UNIVERSITY OF KERALA

POST GRADUATE DIPLOMA IN

MOLECULAR DIAGNOSTICS

Scheme and Syllabus

w.e.f. 2015 admission
About the course

Molecular Diagnostics are the tools based on the principles of Molecular Diagnosis. It is the process of identifying a disease by understanding the molecules, such as proteins, DNA, and RNA, in a tissue or fluid, which forms the markers of the diseases directly or indirectly. Molecular diagnostics is a new discipline that captures genomic and proteomic expression patterns and uses the information to distinguish between two or more conditions at the molecular level. The conditions under investigation can be human genetic disease or infectious diseases. Molecular diagnostics is not confined to human diseases but can be used in animals and plants also. Molecular diagnostics can also be used to identify foodstuffs, vegetables, meet types and food processing methods etc., and can be also used in environmental monitoring, detect the presence of specific microorganisms in various samples including food materials. The course will provide the theory and use of molecular techniques in the diagnostic techniques, with more importance on nucleic acids based techniques. Common analytical techniques and molecular techniques related to the development and use of diagnostics such as polymerase chain reaction (PCR), quantitative real time PCR (qRT-PCR), microarray analysis, chromosomal techniques, hybridization techniques and DNA bioinformatic tools will be emphasized. The laboratory exercises are designed to provide a hands-on context for the topics being presented in the syllabus.

Objectives of the Course

The course focuses on learning and understanding how the various molecular techniques that were studied in other classes can be developed and utilized in diagnosis and sold in diagnostic kits. Many of the diagnostic techniques are developed and marketed as Diagnostic Kits by biotechnology companies. The main source of information is web sites of such companies that develop and market the molecular diagnostic kits, which they continuously develop.

Eligibility for admission

A first class with 60% M. Sc., Degree in Biotechnology, Botany, Zoology, Biochemistry, Microbiology, Genomic Sciences, Agriculture, Veterinary Sciences, Fisheries Sciences, Pharmacy or M.Tech., in Biotechnology, or Post graduate degree in a branch of Life Sciences, Medical Sciences or equivalent. A minimum of 50 % marks or equivalent for SC/ST candidates.

Mode of Selection

Selection will be made based on the basis of a National entrance test conducted by the University and Reservation Policy stipulated by the Government from time to time. A total of 10 students will be selected each year.

Course structure and Duration

The course consists of two semesters and shall extend over a period of 12 months, including the examinations.

5. Examination and Evaluation

The conductance of the examinations and evaluations will be as per the general regulations of the University adopted for the PG Diploma Courses. Each Theory course carries 3 credits and practical course carries 5 credits.
For each course, 40% of the total marks will be allotted to continuous assessment or internal assessment, and 60% for End semester examination by the University. The internal assessment (CA) for each course will be carried out by the concerned faculty member or the course instructor.

The break up for Continuous Assignment will be as follows:

a) **For Theory Paper**

<table>
<thead>
<tr>
<th>Component</th>
<th>Marks</th>
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<tbody>
<tr>
<td>Mid Sem Examination</td>
<td>15</td>
</tr>
<tr>
<td>Seminar / Tests / Quiz</td>
<td>10</td>
</tr>
<tr>
<td>Assignment</td>
<td>10</td>
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<tr>
<td>Attendance</td>
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b) **For Practical Course**

<table>
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<tbody>
<tr>
<td>Mid Sem Examination</td>
<td>15</td>
</tr>
<tr>
<td>Performance of experiments And Results</td>
<td>10</td>
</tr>
<tr>
<td>Viva</td>
<td>10</td>
</tr>
<tr>
<td>Maintenance of records</td>
<td>05</td>
</tr>
<tr>
<td><strong>Total</strong></td>
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</table>

**Project Work evaluation**

a) Project Presentation and Viva 100 Marks (50+ 50)

c) Dissertation 100 Marks

**Total** 200 Marks

Project Presentation, Viva and evaluation of the Dissertation will be done by a committee composed of all Faculty members of the course Evaluation and External Examiner.

**Declaration of final results**

1. The marks obtained in each course, CA (Internal) and ESA (Final University Examination) will be entered separately as general procedures of the University.

2. A candidate shall be declared to have passed in each course if he/she secures not less than 40% marks in the ESA (University Examination) and 40% marks in the CA (Internal Assessment) and not less than 50% in the aggregate, taking CA and ESA marks together.

