

1st Semester Syllabus (Theory)

◆ PGD-101: Biochemical Techniques in diagnostics (4 Credits=100 Marks Each)

Unit-I: Introduction and History of diagnostics:

Biochemical tests for detection and quantification of sugar, albumin, urea, protein, globulin, and vitamins. Importance of molecular biology in diagnostics. Various types of storage and shipping/ transport procedures for clinical samples. Collection procedure for clinical samples.

Unit-II: Isolation and Purification of Nucleic acids:

Principles and Methods of Nucleic Acid isolation. Role of different reagents in isolation process of DNA and RNA. Isolation of bacterial DNA. Isolation of DNA and RNA from eukaryotic cells (Human). Complementary (c) DNA synthesis from RNA. Analyze the migration pattern of various types of DNA (genomic and plasmid) on Agarose gel.

Unit-III: Molecular cloning:

Gene cloning, labelling of nucleic acids, and hybridization. Introduction of restriction enzymes, palindromic sequence, isoschizomers. TA cloning, blunt-end cloning, staggered end cloning. Types of labels for nucleic acid probes: radioactive and fluorescent labels. Body and end labelling of nucleic acid probes. Northern blotting: experimental set-up, reagents used and its application. Southern blotting: experimental set-up, reagents used and its application. Western blotting: primary and secondary antibody, detection methods and its application.

Unit-IV: Molecular Techniques for Nucleic Acid and Protein Detection:

PCR, Multiplex-PCR, RFLP, DNA fingerprinting, Southern Blotting, Electrophoresis techniques, HPLC, MALDI- TOF, DNA Sequencing. Introduction to primer designing. PCR and qPCR principle, components and procedure. Concept of T_m (melting temperature of DNA), hybridization, annealing, extension by Taq polymerase. Proof reading activity of DNA polymerases. Sanger sequencing.

Concept of multiplexing: using more than one primer pair in PCR reaction. Sybr green and TaqMan probe principle and applications. Agarose and SDS-PAGE electrophoresis. Chromatography method of separation and detection: high performance liquid chromatography. Identification of a molecules by mass spectrometry: introduction to the technique, its applications in diagnostics and basic interpretation of the mass spectrometer graphs.

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Text Book:

1. Genomes 4, 4th Edition, T. A. Brown (2017). CRC Press. ISBN: 9780815345084.

Reference Books:

1. Parasitology (1997). Chatterjee K.D, Chatterjee Medical Publishers. ISBN: 108123918100.
2. Medical Microbiology (1997). Edited by Greenwood, D, Slack, R and Peutherer, J, ELST Publishers. ISBN: 9780702040894.
3. Bailey & Scott's Diagnostic Microbiology (2002). Betty A. Forbes, Daniel F. Sahm, Alice S. Weissfeld, Ernest A. Trevino, Published by C.V. Mosby. ISBN: 9780323075022.
4. Fundamentals of Molecular Diagnostics (2007). David E. Bruns, Edward R. Ashwood, Carl A. Burtis. Saunders Group. ISBN-13: 978-1-4160-3737-8.

◆ PGD-102: Molecular Genetics and Immunodiagnostics (4 Credits=100 Marks)**Unit-I: Introduction to Human Genetics:**

Chromosome and DNA structure and function. Cell cycle and Cell division: G1, S, G2, M phases, and Check points. Mitosis, Stages, mitotic apparatus, cytokinesis, mitogens and inhibitors, and significance. Meiosis, Stages, synaptonemal complex, crossing over and chiasma formation, and significance. Laws of inheritance: Mendel's Laws, concept of dominance, segregation, independent assortment, Concept of alleles, types of dominance, lethal alleles, multiple alleles. Linkage, concepts of linkage, recombination, gene mapping, sex-linked inheritance, quantitative inheritance, and cytoplasmic inheritance, mutations, diseases and disorders.

Changes in Chromosome number and structure: Polyploidy, aneuploidy, chromosomal rearrangements - deletion, duplication, inversion, and translocation.

Unit-II: Cell culture and Chromosome Study:

Cells culture, induction of metaphase, cell cycle arrest, chromosome staining and visualization of chromosome. Chromosome karyotyping, chromosome banding (G-banding, C-banding, R-banding etc), chromosome labeling, in situ hybridization (Fluorescence in situ hybridization), chromosome painting, comparative genome hybridization (CGH).

