between individuals. CNVs may involve either copy number gains (insertions/ duplications) or losses (deletions/ null genotypes). *De novo* locus specific mutation rates for CNVs are estimated to be in the range of 1.7×10^{-6} to 1.0×10^{-4} per locus per generation which is 100-1000 times higher than nucleotide substitution rates. CNVs may represent benign polymorphic variations or convey clinical phenotypes either by disrupting genes or by altering gene dosage. Inherited and *de-novo* CNVs have increasingly being linked not only to sporadic birth defects, sporadic and mendelian diseases but also to complex human traits such as susceptibility to Alzheimer, Parkinson's, mental retardation, cleft lip/palate, autism, schizophrenia, HIV infection and anatomical deformaties. Germline CNVs have been implicated with increased cancer risks including neuroblastoma, breast and prostrate cancers. Numerous genome analysis platforms have been used for the identification of CNVs including single nucleotide polymorphism (SNP) genotyping platforms, array comparative genome hybridization (aCGH) and next generation sequencing (NGS) platforms. Over 41% of all CNVs identified overlap with known genes, emphasising the important role played by them in modulating expression of genes. CNVs have thus, opened up new analytical avenues for clinical cases which had previously eluded diagnosis. However, pathogenicity of a given CNV can be difficult to establish and the connection between genomic observation and clinical implication is not always clear. The real challenge for laboratories and clinicians therefore, lies in using this knowledge of CNVs not only for prognosis but also in formulating a pharmacological strategy for prevention and treatment of genomic disorders.

Nuclear Magnetic Resonance Study for Drug and Biological Molecules

Dr. Ashes Ganguly

Cryogen Instruments India Pvt. Ltd.
Thane – 400602, MH. India
(Website: www.cryogen.co.in)

Nuclear Magnetic Resonance (NMR) technique is widely used for structural elucidation for drug and biological molecules. For the molecular weight of upto >2000, the medium field NMR (1H/13C) are used upto 400MHz (9.4 T) or so. But the molecular weight more than 2000 i.e even upto 10-15 Kilodaltons, the high field NMR (13C/15N/31P) like from 500MHz (11.7 T) to 900MHz (21.2 T) are generally used, now a days.

In general, the biological molecules are being complex as the sizes are bigger, so, the interpretation of structural elucidation are also bit tedious. In these cases high field NMR with special techniques and cryo probes are being used based on the nature, size and complexity of molecules.

In the coming days, the work in above direction will be continued with new sequences of biological relevance using a variety of NMR techniques, computational methodologies with high field NMR.

Independent Risk Factors for Early Detection of Coronary Heart Disease

Dr. Rekha Bhagwat

**Associate Professor, Department Of Biochemistry,
Padamashree Dr. D. Y. Patil Medical College, Nerul**

In Indian population highest numbers of patients are suffering from Coronary heart disease (CHD). It is projected to be 40 million Indians may suffer due to Coronary heart disease by the year 2020. Present study revealed that conventional markers for CHD are insufficient to predict the risk. The measurement of hs-CRP and Lp-PLA$_2$ level & clinical indication may predict as a diagnostic marker for early detection of coronary heart disease. The present study was carried at Padamashree Dr. D. Y. Patil Hospital & Research Centre, Navi Mumbai. Fasting blood samples were collected from male and female patients attending Cardiology & Diabetic Clinics. The study was designed between the different groups as diabetic, diabetic with hypertension and Myocardial Infarction & normal healthy individuals between the ages 25-60 years. It was concluded that hs-CRP and Lp-PLA$_2$ level increased significantly in diabetic, diabetic with hypertension and MI, though their lipid levels were within normal range.

Key words: hs-CRP- Lp-PLA$_2$ – diabetes, diabetes with hypertension - lipid profile- myocardial infarction

Expressions" matter:
RNAs in diagnosis and molecular medicine

Dr. Bhakti Pathak

Scientist C, Structural Biology, NIRRH, Mumbai.

Apart from acting as an intermediate molecule linking the genome and the proteome, Ribonucleic acid (RNA) is also performing an important task of controlling the genome and the proteome. Only a small fraction of the RNA species found in eukaryotic cells has protein-coding function- the traditional role for RNA. During the past decade, a multitude of RNAs arising from the huge non-coding part of the genome was discovered to exert regulatory functions of fundamental importance to maintain normal cell function. Understanding this regulatory role of RNA in gene expression is gaining importance and is being exploited in the field of molecular medicine.

At the same time RNAs as messengers of genetic information for protein synthesis, are being utilized for the diagnostic purposes in cases where detection of the key protein is not possible. Specific examples with reference to prostate cancer diagnosis would be discussed in detail. From the point of regulation of gene expression, discovery of RNAi technology has not only provided a tool for fundamental loss-of-function studies but it has also opened up new possibilities in the field of molecular medicine. The rational design of RNA sequences that can specifically block the expression of selected genes responsible for various diseases, including cancer, viral infection and genetic disease has received major attention in recent years. Multiple challenges, such as optimization of selectivity, stability, delivery, and long-term safety, have to be addressed in order for 'RNA drugs' to become successful therapeutic agents. Progress made in this field will be reviewed with some examples.

Application of Bioinformatics in Medical Diagnostics

Dr. Suruchi Jamkhedkar

B-55/535, MIG Colony, Bandra (E), Mumbai 400051, India
Email: suruchi.jamkhedkar@gmail.com

Bioinformatics is an interdisciplinary field, which encompasses branches like biology, computer science, engineering, information technology, statistics and mathematics. It is science of managing and analyzing vast biological data using advanced computational techniques. With the advent of Bioinformatics the basic concept of Central Dogma has changed from traditional: Gene to mRNA to Protein to newer version of Genome to Transcriptome to Proteome with direct application to bench research work.

In 2001, an NIH working group standardized the definition of a biomarker as "a characteristic that is objectively measured and evaluated as an indicator of normal biological processes, pathogenic processes, or pharmacologic responses to a therapeutic intervention". In the post- genome era, efforts are focused on biomarker discovery and the early diagnosis of a disease through the application of various omics technologies like transcriptomics, proteomics, metabonomics (genetic and metabolic network)- on body fluids and tissue samples. For biomarker development, bioinformatics tools are required to extract the diagnostic or prognostic information from the complex data like cell expression profile, microarray and high- through output sequencing data. Thus, medical applications of Bioinformatics would be in:

(a) understanding genetic diseases like cystic fibrosis, sickle cell anemia (SNPs), cancer, cardiovascular diseases

(b) gene therapy

(c) pharmacogenetics and pharmacogenomics to personalize drugs for better bioavailability, to design new and better drugs, to develop better drug delivery system.