3. The final results will be based on total marks obtained by adding CA and ESA together for each Candidates. Successful candidates passing the examinations and earning 80 % or above shall be declared to have passed in First Class with distinction and those who earned marks 60 % or above will be declared to have passed in First class. Those who have scored below 60 % shall be declared to have passed in Second Class. Candidates earning Marks below 50 will be declared Failed.
Course Structure and Scheme of evaluation

<table>
<thead>
<tr>
<th>Sl. No.</th>
<th>Course Code</th>
<th>Course Title</th>
<th>Credits</th>
<th>CA</th>
<th>ESA</th>
<th>Total</th>
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<tr>
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<td>MD 511</td>
<td>Introduction to Molecular Diagnostics</td>
<td>3</td>
<td>40</td>
<td>60</td>
<td>100</td>
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<tr>
<td>2</td>
<td>MD 512</td>
<td>Human Genetics and Genomics</td>
<td>3</td>
<td>40</td>
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<td>100</td>
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<td>3</td>
<td>MD 513</td>
<td>Biochemistry, Molecular Biology and Immunology in Diagnosis</td>
<td>3</td>
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<td>60</td>
<td>100</td>
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<td>4</td>
<td>MD 514 (MD 521)</td>
<td>Molecular diagnostics- I (Nucleic acid and Protein based diagnostics)</td>
<td>5</td>
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<td>100</td>
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<tr>
<td>5</td>
<td>MD 515 (MD 522)</td>
<td>Molecular diagnostic II (Genetic &amp; Cytologic techniques)</td>
<td>5</td>
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<td>7</td>
<td>MD 524</td>
<td>Viva Voce</td>
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<td><strong>25</strong></td>
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<td><strong>700</strong></td>
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</tbody>
</table>

Detailed Syllabus

Semester I

MD 511 Introduction to Molecular Diagnostics

Module 1

- Philosophy and general approach to clinical specimens, Sample collection- method of collection, transport and processing of samples, Interpretation of results, Normal microbial flora of the human body, Host - Parasite relationships.

Module 2

- Diagnosis of DNA and RNA viruses- Pox viruses, Adenoviruses, Rhabdo Viruses, Hepatitis Viruses and Retroviruses.

Module 3

Cancer- different types of cancers, genetics of cancer- oncogenes, tumour suppressor genes. Methods available for the diagnosis of genetic diseases and metabolic disorders.

Genetic disorders- Sickle cell anemia, Duchenne muscular Dystrophy, Retinoblastoma, Cystic Fibrosis and Sex – linked inherited disorders.


Blood (formation, composition, function and pathology of blood disorders (haemoglobinopathies, sickle cell anemia, hemophilia), Muscle disorders (Duchene muscular dystrophy-DMD, Becker’s muscular dystrophy-BMD, spinal muscular atrophy-SMA), Bone disorders (Osteogenesis imperfecta, Rheumatoid arthritis), Skin disorder (Albinism), Eye disorder (Retinitis pigmentosa)

References
11. Expert Review of Molecular Diagnostics

Semester 1

MD 512 Human Genetics and Genomics

Module 1

Principles of inheritance- Mendelian and Non-Mendelian inheritance, Polygenic inheritance, Multifactorial trait, Threshold trait, Genetic Susceptibility & risk factors, Mitochondrial genome and disorders.

Medical Genetics: Human genome Project, Genome Organization, Genome Annotations and databases, Identifying human disease genes. Genetic markers for diseases.( microsatellites, SNPs), Pharmacogenomics, Ecogenomics, Metabolomics, Teratogenetics
- Mapping and identification of disease genes (linkage analysis, LOD score, association study) SNPs in diagnostics

Module 2

- Human Cytogenetics - Chromosomal basis of inheritance, sex chromosome, X-chromosome inactivation, Basics of cell culture, Techniques of cell cultures (short term lymphocyte, primary and secondary cell cultures, maintenance of cell lines) Techniques of chromosome analysis
- Chromosome preparation from cultured lymphocytes, cell lines and solid tumors, Karyotyping, C-, G-banding and fluorescence banding, nomenclatures of bandings, In-situ hybridization techniques – FISH and GISH,
- Meiotic chromosomes- Chromosomal anomalies and disorders, Numerical variations (polyploidy, aneuploidy, autosomal, sex-chromosomal) Structural variations (deletion, duplication, translocation, inversion, isochromosome, ring chromosome) Chromosomal abnormalities in cancer.