Unit-III: Introduction to Immunology:

Overview of immune system. Antigens: mitogens, hapten, immunogen, adjuvants. Antigen presenting cells, Natural Killer cells, Mast cells, Dendritic cell. Antibodies: structure of

antibody, Classification, Isotypes. B and T cells. Innate and acquired immunity. Humoral and cell mediated immune response. Major Histocompatibility Complex (MHC): Role of MHC, Structure of MHC molecule, binding of peptides to MHC molecules, MHC restriction. Human leukocyte antigen (HLA) typing, Immunotherapy and immunodiagnostics.

Unit-IV: Immunodiagnostics:

Immunodiagnostics – Introduction, antigen-antibody interaction, polyclonal and monoclonal antibodies. Immunoassays – types [Radioimmunoassay (RIA), ELISA (enzyme-linked immunosorbent assay). Chemiluminescent Immunoassays, Fluorescent Immunoassays (FIA)] and specific applications. Immunohistochemistry – Principle and techniques. Good Laboratory Practices.

Text Book:

1. Basic Concepts of Molecular Pathology Series: Molecular Pathology Library (2009). Cagle, Philip T. Allen, Timothy C. (Eds.); Springer, Softcover. ISBN: 9780387896250.

Reference Books:

2. Molecular Pathology: The Molecular Basis of Human Disease (2009). William B. Coleman, Gregory J. Tsongalis (Eds.); Academic Press 1 edition. ISBN 10: 0123744199 ISBN 13: 978- 0123744197.
3. Medical Genetics (2009). Lynn B. Jorde, John C. Carey, and Michael J. Bamshad, Mosby. 4th Edition. ISBN: 9780323053730.
4. Molecular Microbiology: Diagnostic Principles and Practice (2003). David H. Persing, Fred C. Tenover, James Versalovic, Yi-Wei Tang, Elizabeth R. Unger, David A.; Relman, and Thomas J. White, (Eds.) ASM Press. ISBN: 155581221X.
5. Proteins: Biochemistry and Biotechnology (2014). Gary Walsh. Wiley-Blackwell 2nd Edition. ISBN: 978-0-470-66985-3.
6. Protein Electrophoresis, Methods and Protocols (2012) Editors: Kurien, Biji T., Scofield, R. Hal (Eds.), Springer Protocols. ISBN 978-1-61779-821-4.
7. Principles of Immunology and Immunodiagnostics (1988). Ralph Michael Aloisi. Lippincott Williams and Wilkins. ISBN-10: 0812111338; ISBN-13: 978-0812111330

◆ **PGD-103: Data management, Analysis and Statistics (4 Credits=100 Marks)**

Unit-I: Introduction to Bio-statistics:

Descriptive Statistics. Measures of central tendency: Mean (Sample mean, Population mean and Weighted mean), Median, Mode. Measures of dispersion: Range, Variance

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(Sample and population), Standard deviation, Standard errors, Covariance. Displaying Descriptive statistics: Histogram and Frequency distributions. Probability distributions: Normal (Dispersion percentages & Z- score) and Binomial distribution. Inferential statistics: Hypothesis testing (Null hypothesis), p-value, χ^2 test, t-test (one sample t-test and paired sample t-test), Principal component analysis.

Unit-II: Data analysis:

Curve fitting: Straight line, Exponential and Logarithmic fitting and its application. Linear regression and Pearson correlation coefficient.

Unit-III: Programming for data analysis:

Shell scripting – File sorting, logical & mathematical operators. Python programming for data handling and curve fitting.

Unit-IV: Application of statistics in biological data:

Hands on sessions for handling and analysing the data sets acquired using following assays. Protein quantification assay through Western blotting. Quantitative real time PCR (qRT-PCR). Pair t-test analysis for Cytotoxic assay.

Text Book:

1. The Complete Idiot's Guide to Statistics (2007). Robert A. Donnelly Jr., Ph.D. Alpha publishing. 2ndedition. ISBN:1592576346.

Reference Books:

1. Medical Biostatistics (2017). Abhaya Indrayan & Rajeev Kumar Malhotra. Chapman and Hall/CRC. 4thedition. ISBN: 9781498799539.
2. Essential Biostatistics: A Nonmathematical Approach (2015). Harvey Motulsky. OXFORD University Press. 4thedition. ISBN: 0199365067.
3. Programming python. Mark Lutz (2010). O'Reilly Media. 4thedition. ISBN:9780596158101.
4. Classic Shell Scripting: Hidden Commands that Unlock the Power of Unix (2005). Arnold Robbins and Nelson H. F. Beebe. 1stedition. ISBN: 059600595-4.

◆ PGD-104: OMICs based diagnostics (4 Credits=100 Marks)

Unit-I: Introduction to Genomics:

Genomes of Prokaryotes & Eukaryotes; Prokaryotic and Eukaryotic gene; Open Reading Frame (ORF); Genetic Code; Genome organization; Human Genome Project; Common, Rare and Structural variation of genome; Sequence alignment methods (PUBMED, BLAST, CLUSTALW).