Biosensors and biochips: advances in biological and medical diagnostics

Dr. Vandana Singh

Faculty, Department of Biochemistry, Govt. Holkar Science College, Indore (M.P.).

The development of biochips is a major thrust of the rapidly growing biotechnology industry, which encompasses a very diverse range of research efforts including genomics, proteomics, and pharmaceuticals etc. The merging of semiconductor industry and biotechnology has enabled biotechnologists to begin packing their traditionally bulky sensing tools of biotechnology into smaller and smaller spaces, onto so-called biochips. Easy sample preparation, standard operating protocols and a portable biochip reader make the system suitable for use in the various fields. Advances in these areas are giving scientists new methods for unraveling the complex biochemical processes occurring inside cells, with the larger goal of understanding and treating human diseases.

A biosensor can be generally defined as a device that consists of a biological recognition system, often called a bioreceptor, and a transducer. In general, a biochip consists of an array of individual biosensors that can be individually monitored and generally are used for the analysis of multiple analytes. The interaction of the analyte with the bioreceptor is designed to produce an effect, such as an electrical signal. Biosensors and biochips can be classified either by their bioreceptor or their transducer type. A bioreceptor is a biological system (e.g., cells, tissue, or whole organisms) that utilize a biochemical mechanism for recognition. The sampling component of a biosensor contains a bio- sensitive layer. The layer can either contain bioreceptors or be made of bioreceptors covalently attached to the transducer. The most common forms of bioreceptors used in biosensing are based on 1) antibody/antigen interactions, 2) nucleic acid interactions, 3) enzymatic interactions, 4) cellular interactions and 5) interactions using biomimetic materials (i.e., synthetic bioreceptors).For transducer classification, conventional techniques include: 1) optical measurements 2) electrochemical and 3) mass-sensitive measurements.

Biosensors have seen a wide variety of applications primarily in two major areas, environmental sensing and biological monitoring. Biosensors are powerful tools aimed at providing selective identification of toxic chemical compounds at ultra trace levels in industrial products, chemical substances, environmental samples (e.g., air, soil, and water) or biological systems (e.g., bacteria, virus, or tissue components) for biomedical diagnosis.

Cancer Diagnosis :-Biochips can detect cancers before symptoms develop.Laser-induced fluorescence (LIF) is used for in vivo cancer diagnosis (LIF).

Biochip Array Technology is used for Blood Analysis as well as in detection of blood Glucose level.

The biodetectors in the SIMBAS (Self-powered Integrated Micro fluidic Blood Analysis System)chip provided a readout of the biotin levels in 10 minutes. The biochip system can identify infectious disease strains in less than 15 minutes when testing protein arrays and in less than two hours when testing nucleic acid arrays.

Diagnosis of ASPV (Apple stem pitting virus):- A direct method of detecting plant viruses using an aptamer based biochip. ASPV is a worldwide virus that has been associated with complex growth disorders in fruits.

Biochip technology is a powerful tool for studying the specificity and pathogenesis of autoantibody responses in autoimmune diseases.

Routine analytical measurement of folic acid, biotin, vitamin B12 and pantothenic acid as an alternative to microbiological assay.

Determination of drug residues in food, such as antibiotics and growth promoters. Drug discovery and evaluation of biological activity of new compounds.

The biochip offer a chance to determine the "signatures" of weaponizable biological agents, most notably anthrax.

Biochips technology are most attractive in the area of human health. Biochips enable researchers to quickly screen large numbers of biological analytes for a variety of purposes, from disease diagnosis to detection of bioterrorism agents.

Pharmacogenomics: Towards Personalized Medicine

Dr. Supriya Bhalerao, Renuka Munshi, Falguni Panchal

Dept. of Clinical Pharmacology, TNMC & BYL Nair Ch. Hospital

Technology and resources promoted by the Human Genome Project have profound impacts on biomedical research. As a major outcome of this research, a new branch of clinical medicine, Pharmacogenomics, a combination of functional genomics and molecular pharmacology, has been emerged recently. Pharmacogenomics is defined as the study of the effect of variation in multiple genes that influence drug metabolism and response.

There are often large differences among individuals in the way they respond to medications, both in terms of efficacy and safety. Although there can be various potential causes for this variability in drug effects viz. nature and severity of the disease being treated, individual's age and race, organ function, drug interaction and concomitant illnesses etc., genetic variation in drug metabolizing enzymes is one of the major causes of inter-individual variation of drug effects.

Genetic variations i.e. genetic polymorphisms of drug-metabolizing enzymes give rise to distinct subgroups in the population that differs in their ability to perform certain drug biotransformation reactions. Polymorphisms are generated by mutations in the genes coding these enzymes, which lead to decreased, increased, or absent enzyme expression or activity by multiple molecular mechanisms.

Since Pharmacogenomics aims at discovering association between specific genotypes and phenotype (drug metabolism), it helps physicians to select a right drug and right dose for an individual patient. This ultimately facilitates effective management of the patient's disease condition without producing side effects (or personalized therapy). The present paper discusses the concept of Pharmacogenomics in detail with the examples of the work carried out at the Molecular Biology lab, Dept. of Clinical Pharmacology, BYL Nair Hospital.

Metabolomics as a tool for Diagnostics

Dr. Ajit Datar

Shimadzu Analytical (India) Pvt. Ltd.

In the field of Biology, the functional analyses at "Omics" levels have been growing very rapidly from the beginning of 21st century. These include Genomics, Transcriptomics, Proteomics, Metabolomics, and Lipidomics etc. For disease diagnosis, Metabolomics offers unique advantages over other 'Omics' techniques. Hence Metabolic Fingerprinting has become one of the very important tools. In diagnostics there are several techniques used for Metabolite Fingerprinting. Most commonly used analytical techniques are based on Spectroscopy or Chromatography or Hyphenation of these techniques. These include Infra-Red (IR) Spectroscopy, Raman micro spectroscopy, Nuclear Magnetic Resonance (NMR) Spectroscopy, Mass Spectroscopy (MS) and Chromatographic Techniques like Gas Chromatography (GC), High Performance Liquid Chromatography (HPLC) and Hyphenated Techniques like GC-MS or LC-MS. Tandem MS is also used in most of the applications, e.g. GC-MS/MS or LC-MS/MS. The MS methods are used predominantly because MS offers exquisite sensitivity and the great selectivity in identifying many hundreds of metabolites in a single run. Tandem MS has proven itself in the Diagnosis of Neonatal Screening for Inherited Disorders of Intermediary metabolism, in the field of identification of biomarkers from Type 2 diabetes. There are several examples in literature for the disease diagnostics using MS, e.g. cancer, coronary heart disease, neurological disease, many infectious diseases and so on.

