

Teacher's Manual

Outline with descriptions, objectives, readings, and URLs

Forward

To program directors, educators, students, and others interested in the education of cytotechnology students:

This project grew out of the meeting of educators sponsored by the ASCT and the ASC in April of 2009. There was a concern at that time regarding a number of issues to be addressed by the educators of Cytotechnology Programs. Our group was charged with helping all of the programs incorporate the new advances in molecular technology into our previously traditional curriculum. At the time, there was probably an enormous variety among the Programs, with some Programs teaching a very traditional curriculum and others including many cutting edge topics from molecular biology. Most Programs probably fell between these two extremes.

With the assistance of educators from some of the finest institutions in the country, we have assembled a core curriculum that leads the student from a detailed examination of the basics of molecular science and the molecules of DNA and RNA, through the mechanisms of replication, transcription, and translation, and from there into the analysis of these mechanisms, their products, and how these techniques may be used in the laboratory to aid in the detection and diagnosis of cancer and other diseases. Included in these units are many online sources of animations, video clips, text, interactive tests, etc. In addition, many PowerPoint presentations, developed by the educators of this group, are also provided for use by the Cytotechnology Programs.

Where appropriate, both virtual and suggested actual laboratories are provided to the programs, to be utilized at their discretion. Additionally, readings from a basic textbook are provided to assist the Program Directors in assigning appropriate topics as the students move through the curriculum.

While this project may not be a perfect match for all Programs and their goals, nevertheless, we feel that it represents a huge leap forward for most Programs, and provides a basic education in these topics, which may then be further elaborated according to the needs of each region and each Program.

November 12, 2010

CHAPTER 1 – MOLECULAR BASIS OF CANCER

1. Molecular Basis of Cancer: *Presentation 1, Marilee Means*

This presentation discusses some of the competing theories of the development of cancer and how many of them are related to a molecular view of changes within the cells. (46 slides)

Objectives:

- Discuss how various theories regarding the development of cancer have a common thread regarding genetic abnormalities.

Inside Cancer – Website – <http://www.insidecancer.org/>

1. Hallmarks of Cancer
2. Causes and Prevention
3. Diagnoses and Treatment
4. Pathway to Cancer

Going through this entire website gives a good overview of what cancer is, how it develops, and some of the diagnostic and treatment alternatives. Also, it discusses some of the genetics of the development of cancer. You may assign it for students to review on their own, or do it in class. It takes about 3 hours to complete the whole website.

2. Molecular Oncology – *Presentation 2, Keisha Brooks*

This presentation discusses the differences between benign and malignant tumors, the multifactorial and multistage causes of cancer, oncology markers used to detect genetic mutations, oncogenes, tumor suppressor genes, the cell cycle, targets and methods for the molecular detection of disease, proto-oncogenes, HER2/neu, FISH, ISH, p53, BRCA1, BRCA2, and other molecular markers for cancer. (51 slides)

Objectives:

- Define "tumor" and "cancer", and be able to distinguish between benign and malignant neoplasms.
- Gain an understanding of cancer development.
- Differentiate between tumor suppressor genes and oncogenes in terms of normal and abnormal functions and how they contribute to the development of tumorigenic cells.
- Identify the common oncogene and tumor suppressor gene mutations in the development of human cancer.

Readings: MD*, Chapter 14 (This reading may be too advanced for this point in the curriculum. It could be done later, after the students have been introduced to the methods.)

CHAPTER 2 – MOLECULAR SCIENCE

3. Nucleic Acid Biochemistry: *Presentation 3, Stephanie Hamilton*

This section introduces the basic structures of nucleic acids and includes a discussion of chromosomes, genes, bases, sugars, base pairing, histones, nonhistones, and chromatin. (34 slides)

Objectives:

- Discuss the structure and chemistry of DNA, RNA, their components and respective functions
- Discuss difference between DNA and RNA; nucleotide and nucleoside; purine and pyrimidine; genotype and phenotype; haploid, diploid, euploid and aneuploid; heterochromatin and euchromatin
- Describe features of the various forms of DNA structure

<http://learn.genetics.utah.edu/> - Tour the Basics

Readings: MD, Chapter 1

4. Basic Molecular Theory: *Presentation 4, Stephanie Hamilton*

This section discusses the cell cycle, mitosis, meiosis, DNA replication, transcription, translation, gene structure, and gene expression. (39 slides)

Objectives:

- Discuss the structure and chemistry of DNA, RNA, their components and respective functions.
- Discuss differences between DNA and RNA; nucleotide and nucleoside; purine and pyrimidine; genotype and phenotype; haploid; diploid; euploid and aneuploid; heterochromatin and euchromatin.
- Describe features of the various forms of DNA structure.