Module 3

- Genetic disorders: Classification of genetic disorders, Single gene Disorders - Sickle cell anaemia, Duchenne muscular Dystrophy, Retinoblastoma, Cystic Fibrosis and Marfan’s syndrome Multifactorial disorders - Diabetes, Atherosclerosis, Schizophrenia
- Sex – linked inherited disorders.
- Cancer genetics- Molecular basis of cancer, oncogenes, tumour suppressor genes. Gene therapy and other molecular based therapeutic approaches.. Genetic Counseling, Ethical and legal issues in genetic counseling

Module 4

- Molecular Techniques for diagnosis - PCR- RFLP, ARMS-PCR, ELISA, Multiplex-PCR, SSCP, CSGE, DGGE, DHPLC, MALDI-TOF, DNA Sequencing
- Disease identification and Genetic tests for following disorders- Thalassemia, Fanconi anemia, Sickle Cell anemia, Fragile-X syndrome, Alzheimer’s disease, Duchenne Muscular Dystrophy/ Becker’s Muscular Dystrophy, Huntington’s disease
- Allelic susceptibility test for multifactorial disorders (Neural Tube Defect, Cleft Lip and Palate, Cardio Vascular Disorder, Male infertility)
- Risk evaluation (Mendelian risk, empirical risk), Prenatal and pre-implantation diagnosis. Non-invasive: Triple test, Ultrsonography (USG), Invasive: Amniocentesis (AC), chorionic villi sampling (CVS), Fetal blood sampling (FBS), Population screening for genetic disorders,
- Treatment and management of genetic disorders.

References

5. DNA from A to Z & Back Again; Carol A. Holland and Daniel H. Farkas; AACC Press 2008 159425088X $30.00
16. Principles of Molecular Pathology; Anthony A. Killeen; Humana Press 2003 ISBN: 1588290859 $120.00
18. Human Molecular Genetics 3; Tom Strachan and Andrew P. Read; Garland Science 2003 ISBN: 0815341822 $104.95

**Semester 1**

**MD 513. Biochemistry, Molecular Biology and immunology in Diagnosis**

**Module 1**
- Biochemical tests for detection and quantification of sugar, albumin, urea, protein, globulin, vitamin
- Biochemistry and diagnostic tests of following diseases - Duchenne Muscular Dystrophy (DMD) (Creatine phosphokinase-CPK) , Phenylketonuria-PKU (phenylketone), G6PD deficiency syndrome (G6PD) , Mucopolysaccharidosis, Endocrine disorders related to thyroid and reproduction (TSH, T3, T4, Estradiol, Testosterone, LH, FSH)

**Module 2**
- Nucleic acid amplification methods and types of PCR: Reverse Transcriptase-PCR, Real-Time PCR, Inverse PCR, Multiplex PCR, Nested PCR, Alu-PCR, Hot-start, In situ PCR, Long-PCR, PCR-ELISA, Arbitrarily primed PCR, Ligase Chain Reaction.
- Proteins and Amino acids, Qualitative and quantitative techniques: Protein stability, denaturation; amino acid sequence analysis.
- Hybridization techniques – Southern, Northern, in-situ (including FISH), microarrays – types and applications; Protein extraction and analysis (including PAGE and its variations); Western Blot

**Module 3**
- Applications of PCR- PCR based microbial typing: Eubacterial identification based on 16S rRNA sequences- Amplified Ribosomal DNA Restriction analysis (ARDRA)-Culture independent analysis of bacteria- DGGE and TRFLP. Molecular diagnosis of fungal pathogens based on 18S rRNA sequences- Detection of viral pathogens through PCR. RAPD for animal and plants.
- PCR in forensic science- AmpFLP, STR, Multiplex PCR- Determination of Paternity- Human identification and sex determination.
Module 4
- Automated DNA sequencing- Principles, Methods and Instrumentation- Advances in DNA sequencing- New Generation sequencing Methods, Pyrosequencing
- Microarrays- Personalised Medicine- Pharmacogenomics.
- Levels for rDNA experiments. Biosafety aspects of transgenic plants and germplasm.