It is essential then to understand the Tandem MS technique with GC or LC as front end for Metabolite fingerprinting. The power of Chromatography to separate the complex mixture of metabolites from the complex matrices such as blood plasma, urine, tissues etc. and the ability of MS for its accurate mass measurement are essential for understanding the structure and pathways of metabolite formation and thus help in

treatment of disease. Current development in the field of Analytical Biochemistry (or Bio-analytical Sciences) allows simultaneous measurements of multiple analytes in biological matrix. Despite the current advances in this field, the goal of analyzing all the metabolites in the metabolome is still a challenge. It requires continued development of libraries of standards, instrument platforms with higher and higher sensitivities and mass accuracies by MS and integration of MS and NMR methods. Not to forget is the development in computational methods for analysis of complex metabolomic datasets and their integration with equally complex other "Omics" and database available as these techniques grow.

SECTION III
CONCLUDING SESSION

Emerging Strategies for Effective Cancer Treatment Based on Cell Electroporation Technology

Hon'ble Vice Chancellor Dr. K. P. Mishra
Nehru Gram Bharati University, Allahabad 211002
Ex Radiation Biology and Health Sciences Division, Bhabha Atomic Research Center, Mumbai
Email: vc.ngbu@gmail.com, mishra_kaushala@rediffmail.com

Living cells have tight regulation of entering and outgoing molecules through sophisticated barrier provided by cellular plasma membrane. Failure of drug action on cells and, therefore, its ineffectiveness on target disease cells/tissues poses challenge to medical science in clinic. Cancer arises due to transformation of normal cell to cancer cell which is known to escape the built-in control mechanisms of membrane permeability, protein transformation and gene regulation and expression affected by various causative factors. Cancer kills millions of patients each year the world over. There is a critical need for safe, effective, and affordable alternative treatment modalities, especially for inoperable, recurring and chemo-resistant cancers, that do not respond well to current treatment modalities. A physical technique based on exposure of cells to short high voltage electrical pulse, discovered a few years ago, allows impermeant drugs, enzymes and other diagnostic and therapeutic agents enter the target cells opening many new opportunities in medicine and biotechnology. This method of electric pulse-mediated permeabilization of cell membrane known as electroporation permits enhanced drug delivery technique has provided one of the novel approach to effectively treat cancer patients which seems especially suitable for low and middle-income countries, where due to lack of infrastructure and resources, many cancers are diagnosed at late stages and this safe, effective, economical, out-patient-based and quick technique is a boon to these patients for palliative and other care with enhanced quality of life. This talk is designed to

present basic aspects of cell electroporation which has important implications to cell biotechnology and gives examples of clinical applications of electroporation. An attempt is made to discuss novel and recent clinical applications of electrochemotherapy for drug delivery in treating various types of cancers and other diseases. The talk points out possibilities of developing novel technologies with potential applications in medicine, biotechnology, agriculture and food science with the use of novel and effective diagnostic and therapeutic tools.

SECTION IV
POSTER ABSTRACTS

Biomedical Imaging
Swapnil Vichare, Amit Adsule, BhooshanMankame, B. N. Bandodkar College of Science, Thane

Biomedical imaging is the technique and process used to create images of the human body or parts for clinical purposes. Medical imaging constitutes a sub-discipline of biomedical engineering and of medical physics.

Medical imaging is often perceived to designate the set of techniques that non-invasively produce images of the internal aspect of the body. The term non-invasive is a term based on the fact that medical imaging modalities do not penetrate the skin physically. But on the electromagnetic and radiation level, they are quite invasive. There are several techniques under medical imaging like radiography, ultrasound, Magnetic Resonance Imaging (MRI), tomography etc.

Medical imaging has become a major tool in clinical trials since it enables rapid diagnosis with visualization and quantitative assessment. A typical clinical trial goes through multiple phases and can take up to several years. An imaging technique is used as an indicator of pharmacological response to a therapy, obtaining quick results. Imaging is able to reveal change that is indicative of the progression of therapy that may be missed out by, traditional approaches. Thus Biomedical Imaging plays an important role in the medical fields.

Microarray Analysis as a Diagnostic Tool
Sneha Tawde, Humera Bhattiwala, Manali Patel
B.N. Bandodkar College of Science, Thane

The advent of microarray technology has a major impact on the molecular classification and understanding of human diseases. It has revolutionized the study of gene expression and gene regulation.This technology promises to monitor the whole genome on a single chip so that researchers can have a better picture of interactions among thousands of genes simultaneously. There are 2 types of microarray techniques:- 1) DNA microarray and 2) Protein microarray.

In this technique, mRNA is isolated from a given sample; then when cDNA synthesis is initiated the first strand of the cDNA is labeled with the tag to form a pool of target sequences. The next step is to hybridize the labeled cDNA to nucleic acid affixed in a microarray. The process of arraying the cDNA is accomplished using robotics. In case of protein microarray, instead of DNA externally synthesised, purified proteins are attached

to the solid surface like glass slide or nano wells. Taken together, DNA or protein microarray has facilitated the high throughput genomic and proteomic studies and diagnosis of human cancer and other diseases.

Gas Chromatography- Mass Specrometry (GC-MS) in Diagnostics
Anuja Kumbhar, Priyanka Pawar, B. N. Bandodkar College of Science, Thane

Gas chromatography-mass spectrometry (GC-MS) is a hybrid technique which couples the powerful separation potential of gas chromatography with specific characterization ability of mass spectroscopy. Gas chromatography-mass spectrometry (GC-MS) is an important technique for the identification and detection of bacteria and bacterial constituents. Certain classes of structural components, or secreted metabolites, are useful as chemical markers for different groups of organisms. The GC works on the principle that a mixture will separate into individual substances when heated. The heated gases are carried through a column with an inert gas (such as helium). As the separated substances emerge from the column opening, they flow into the MS. Mass spectrometry identifies compounds by the mass of the analyte molecule. The GCMS can detect chemicals in amounts as small as 0.1 to100 ng. A gas chromatography–mass spectrometry (GC–MS) method with minimum sample preparation is described for early diagnosis of tuberculosis(*Mycobacterium tuberculosis*).The technique is also used in detection of Urine d-Arabinitol/l-Arabinitol Ratio in Diagnosis of

Invasive Candidiasis(*Candida spp*) in Newborn Infants. It is used for rapid diagnosis of infectious Diarrhea caused by *Clostridium difficile*& diagnosis of bacterial meningitis by the detection of a fatty acid marker in CSF. GC-MS is the most widely used application in metabolite research & disease diagnosis. It enables the identification of small molecules as amino acids, fatty acids, organic acids in biofluid as blood and urine providing diagnostic information in inherited diseases, including numerous metabolic disorders.