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Unit-II: Introduction to Next-Generation Sequencing (NGS):

Next-Generation Sequencing platform. NGS based methods (ChIP-seq, Metagenomics, Epigenomics & Exome Sequencing). NGS Sequencing Depth and Coverage. Genome Mapping & Annotation. Genome Databases (UCSC, ENCODE, etc.). NGS Data analysis pipelines (Galaxy).

Unit-III: Introduction to Transcriptomics:

Transcription in prokaryotes and Eukaryotes; Gene regulation. Exon and introns. Splicing and Splicing variation. Comparative Genomic Hybridization. RNA-seq method.

Unit-IV: Introduction to Proteomics:

Translation in prokaryotes and Eukaryotes. Overview of Mass spectroscopy. Gene Ontology; Biological pathways database (KEGG); Gene Ontology and Gene enrichment analysis; Network analysis (PANTHER).

Text Books:

1. Introduction to Genomics (2017). Arthur Lesk. Oxford University Press, 3rd Edition. ISBN: 9780198754831.
2. Genomes 4 (2017). T. A. Brown. CRC Press, 4th Edition. ISBN 9780815345084.

Reference Books:

1. Bioinformatics for Omics Data. Methods and Protocols (2011). Mayer Bernd (Ed). Springer. ISBN 978-1-61779-027-0.
2. Next Generation Sequencing, Methods and Protocols (2018). Steven R. Ordoukhanian, Phillip, Salomon, Daniel R. (Eds.). Springer, 4th Edition. ISBN 978-1-4939-7514-3.
3. Metagenomics: Theory, Methods and Applications (2010). Diana Marco. Caister Academic Press. ISBN-13: 978-1904455547.
4. Integration of Omics Approaches and Systems Biology for Clinical Applications (2018). Antonia Vlahou, Harald Mischak, Jerome Zoidakis, Fulvio Magni (Eds). Willy Online Library. ISBN:9781119181149.

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1st Semester Syllabus (Practicals)

◆ PGD-105: Molecular Genetics and Immunodiagnosics (2 Credits=50 Marks)

Unit-I:

- (i) Good Laboratory Practices.
- (ii) Biosafety laboratory (BSL-1 to IV) levels for cell culture.
- (iii) Handling of cells in Laminar flow with negative and positive pressure.
- (iv) Reagent preparation, buffer preparation, micro-pipetting.
- (v) Measurement of pH of solutions, molarity, normality and molality calculation and graph plot.
- (vi) Handling and use of centrifuges, sample collection, handling and storage.

Unit-II:

- (i) Mass spectrometry basics and overview of the instrument.
- (ii) How to perform mass analysis and reagent ion selection.
- (iii) How to perform analyte ionisation analyte quantitation.
- (iv) Methods for interpretation of mass spectra.

Unit-III:

- (i) How to perform Genomic DNA isolation from eukaryotic cells.
- (ii) Quantitation of genomic DNA using Nanodrop method.
- (iii) How to perform total cell RNA isolation from eukaryotic cells.
- (iv) Quantitation of total RNA using Nanodrop method.
- (v) Eukaryotic cell total and gene specific cDNA synthesis.
- (vi) Gel electrophoresis of DNA and RNA.

Unit-IV:

- (i) Hands on training on ENSEMBL for DNA sequence retrieval, exon and intron identification.
- (ii) Transcript sequence identification of a gene using ENSEMBL.
- (iii) Various methods for designing genomic DNA and cDNA primers.
- (iv) Polymerase Chain Reaction (PCR) of genomic DNA and cDNA samples.
- (v) Quantitative Real time PCR (qPCR) of genomic DNA and cDNA samples.
- (vi) Reverse Transcriptase PCR (RT-PCR).
- (vii) Multiplex qPCR for quantitation of gene expression and SNP detection.

Text Book:

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1. Wilson and Walker's Principles and Techniques of Biochemistry and Molecular Biology (2018). 8th edition. Andreas Hofmann, Samuel Clokie. ISBN: 9781108365253.

Reference Books:

2. PCR Technology. Principles and Applications for DNA Amplification (1989). Henry A. Erlich. 978-0-333-48948-2.
3. Mass Spectrometry, A Textbook (2017). 3rd edition. Authors: Gross, Jürgen H. ISBN 978-3-319-54398-7.
4. Good Laboratory Practice Regulations (2007). 4th edition. Sandy Weinberg. ISBN 9780849375835.