Module 5
- Proteomics- Clinical Proteomics.
- Overview of immune system , Antigens and antibodies , Antigen-antibody interactions, Major Histocompatibility Complex (MHC), HLA typing , Immunotherapy and immunodiagnostics
- Immunodiagnostics - Introduction, antigen-antibody binding interactions and assays; antibodies-polyclonal and monoclonal antibodies,
- Immunoassays – types [RIA, ELISA, ChemiluminescentIA, FIA] and specific applications; Immunohistochemistry – principle and techniques. Good Laboratory Practices.
- Different Levels of Biosafety, Containment,

References
1. Genes IX  by B. Lewin, Oxford University Press.
5. rDNA safety guidelines- Government of India, Ministry of Science and Technology, Dept.of Biotechnology, New Delhi.
6. rDNA safety guidelines & regulations-Government of India, Ministry of Science and Technology,Dept.of Biotechnology, New Delhi.
Practical Course

MD 514 Molecular diagnostics- I (Nucleic acid and Protein based Diagnostics)

List of experiments

1. Hormone assay for thyroid (TSH, T3, T4) and
2. Sexual disorders (testosterone, dihydrotestosteron, estradiol, FSH, LH by RIA)
3. Isolation of Genomic DNA from microbe (E.coli), Plant (Bacopa monnieri), Animal (C. elegans) and Human (Peripheral Blood).
4. Isolation of Metagenome (sediment/soil).
5. Plasmid DNA isolation by Alkaline lysis and Boiling method.
6. Quality / Quantity checking of Nucleic acids by a) UV Spectrophotometer and b) Agarose Gel Electrophoresis.
7. Molecular Cloning- ligation
8. Competent cell preparation
9. Transformation- selection of recombinants (Blue white selection)
10. Confirmation of recombinants by gel electrophoresis.
11. Nucleic acid labeling and Southern Hybridization
12. Automated DNA sequencing (Demo).
13. RNA isolation (from rat liver) and Pulsed Field Gel Electrophoresis
14. PAGE - Denaturing gradient gels, Temperature gradient gels
15. and Western Blot
16. DNA sequencing , Automated DNA Sequencing PCR
17. Identification of human bacterial pathogens by Polymerase chain reaction,
18. PCR based diagnosis of plant bacterial pathogen
19. Culture independent analysis of microbes by DGGE (Denatured Gradient Gel Electrophoresis) and T-RFLP (Terminal Restriction Fragment Length Polymorphism).
20. Molecular diagnosis of parasitic disease
21. Detection of transgenes in GMOs
22. PCR amplification using primers that amplify the regions consisting of dinucleotide repeats, trinucleotide repeats and that detects restriction enzyme polymorphic sites, Alu ins/del sites.
23. Amplification of Short Tandem Repeats (STR)/Microsatellites.
24. Multiplex STR PCR (Demo).
26. HLA typing and tissue transplantation matching (Demo)
27. Microarrays for pathogen detection and SNP studies (Demo).
28. Bioinformatic tools for genome and proteome analysis.

**Practical II**

**MD 515 Molecular diagnostic II (Genetic & Cytologic techniques)**

**List of Experiments**

1. Metaphase chromosome preparations from bone marrow of mouse, rat, cultured Lymphocytes
2. Chromosome preparation from lymphocyte culture / mouse bone marrow
3. Karyotype using Human lymphocyte culture
   a. Q-banding
   b. G-banding
   Fluorescence *in-situ* Hybridization (FISH)
4. Automated Karyotyping
5. Sex chromatin (buccal mucosa, hair bud)
6. Comet assay
7. Micronucleus assay – bone marrow of mouse and cultured lymphocytes
8. Chromosome preparation from chorionic villi, stem cells, cell line
9. Sister Chromatid Exchange (SCE)
10. Study of Chromosomal Aberrations in Mice
11. Meiotic Chromosome preparations from testis
12. Flowcytometry.

**Proteome analysis**

15. Characterization of Proteins by SDS-PAGE and 2D Gel Electrophoresis
16. Purification of proteins by HPLC
17. Immunological methods- Agglutination (ABO/Bacterial),
18. Precipitation, Immunodiffusion, Immunoelectrophoresis

**Molecular Diagnosis of human genetic disorders**

19. Beta thalassemia
20. Checking of p53 gene polymorphism for susceptibility to cancer
21. Down’s Syndrome
22. Retinitis pigmentosa
23. Human identification and paternity determination (simulated) by VNTR Probes
24. Mutation detection methods
25. Functional protein identification- 2D gels
26. Forensic Application – Paternity Testing, victim Identification, crime detection,
MD 521 Project Dissertations

Each of the students has to undertake a project during the first semester itself and should be completed in the end of second semester. They have to submit the dissertation at the end of 2nd semester for evaluation. Each student has to present their work before a committee consisting of the faculty members and an external expert as an examiner.

MD 522 General Viva voce

The committee constituted for evaluating the project dissertation also has to conduct the General viva voce examination along with the Project presentation. They have to submit the mark list to the chairman of the examination Board, who will the Department head, or the Director of the Centre or Institute.
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