Molecular Markers in Diagnostics
Nikita Diwan, Pooja Kadhane, Rashmi Joshi, B. N. Bandodkar College of Science, Thane

Recent revolutionary progress in human genomics is reshaping our approach to therapy and diagnosis. No two human individuals have exactly the same genome and this has led to development of DNA typing. DNA typing relies on DNA analysis using molecular markers such as RFLPs, RAPD, AFLP, SNPs, and SSLPs.

Restriction fragment length polymorphism (RFLP) generated when natural mutations at site of restriction enzymes as well as insertion or deletion between the sites differentiate individuals. Random amplified polymorphic DNA (RAPD) markers are generated by PCR amplification of genomic DNA segments using a single, short primer under low annealing temperature. Amplified fragment length polymorphism (AFLP) has been widely used in genome mapping and gene tagging. This versatile method is able to detect the presence of restriction fragments in almost any DNA, regardless of its complexity.This approach can be used for indirect detection of a disease causing mutation or simply distinguishes DNA fragments of different sizes from the same region or also can be used as markers within a family who is likely to carry a disease – causing mutation and who is not . The identification of such markers has shown some important consequences i.e. it may offer a diagnostic procedure for detecting some of the human diseases that are genetically well characterized but ill defined in molecular terms cannot be easily diagnosed . This is a clear indication that as many molecular techniques have made their expected transitions into the clinical arena. Molecular diagnostics is becoming an integral part of clinical practice.

Prognostic Markers
Hemant Shirvalkar, Satish Mirgal, Ruchi Khadtale, B.N. Bandodkar College of Science, Thane

Prognostic markers (biomarkers) are characteristics that help to identify or categorise people with different risks of specific future outcomes. They may be simple clinical measures such as body mass index, but are more often pathological, biochemical, molecular or genetic measures or attributes. Identifying those who are or who are not at risk can facilitate intervention choice, and aid patient counselling.

Prognostic markers help to stratify patients for treatment by identifying patients with different risks of outcome (e.g. recurrence of disease), and are important tools in the management of cancer and many other diseases. Systematic review and meta-analytical approaches to identifying the most valuable prognostic markers are needed because (sometimes conflicting) evidence relating to markers is often published across a number of studies.

In oncology, prognostic markers are clinical measures used to help elicit an individual patient's risk of recurrence of disease after primary treatment. Evidence based results regarding prognostic markers are therefore very important to both clinicians and patients. Cervical cancer remains the leading cause of cancer deaths in women. The researchers of United States National Cancer Institute(NCI), The Gynecologic Cancer Intergroup found out that para-aortic and pelvic lymph node involvement is clearly one of the most important prognostic markers, as well as one of the most important factors to influence treatment decisions. Additional adverse prognostic markers include peritoneal spread as well as presence of supraclavicular nodes in those with more advanced diseases.Tejashri Patil.

Diagnosis of HIV
Priyanka Bhalerao, Anuja Bharati, B. N. Bandodkar College of Science, Thane

Human Immunodeficiency Virus (HIV) was unknown until the early 1980s but since that time it has infected millions of people worldwide. HIV is most commonly diagnosed by testing the blood or saliva for the antibodies to the virus. However, different types of HIV diagnostic techniques have been used over a period of time which include antibody test:-ELISA (rapid test) which detects HIV antibodies & the results are confirmed by Western Blot which is one of the oldest but most accurate confirmatory tests. But it takes time for a person's body to develop these antibodies, antigen tests:- protein P24 most commonly provokes an antibody response for HIV & are now most often used as a component of fourth generation tests. Some of the most modern HIV tests combine P24 antigen tests with standard antibody tests, PCR test which can detect the genetic material of HIV within 2-3 weeks, HIV home sampling & HIV home testing which is done using home sampling kits & home testing kits. Several types of tests help the doctor determine what stage of the disease a person has :-CD4 count, viral load, drug reistance. Along with them come the test for complications for TB, Hepatitis, Toxoplasmosis, STD, Liver or kidney damage, Urinary tract infection.

The period between HIV infection & the production of antibodies is known as ''Window Period''.During this time,an antibody test may give a false negative results which means the test will be negative even though a person is infected with HIV. To avoid false negtive result, antibody test recommended 3 months after potential exposure to HIV infection.

Diagnostic techniques provide prognostic information in patients with estblished disease. The criterion (reference) standard test definatively decides either presence or absence of a disease. However, these test routinely come with drawbacks such as they are usually expensive, less widely available, more invasive & riskier.

This presentation provides an overview of diffrent HIV diagnostic tests describing how they work & the advantages & limitaion of various tests. It also includes what molecular marker these tests used to pinpoint HIV infection

Enzyme Based Diagnostics
Rucha Kulkarni, Mansi Lad, Darshana Sakpal, B.N. Bandodkar College of Science, Thane

The major importance of enzymes in diagnostics is that they are used to determine the specific levels of enzymes in blood, urine, body fluids and tissues. They are also helpful in determining the concentration of substances and metabolites. There is a lot of progress in diagnosticsusing enzymes, as they are comparatively easy to use and are now commercially available. Highly sophisticated equipments and assays are available for enzyme diagnoses that are easy to assay and automate. They are often preferred over older tests as the differences between the normal specimen and the disease specimen are clearly demonstrated and diagnostically significant. The enzymes utilized are sufficiently stable and allow storage for limited but reasonable time. Available diagnostic tests can demonstrate plasma-specific enzymes that are associated with normal functioning of plasma such as blood coagulation components, complement activation components, and lipoprotein metabolism. Newer tests can also determine non-plasma specific enzymes such as those resulting from abnormal cellular metabolism that have no actual function in plasma where coenzymes or substrates are lacking they are present in plasma as a result of other body problems such as liver disease heart attacks, muscle disease, disease of pancreas or cancer. Examples include phosphatases, transaminases, lipases and amylases. There are certain serum enzymes which are released in blood only when the cells to which they are confined are damaged. The presence of these enzymes in blood indicates the site of tissue injury. Some of the examples of such enzymes areAldolase, Creatine phosphokinase, Lipase. There are many other examples likeTransaminases like Glutamic-oxaloacetic acid (GOT), Glutamic-pyruvic transaminase lactic dehydrogenase etc. The common enzymes for clinical diagnosis are Acid phosphatase, alanine aminotransferase, cholinesterase lactate dehydrogenase etc. Enzymes are also used for the prognosis of a disease.