◆ **PGD-106: Molecular Genetics and Immunodiagnosics (2 Credits=50 Marks)**

Unit-I:

- (i) Mammalian cell culture.
- (ii) Preparation of Dulbecco's modified Eagle' medium (DMEM).
- (iii) Activation of Fetal Bovine Serum (FBS).
- (iv) Growing the hepatoma cells and passaging hepatoma cells.

Unit-II:

- (i) Preparation of phosphate buffer saline (PBS).
- (ii) Preparation of NP40 lysis buffer or radioimmunoprecipitation assay buffer (RIPA buffer).
- (iii) Preparation of protein estimation reagents (Lowry/Bradford).
- (iv) Hepatoma cells washing and harvesting.
- (v) Centrifugation of cells followed by washing the pellets with PBS buffer.

Unit-III:

- (i) Cellular protein lysates preparation in lysis buffer.
- (ii) Standard graph preparation using different concentration of bovine albumin serum (BSA).
- (iii) Cellular protein quantification with the help of standard BSA graph.
- (iv) Preparation of acrylamide and bis-acryamide solution.
- (v) Preparation of Tris buffer and sample buffer.
- (vi) Sodium Dodecyl Sulfate Poly Acrylamide Gel preparation.
- (vii) SDS-PAGE running buffer preparation.
- (viii) SDS gel running and Coomassie Brilliant Blue Dyes (CBB) staining.

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Unit-IV:

- (i) Western Blotting: Sodium dodecyl sulphate-polyacrylamide gel electrophoresis (SDS-PAGE) for the separation of proteins based on their molecular weight.
- (ii) Protein transfer from gel to nitrocellulose membrane followed by blocking in BSA or milk.
- (iii) Primary antibody incubation followed by secondary antibody incubation.
- (iv) Desired protein detection and analysis.

Text Book:

1. Basic Concepts of Molecular Pathology Series: Molecular Pathology Library (2009). Cagle, Philip T. Allen, Timothy C. (Eds.); Springer, Softcover ISBN: 9780387896250.

Reference Books:

1. Molecular Pathology: The Molecular Basis of Human Disease (2009). William B. Coleman, Gregory J. Tsongalis (Eds.); 1st edition. ISBN 10: 0123744199 ISBN 13: 978-0123744197.
2. Medical Genetics (2009). Lynn B. Jorde, John C. Carey, and Michael J. Bamshad, Mosby. 4th Edition. ISBN: 9780323053730.
3. Molecular Microbiology: Diagnostic Principles and Practice (2003). David H. Persing, Fred C. Tenover, James Versalovic, Yi-Wei Tang, Elizabeth R. Unger, David A. Relman, and Thomas J. White, (Eds.). ISBN: 155581221X.
4. Proteins: Biochemistry and Biotechnology (2014). 2nd Edition (Wiley-Blackwell), Gary Walsh. ISBN: 978-0-470-66985-3.
5. Protein Electrophoresis, Methods and Protocols (2012). Editors: Kurien T. Biji, Scofield R. Hal (Eds.), Springer Protocols, ISBN 978-1-61779-821-4.

◆ PGD-107: Data management, Analysis and Statistics (2 Credits=50 Marks)

Unit-I:

- (i) Bio-statistics using Excel.
- (ii) Hands on training to use Microsoft excel for data analysis.
- (iii) File handling (creating, exporting the data from text to excel spreadsheet and saving the file).
- (iv) Data analysis based on measures of central tendency: Mean, median and mode for the different sets of data.
- (v) Data analysis based on measures of Dispersion: Variance, Standard deviation, Standard error for the different sets of data.
- (vi) Pearson correlation coefficient for finding out association between variables.

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Unit-II:

- (i) Data representation and analysis using Excel.
- (ii) Creating and analyzing histogram for different sizes of bins and ranges.
- (iii) Data (scatter) plotting and curve fitting (linear, exponential and logarithmic).
- (iv) Statistical tests – t-test, χ^2 test.

Unit-III:

- (i) Using bioinformatics tools.
- (ii) Familiarizing with EMBL-EBI software suites.
- (iii) Hands on sessions on sequence alignment and analysis using ClustalW, BLAST and MUSCLE.
- (iv) Introduction to data bases such as KEGG, NCBI for biological data analysis.

Unit-IV:

- (i) Data handling and analysis using Linux and Python.
- (ii) Shell scripting – File sorting, usage of logical & mathematical operator for data analysis.
- (iii) Python for Data handling, plotting, fitting.

Text Book:

1. The Complete Idiot's Guide to Statistics (2007). Donnelly Jr., Ph.D. Alpha publishing. 2ndedition Robert A. ISBN:1592576346.