Differential Staining Of Blood
Dr. Kalpita Mulye, Anjali Tidke, Priyanka Barmukh, B.N. Bandodkar college of Science

Hematology encompasses the study of blood components and coagulation. It includes, analysis of the concentration, structure &functions of the cells & repair their precursors in the bone marrow, analysis of chemical constituents of plasma or serum intimately, intimately, linked with blood cell structure & functions, Study of functions of the platelets & proteins involved in blood coagulation.

Importance of Differential Staining :-. Differential count is useful to identify changes in the distribution of WBCs which may be related to specific types of disorders. It also gives idea regarding the severity of the disease & the degree of response of the body.

Principle :- Giemsa stain contains methylene blue & eosin. These basic and acidic dyes includes multiple colors applied to cells. Methanol acts as a fixative and also as a solvent. The basic component of WBCs i.e. cytoplasm is stained by acidic dye & they are described as eosinophilic. The acidic component i.e. nucleus with nucleic acid take blue colour by basic dye & they are called as basophilic. The neutral component of the cells are stained by both dyes.

Screening of blood smears :- 25 blood smears were taken & screened for White Blood Cells count with Giemsa staining. Different abnormalities detected are listed below :-Toxic Granulation which may be due to severe bacterial infection & also in some hereditary disorders. Hypersegmentation which may be due to inherited disorder also seen in macrocytic anemias.

Cholescintigraphy (HIDA Scan)
Shrikant Sonawane, Uttara Patil, Noopur Patil, B. N. Bandodkar College of Science, Thane

HIDA(Hepato Iminodiacetic Acid) Scan is an imaging procedure that helps track the production and flow of bile from liver to small intestine. Bile, fluid produced by liver that helps digestive system breakdown fats into the food we eat. HIDA scan, one of the nuclear medicine scan, uses a radioactive chemical or tracer that helps highlight certain organs like liver, gallbladder, bile ducts and small intestine on the scan.

HIDA scans are ordered for patients who are suspected of having an obstruction in biliary tract, most commonly those who are thought to have a stone blocking the cystic duct leading out of the gallbladder. Such a scenario is consistent with acute cholecystitis, which often requires surgical removal of gallbladder. In cholecystitis, HIDA will appear in the bile ducts, but will not enter the cystic ducts or gallbladder- a finding that indicates obstruction. If HIDA enters bile ducts but does not enter the small intestine, then an obstruction in bile duct is suspected.

The patient receives an intravenous injection of radioactive material i.e. technetium 99m iminodiacetic acid derivative, taken up by the liver and excreted into the biliary tract. HIDA imaging is done by a nuclear scanner, which takes pictures of patient's biliary tract over the course of about an hour or two. The images are then examined by a radiologist, who interprets the results.

HIDA scan for acute cholecyctitis has a sensitivity of 97%, specificity of 94%. It is generally a very safe test and well tolerated by most patients.

Polymerase Chain Reaction
Pranali Chowre, Tejal Niphade, Sudha Shukla, Vaibhavi Ghag,
B. N. Bandodkar College of Science, Thane

Polymerase chain reaction is a method for producing many copies of a specific DNA sequence from a DNA mixture without having to clone the sequence in host organism. The three main steps involved in the PCR techniques are repeated denaturation, annealing and extension cycle.

PCR is mainly used for amplifying small (generally less than 10 kb) region of DNA. It is much more

sensitive and quicker method than cloning.

PCR is mainly used for mapping DNA sequence, DNA fingerprinting, DNA sequencing, forensics, disease diagnosis, sex determination of embryos, tissue typing. PCR is a key point in the process of DNA sequencing.PCR is routinely used tool in the laboratories of molecular geneticists.

PCR technology has become an essential research and diagnostic tool for improving human health and quality of life.PCR based diagnostics tests are available for detecting and quantifying several hazardous pathogens. In diagnosis, it is used to detect the bacterial pathogens or viral pathogens such as HIV and Hepatitis B. it is also widely used in the genetic disease diagnosis.

Fluorescence *in situ* Hybridization
Ruchita more, Priyanka Arekar, Chaitali Chachad, B. N. Bandodkar College of Science, Thane

Fluorescence in-situ hybridization (FISH) is a cytogenetic technique developed by biomedical researchers in the early 1980s that is used to detect and localize the presence or absence of specific DNA sequence on chromosomes. FISH is a process which 'paints' chromosomes or parts of chromosomes with fluorescent molecules. Fluorescence in-situ hybridization involves the use of probes (short sequence of single-stranded DNA) which are labeled with fluorescent tags to hybridize or bind to only those parts of the chromosome with which they show a high degree of sequence complementarity within the intact cell.

Fluorescence microscopy can be used to find out where the fluorescent probe is bound to the chromosomes. FISH is a technique which helps in identifying chromosomal abnormalities. FISH also aids in gene mapping, toxicological studies, analysis of chromosome structural aberrations, and ploidy determination. It is also used to identify the presence and location of a region of DNA or RNA within morphologically preserved chromosome preparations, fixed cells or tissue sections. A variety of procedures are available to cytogeneticists, who use them to diagnose many types of chromosomal abnormalities in patients.

The methodology is being continuously improved so far however, microscopic analysis by FISH has not been automated sufficiently which would be desirable in many investigations. Accurate quantification still remains a challenging task and study needs careful controls.

Cancer Diagnostics
Sonam Patil, Nayana Choudhari, Pooja Mandluskar, B. N. Bandodkar College of Science

Cancer is a group of diseases in which abnormal body cells in one part of the body start to grow out of control. Oncology is the study of cancer and tumors.

Diagnostic procedures for cancer may include imaging, laboratory tests (including tests for tumor markers), tumor biopsy, endoscopic examination, surgery, or genetic testing.

Blood test: A complete blood count (CBC) to measures the size, number, and maturity of the different blood cells. A variety of blood tests are used to check the levels of substances in the blood

Urinalysis: Urinalysis breaks down the components of urine to check for the presence of drugs, blood, protein, and other substances.

Tumor markers: Tumor markers are substances either released by cancer cells into the blood or urine or substances created by the body in response to cancer cells. Some of useful tumor markers are prostatic acid phosphatase (PAP), CA 125, carcinoembryonic antigen(CEA), alpha-fetoprotein (AFP), human chorionic gonadotropin (HCG), CA 19-9, CA15-3, CA 27-29.

Imaging: Imaging is the process of producing valuable pictures of body structures and organs. It is used to detect tumors and other abnormalities, to determine the extent of disease, and to evaluate the effectiveness of treatment .There are three types of imaging used for diagnosing cancer: transmission imaging, reflection imaging, and emission imaging.

Transmission Imaging: X-ray, computed tomography scan (CT scan or CAT scan),bone scan, lymphangiogram (LAG), mammogram, Reflection Imaging: ultrasound Emission imaging, Magnetic Resonance Imaging (MRI)

Haematological Diagnostics
Swati Kolte, Poonam Shukla, B. N. Bandodkar College of Science, Thane

Haematology encompasses the study of blood components and coagulation. It includes, the analysis of the concentration, structure and functions of the cells and their precursors in the bone marrow, analysis of chemical constituents of plasma or serum intimately linked with blood cell structure and functions, study of functions of the platelets and proteins involved in blood coagulation. Changes in one or more of the characteristics mentioned above may produce haematological disease or manifestations.

Some of the Haematological disorders are haemoglobinopathy, and haemolytic anemias. Haemoglobinopathy is a kind of genetic defect that results in abnormal structure of one of the globin chains of the haemoglobin molecule. Haemoglobinopathies are inherited single-gene disorders; in most cases, they are inherited as autosomal co-dominant traits. Common haemoglobinopathies include sickle-cell disease.

Methods of detecting Haemoglobinopathy are alkaline and acid gel electrophoresis, Isoelectric focusing (IEF), High Pressure Liquid Chromatography (HPLC) and Capillary electrophoresis (CE)

Haemolytic anaemia is a form of anaemia due to haemolysis, the abnormal breakdown of red blood cells (RBCs), either in the blood vessels (intravascular haemolysis) or elsewhere in the human body (extravascular). The general classification of haemolytic anaemia is either inherited or acquired. Treatment depends on the cause and nature of the breakdown. Methods of detecting haemolytic anaemia are absolute reticulocyte count, red blood cell count (RBC), haemoglobin, and haematocrit (HCT), Serum haptoglobin levels, Serum indirect bilirubin levels and Serum LDH determination.

Use of monoclonal antibody-based biosensors in diagnostics tests
Sandhya Dumbre, K. B. P. College, Vashi

Biosensors are analytical tools of bio-material samples to gain an understanding of their bio-composition, structure and functions by converting a biological response into an electrical signal. The analytical devices composed of a biological recognition element directly interfaced to a signal transducer which together relates the concentration of an analyte (or a group of related analytes) to a measurable response.Biosensor provides similar sensitivity as provided by other conventional detection instruments & techniques.

Some biosensors reached even detection limits similar to the PCR techniques. Typical time of immunodetection is 15 min, but some devices like resonant mirror, quartz crustal microbalance are able to provide signal within 5 min. A new generation of biosensors discussed in this presentation uses antibody and DNA probes, like Antibody-based fluoroimmunosensors (FISs), Biochips, NANOSENSORS, etc. Monoclonal antibodies are proving to be very useful as diagnostic, imaging, & therapeutic reagents in clinical medicine. Among the many monoclonal antibody diagnostic reagents now available are products for detecting pregnancy, diagnosing numerous pathogenic microorganisms, matching histocompatibility antigens, & detecting antigens shed by certain tumors. Thus, by combining the properties of monoclonal antibodies & principle behind the biosensors, various biosensors can be developed which would be useful in diagnosing diseases/ infections whose cure is still a mystery.Due to their small size & low cost, biosensors are convenient not only for laboratory routine but also for mobile laboratories & portable systems in the field. In the future, one can expect development of new biosensors giving reliable detection results with amount of individual agents under their infectious doses, with multi-channel arrangement for several agents, & with further miniaturized designs

Karyotype Analysis
Prathamesh Chaughule, K. B. P. College, Vashi

Organisms may be identified by using the size and shape of their genetic material at a particularpoint in the cell division cycle, termed metaphase. At this point DNA condenses to form anumber of very distinct chromosome structures. Various morphological characteristics ofchromosomes may be identified at this stage including the centromere and the telomere. Thedisplay of the 46 human chromosomes at mitosis is called the human karyotype. If parts ofchromosomes are lost or are switched between chromosomes, these changes can be detectedby changes in the banding patterns or by changes in the pattern of chromosome painting.Using chromosome banding alone, a disturbingly high number of chromosomal aberrationscannot be characterized comprehensively. Thus, a special approach is applied, called spectralkaryotyping, that is based on the simultaneous hybridization of 24 combinatorially labeledhuman chromosome painting probes. Spectral karyotyping was developed for the detection ofnon-homologous structural and numerical chromosomal aberrations on the single metaphaselevel hybridizing a 24-color whole chromosome probe mix. Each homologue chromosome isdisplayed in a different color, based on the use of computer-generated false-color chromosomeimages and karyotyping. Spectral karyotyping combines Fourier transform spectroscopy, CCDimaging, and optical microscopy to measure simultaneously at all points in the sample emissionspectra in the visible and near-infrared spectral range. This technique is widely used to studyconstitutional chromosome abnormalities is particularly useful for identifying de novo balancedand unbalanced translocations. Spectral karyotyping is also used in multiparameter analysis ofcytological preparations to routine diagnostic preparations.

Diagnostic Techniques for Human Diseases caused by Fungal Pathogens: A Brief Review
Moses Kolet, Amit Bendre, Sohil Pawar, B. N. Bandodkar College of Science, Thane

Fungi constitutes a large and diversified group of heterotrophic organisams comprising parasites as well as saprophytes. Their occurance can be described as both overt and covert. Of approximately 100,000 fungi known to mankind, less than 500 have been implicated in various cases of mycosis affecting man and animals. Some of these are capable of affecting exposed individual while others are opportunistic pathogenes. The infections themselves vary in degree of seriousness; some apperently ignorable while others may prove fetal.

The present work deals with an overview of epidemiology of the diseases of fungal origin, their incidence and different types of fungal infections affecting human beings. A brief review of classical and modern diagnostic techniques inclusive of latest serological techniques and application of Polymerase chain reaction for diagnosis of fungal infection is undertaken.