References Books:

1. Medical Biostatistics (2017). Abhaya Indrayan & Rajeev Kumar Malhotra. Chapman and Hall/CRC. 4thedition. ISBN: 9781498799539.
2. Essential Biostatistics: A Nonmathematical Approach (2015). Harvey Motulsky. OXFORD University Press. 4thedition. ISBN: 0199365067.
3. Programming python (2010). Mark Lutz. O'Reilly Media. 4thedition. ISBN:9780596158101.
4. Classic Shell Scripting: Hidden Commands that Unlock the Power of Unix (2005). Arnold Robbins and Nelson H. F. Beebe. 1stedition. ISBN: 059600595-4.

◆ PGD-108: OMICs based diagnostics (2 Credits=50 Marks)**Unit-I:**

- (i) How can you identify Open Reading Frame in a stretch of DNA sequence using NCBI ORF finder?
- (ii) What is FASTA format and how can you generate a FASTA sequence file from a given stretch of DNA or Amino acid sequence.

- (iii) Sequence Manipulation (a) combine FASTA file, (b) Filter DNA and Protein sequence, (c) Gene Bank to FASTA file, (d) Split FASTA file and (e) Generate complementary DNA strand using bioinformatics resources.
- (iv) Sequence Analysis (a) Codon Usage (b) CpG island (c) DNA stat (d) Identity and Similarity between sequence (e) Translate a DNA sequence and (f) shuffle a DNA sequence using bioinformatics resources.
- (v) Performing comparative pairwise sequence analysis of DNA or Amino acid sequence using BLAST and CLUSTALW.
- (vi) How to access information about genome, gene, variation in genome including SNP using PUBMED database.

Unit-II:

- (i) How do we calculate sequencing Depth and Coverage for DNA and RNA samples using different NGS platform?
- (ii) How sample DNA is prepared for whole genomic 16srRNA or whole metagenome analysis for an NGS sequencing Platform.
- (iii) How do we differentiate FASTA vs FASTq file and what are raw data file ((R1) FASTQ and (R2) FASTQ) of NGS?
- (iv) How do we perform quality control of NGS raw data (FASTQ) file using FastQC tool?
- (v) What are the quality parameters to check for a dataset or FASTQ file using Galaxy server?
- (vi) How to improve the quality of a FASTQ file using Galaxy server.
- (vii) How do we perform genome mapping and annotation using UCSC genome browser?
- (viii) How to generate a PCoA plot and hierarchal clustering using ClustVis using differentially expressed gene.

Unit-III:

- (i) How do we convert DNA sequence in RNA sequence manually as well as using online resources?
- (ii) How do analyze a promoter, transcription start site and ribosome binding site in a given DNA sequence.
- (iii) How do we identify introns and exons in given DNA sequence using bioinformatics tools?
- (iv) How do we identify potential sequence variants for a given DNA sequence/gene using Ensemble genome browser?
- (v) How do we store transport various biological specimens for RNA seq analysis?
- (vi) How do we perform Gene Ontology, gene enrichment analysis using KEGG database?

Unit-IV

- (i) How do we isolate protein from bio-specimens sample from Cell-lysate?
- (ii) Determine peptidase cleaving site as well as predict size of fragments in a given peptide sequence using ExPASy PeptideCutter.
- (iii) How Liquid Chromatography separate and isolate trypsin digested peptide fragments.
- (iv) Determine peptide mass in a given peptide sequence using ExPASy PeptideMass.
- (v) How to perform Gene Ontology analysis and network pathway analysis from identified protein sequence using bioinformatics tools.

Text Books:

1. Bioinformatics: Sequence and Genome Analysis (2004). David Mount. Cold Spring harbor laboratory Press, US Revised Edition. ISBN-10: 0879697121.
2. Bioinformatics for Omics Data. Methods and Protocols (2011). Mayer Bernd. Springer. ISBN 978-1-61779-027-0.

Reference Books:

1. Sequence Analysis in a Nutshell – A Guide to Common Tools & Databases (2003). Scott Markel. O'Reilly, 1st edition. ISBN-13: 978-0596004941.
2. Metagenomics: Theory, Methods and Applications (2010). Diana Marco. Caister Academic Press. ISBN-10: 1904455549.
3. From Molecular Genetics to Genomics: The Mapping Cultures of Twentieth-Century Genetics (2004). Jean-Paul Gaudillière, Hans-Jörg Rheinberger. Routledge 1st Edition. ISBN-13: 978-0415328500.
4. Microbial Metagenomics, Metatranscriptomics, and Metaproteomics (2013). Ed DeLong. Elsevier, 1st edition. ISBN: 9780124078635.

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