SECTION V
PRE CONFERENCE WORKSHOPS
ABSTRACTS

Conventional Diagnostic tests

Rajitha Radhakrishnan
Lecturer, Department of Microbiology and Biotechnology, B. N. Bandodkar College of Science, Thane

Diseases are on rise all over the world. The ability to control and manage diseases is largely dependent on the ability to detect the etiological agents in the clinical microbiology laboratory. Hence diagnostic plays very important role in disease management.

Though the last few years of the twentieth century have seen an explosion of molecular biology techniques in diagnostics, the underlying principle of many such techniques still lies in conventional diagnostics methods. Conventional Diagnostic technique includes techniques like microscopy, culturing of sample, antigen or antibody detection, immunoserology as well as Enzymatic test. Conventional identification of microbial pathogens using microscopy relies on phenotypic characteristics of the organisms, morphology, bacterial metabolic characteristics, fungal structures, parasitic morphology and viral cytopathic effect .These primarily includes Gram staining, differential staining using Giemsa stains, HE stains, ZNCF, wet mounts of sample as in KOH/ fungal wet mount. Culturing of sample includes collection of Sample, followed by macroscopic and microscopic observation of sample, isolation on suitable selective media, and based on colonies obtained further detection of etiological agent is done using various biochemicals. A variety of test systems have been developed to identify the antigens of infectious diseases as well as antibodies secreted as part of protective mechanisms of body. Test systems used in immune serology have classically included methods of detecting antigen-antibody reactions which range from complement fixation to immunoassay methods. Similarly many diseases like sickle cell anemia, phenylketonuria (PKU) and Tay-Sachs disease are detected by checking abnormal enzymatic levels in blood or tissues.

In many developing countries even now many diseases are detected using conventional methods as they are simple, affordable and accurate. Although conventional diagnostic methods are dependable and reliable too, most approaches still have certain limitations. Thereby for improved sensitivity and specificity one goes for advanced molecular techniques.

Immunological Diagnostics

Nilambari Daripkar
Lecturer, Department of Microbiology and Biotechnology, B. N. Bandodkar College of Science, Thane
E-mail: nilambarib6@gmail.com

Immunity is a biological term that describes a state of having sufficient biological defenses to avoid infection, disease, or other unwanted biological invasion. The human immune system is a truly amazing constellation of responses to attacks from outside the body. It has many facets, a number of which can change to optimize the response to these unwanted intrusions. The system is remarkably effective, most of the time. An immune system is a system of biological structures and processes within an organism that protects against disease. In order to function properly, an immune system must detect a wide variety of agents, from viruses to parasitic worms, and distinguish them from the organism's own healthy tissue.

An antigen is any substance that elicits an immune response, from a virus to a sliver. Detection of particular antibodies raised against these antigens is a very common form of medical diagnostics. For example, in biochemical assays for disease diagnosis, a titer of antibodies directed against Epstein-Barr virus or Lyme disease is estimated from the blood. If those antibodies are not present, either the person is not infected, or the infection occurred a very long time ago. Practically, several immunodiagnostic methods based on detection of complex antigen-antibody are used to diagnose infectious diseases, for example ELISA, Immunofluorescence,Westernblot, Immunodiffusion, Immunoelectrophoresis and Magnetic immunoassay.

Example, Antibodies raised against Human Chorionic Gonadotropin is **used in Pregnancy detection tests.**

Modern Immuno-Diagnostic Techniques

Kalpita Mulye
Assistant professor, Department of Biotechnology and Microbiology
B. N. Bandodkar College of Science, thane

Modern methods of immunodiagnostics are based on the much familiar phenomenon of Antigen – Antibody interaction viz. Specificity, Affinity and avidity, Reversibility and Cross reactivity.

High Sensitivity, Precision, Rapidity, Reproducibility and sophisticated Automation render modern immunodiagnostic techniques their much deserving popularity.

The milestone discovery "ELISA" revolutionized diagnostics by attaining detection sensitivity up to 0.0001–0.01 micrograms of antibody from the sample. The technique is based on the use of specific enzyme tagged antibodies which will bring about the conversion of substrate to chromogenic product that can be analyzed by spectrophotometer.

The similar technique which uses the radioactive label instead of an enzyme label is Radio Immuno Assay (RIA). Principle of RIA involves competitive binding of radio-labeled antigen and unlabeled antigen to a high-affinity antibody.

Variation to the conventional ELISA, which is used for detection of specific biomolecules e.g. Cytokines *in situ* is ELISPOT. Most of the cytokine molecules secreted by a particular cell react with nearby well-bound antibodies and is in wide use to track the cytokine levels as one of the prognostic markers for diseases like HIV/AIDS.

Automated analysis and separation of cells is possible now with help of flow cytometry, which make the use of fluorescent monoclonal antibodies developed for specific cell surface markers.

Western blotting, Immuno-electron microscopy, Immuno fluorescence, Immuno-histochemistry (IHC) are few more important techniques based on use of specific antibodies tagged with different moieties for varied diagnostic goals.

Molecular Diagnostics - Polymerase Chain Reaction

Jayashree Pawar
Assistant Professor, B. N. Bandodkar College of Science, Thane

Polymerase chain reaction, PCR, the quick, easy method for generating unlimited copies of any fragment of DNA, is one of those scientific developments that actually deserves time-worn superlatives like "revolutionary" and "breakthrough." PCR has transformed the diagnostic methodology utterly. PCR takes analysis of tiny amounts of genetic material—even damaged genetic material—to a new level of precision and reliability.

The central scientific fact that makes PCR so useful is this: the genetic material of each living organism possesses sequences of its nucleotide building blocks that are uniquely and specifically present only in its own species. These unique variations make it possible to trace genetic material back to its origin, identifying with precision at least what species of organism it came from, and often which particular member of that species.

PCR requires a template molecule—the DNA or RNA one wants to copy—and two primer molecules to get the copying process started. Primer designing is an important aspect of polymerase chain Reaction. The reaction also requires dNTPs, a thermostable DNA polymerase, e. g., *Taq* DNA polymerase and buffer for the enzyme with correct magnesium ion concentration.

There are three basic steps in PCR. Denaturation of the template DNA molecules, primer annealing, and extention of annealed primers by polymerase. The result is two new helices in place of the first, each composed of one of the original strands plus its newly assembled complementary strand. The three steps are repeated 25-30 times to get millions of copies of the template DNA flanked by the primers. The reaction is perfomed in a 'Themal Cycler'. Use of capillry systems instead

of tubes and forced turbulant air systems have dramatically lowered the time required for a round of PCR.

Various modifiactions of the basic PCR are carried out for special applications e. g., Reverse Transcriptase PCR is used to amplify RNA templates. Nested PCR is used to assure specificity of the reaction as two primer pairs in two rounds of PCR cycling are used.

Medical research and clinical medicine are profiting from PCR mainly in two areas: detection of infectious disease organisms, and detection of variations and mutations in genes, especially human genes. The method is especially useful for searching out disease organisms that are difficult or impossible to culture, such as many kinds of bacteria, fungi, and viruses, because it can generate analyzable quantities of the organism's genetic material for identification

Techniques in Animal Cell Culture

Dr. Sharmila Raikar
Co-ordinator, M. Sc. Neutraceuticals, G. N. Khalsa College, Matunga, Mumbai.

Animal tissue culture (ATC) deals with removal of cells, tissues, or organs (organ culture) from an animal and their growth *in vitro*. ATC finds many applications in cell and molecular biology. Cell cultures provide a good model system for studying basic cell biology and biochemistry, interactions between disease-causing agents and cells, drug pharmacokinetics and pharmacodynamics, ageing and nutritional studies. Mechanisms of carcinogenesis can be studied and novel anti-cancer drugs can be screened using cell lines. Some other applications include vaccine production, protein engineering, stem cell therapy, gene therapy, drug screening and development etc. In vitro conditions for animal cell growth are more complex in terms of medium (serum requirement) and environmental factors such as temperature, carbon-dioxide requirements etc. Normal cell lines display certain characteristics such as anchorage dependence (certain exceptions are blood cells that grow as suspension cultures) and contact

inhibition. Transformed cell lines display no contact inhibition and have lower serum requirement than normal cells.

Reliable cell quantitation methods must be employed in order to ensure accuracy and reproducibility of any assay. Methods for animal cell quantitation include cell counting (hemocytometer, cell counters, FACS), proliferation assays (thymidine uptake assays), metabolic assays such MTT reduction assay, colorimetric assays (using dyes such as Crystal Violet, or fluorescence DNA binding assays using dyes such as Propidium iodide, Hoechst dyes etc.). Cytotoxicity is an important parameter for studying the effect of environmental factors (e.g. drugs) on viability and survival of cells. Cytotoxicity may studied as a function of change in cell viability (uptake/exclusion of vital dyes), cell survival (by testing plating efficiency), metabolic ability (MTT reduction) etc.

Biotechnology in Diagnosis of Genetic Disorders

Sonal Mathias
Lecturer, Dept. of Biotechnology, B. N. Bandodkar College of Science, Thane

A genetic disorder is an illness caused by abnormalities in genes or chromosomes, especially a condition that is present from before birth. Most genetic disorders are quite rare and affect one person in every several thousand or millions. A genetic disorder may or may not be a heritable disorder. Some genetic disorders are passed down from the parents' genes, but others are always or almost always caused by new mutations or changes to the DNA. The four different types of genetic disorders are (1) single-gene, (2) multifactorial,

(3) chromosomal, and (4) mitochondrial. Gene testing is carried out only after informed consent of the individuals. Samples used are blood, bone marrow, amniotic fluid, skin , semen and hair. If the disorder is chromosomal in nature then KARYOTYPING is advised.Genetic tests are used for several reasons, including: carrier screening, which involves identifying unaffected individuals who carry one copy of a gene for a disease that requires two copies for the disease to be expressed, preimplantation genetic diagnosis, prenatal

diagnostic testing (amniocentesis &CVS),new-born screening, presymptomatic testing for predicting adult-onset, presymptomatic testing for estimating the risk of developing adult-onset cancers and Alzheimer's disease, confirmational diagnosis of a symptomatic individual. Diagnostic test are of two types: Non-invasive techniques: Fetal echocardiography, Fetal visualization Ultrasound, Screening for neural tube defects (NTDs) - Measuring maternal serum alpha-fetoprotein (MSAFP),Screening for fetal Down syndrome Measuring MSAFP, measuring maternal serum beta-human chorionic gonadotropin (HCG) and Invasive techniques :Fetal tissue sampling Amniocentesis,

Chorionic villus sampling (CVS),Percutaneous umbilical blood sampling (PUBS),Percutaneous skin biopsy, Preimplantation biopsy of blastocysts obtained by in vitro fertilization, Cytogenetic investigations, Detection of chromosomal aberrations, Fluorescent in situ hybridization, Restriction fragment length polymorphisms (RFLPs),Single nucleotide polymorphisms (SNPs) .Many of the risks associated with genetic testing involve the emotional, social, or financial consequences of the test results. Major limitation is the lack of treatment strategies for many genetic disorders once they are diagnosed.

Diagnosis of Diabetes

Dr. Sangeeta Bhagat
Head, Dept. of Biotechnology, Modern college, Shivajinagar, Pune

Diabetes is defined as a state in which homeostasis of carbohydrate and lipid metabolism is improperly regulated by insulin. This results in elevated fasting and postprandial glucose levels. If this imbalance does not return to normalcy and continues for protracted period of time, it leads to hyperglycaemia that in due course turns into a syndrome called "Diabetes mellitus". There are two main categories of this disease. Type one diabetes mellitus called as insulin dependent diabetes mellitus (IDDM) and type two the non-insulin dependent diabetes mellitus (NIDDM).Diabetic hyperglycaemia causes variety of pathological changes in small vessel, arteries and peripheral nerves. Symptoms of diabetes are Polydipsia, polyuria and polyphagia.

Diabetes is diagnosed using different methods, by measuring plasma glucose level, by oral glucose tolerance test (OGTT) and glycosylated haemoglobin. If fasting BGL is >100 but <126 mg/ml condition is called impaired fasting Glucose (IFG). This condition is called prediabetes. Oral glucose Tolerance Test measures blood glucose after a person fasts at least 8 hours and 2 hours after the person drinks 75 gm. of a glucose-containing beverage. If BSL after a drink is around 200 mg/dl, patient is diabetic. Glycosylated Hb is best for diabetes follow up than to diagnose the diabetes good indicator of blood glucose control. 8% or greater indicate BSL is too high.

Research area of diabetes are Oxidative stress, Measurement of AGEs, Measurement of free radicals, Use of natural dietary antioxidants and herbal medicine in prevention of diabetic complications.