<http://www.dnalc.org/resources/3d/index.html> - 3-D animations: DNA molecule, DNA has four units, Chargaff's ratios, basepairing, how DNA is Packaged (advanced), DNA Unzip, Chromosome map, How much DNA codes for protein, Transcription, Translation, Triplet Code, mRNA splicing, replicating the helix, Mechanism of Replication (advanced).

<http://learn.genetics.utah.edu/> - DNA to Protein

Molecular biology – Hologic website

<http://www.cytologystuff.com/indexmolecular.htm>

Download text and view videos and descriptions of each of the following:

Replication, transcription, translation, DNA, RNA, codon, mRNA, tRNA, enzymes, chromosomal abnormalities

Readings: MD, Chapter 2 and 3

5. Nucleic Acid Enzymes (Biochemical Reagents): *Presentation 5, Keisha Brooks*

This section contains information on polymerase enzymes, endo and exonucleases, reverse transcriptase, DNA ligase, and assay development and design. (19 slides)

Objectives:

- State the function of the following: ligase, exonuclease, restriction endonuclease, polymerase, reverse transcriptase.

Readings: MD, Review Chapter 1, 7-14, Chapter 2, 36-38

6. Assay Development and Design: *Presentation 6, Keisha Brooks*

This section discusses various considerations in the design and validation of molecular based assays. These include sample preparation, extraction methods used, the template preparation, experiment setup, and how to optimize the primers and probes. The type of sample, contaminants, the template DNA and RNA, and general guidelines for selection of primers and probes is explored. Quality assurance measures including validation of "home brews" for use in the clinical laboratory, controls, definitions of precision and sensitivity, and other considerations are presented. (22 slides)

Objectives:

- Discuss various factors in the design of a molecular assay including the sample, the extraction method, the preparation of a template, and the optimization of primers and probes.

- Determine possible contaminants resulting from the extraction method.
- Discuss at least five factors that may need to be considered in designing a primer or probe.
- Determine how one would validate a "home brew" molecular test.
- Discuss the appropriate use of controls in the molecular laboratory.
- List at least three other factors other than controls, which should be monitored for quality assurance.

Readings:

Molecular Diagnostics, Buckingham and Flaws, Chapter 6

7. Genetics: *Presentation 7, Dr. Mary Tang*

This presentation discusses chromosomes, genetic coding of protein production, uses of karyotypes, steps to perform karyotyping, chromosomal abnormalities, and FISH analysis. (33 slides)

Objectives:

- Define karyotype and state the karyotype of a normal male and female
- List the basic steps for the cytogenetic chromosomal analysis of blood
- Describe the clinical utility of karyotyping
- Distinguish between structural and numeric chromosomal abnormalities and give examples
- Describe the basic process involved in FISH

<http://www.dnafb.org/dnafbref.html>

Concepts 15-18, Molecules of Genetics: Concepts, animation, and problems for each unit, depending on students' previous background

<http://learn.genetics.utah.edu/> - Heredity and Traits

<http://www.dnalc.org/resources/3d/index.html> - Dolan DNA Learning Center –

DNA from the Beginning: Classical Genetics, Molecules of Genetics, Genetic Organization and Control

Resources: Roche Genetics CD: Introduction, Journey Through the Cell

Readings: MD, Chapter 3 and Chapter 8

CHAPTER 3 – MOLECULAR TECHNIQUES

8. Introduction to Molecular Pathology: *Presentation 8, Marilee Means*

This presentation introduces some of the uses for the main molecular tests used in the laboratory. (16 slides)

Objectives:

- Discuss the uses of molecular pathology in genetics, infectious organisms, lymphoma typing, solid tumors, and identification.

9. Molecular Methods in the Diagnostic Laboratory: *Presentation 9, Marilee Means*

This section goes into further detail regarding some of the main molecular tests used in the laboratory. (23 slides)

Objectives:

- Discuss the ways molecular pathology can be used to detect genetics diseases, track infectious outbreaks, detect subtypes of lymphoma, direct therapeutic decisions, and identify human samples.

10. Nucleic Acid Extraction Methods: *Presentation 10, Keisha Brooks*

This presentation discusses the types of specimens that may be used to isolate DNA, various methods for extraction of both DNA and RNA, methods to determine the quantity and quality of nucleic acid preparations, methods to calculate the concentration and yield of DNA and RNA from a given preparation, and trouble shooting techniques to address common DNA extraction problems.

(32 slides)

Objectives:

- List the types of specimens that are acceptable for isolating nucleic acids
- Compare and contrast organic, inorganic, and solid phase approaches for DNA isolation.
- Compare and contrast organic and solid phase approaches for isolating total RNA.
- Describe the gel-based, spectrophotometric fluorometric methods used to determine the quantity and quality of DNA and RNA preparations.
- Calculate the concentration and yield of DNA and RNA from a given nucleic acid preparation.
- Identify trouble-shooting techniques used to address common problems involving DNA extraction

Actual Lab: Website: <http://learn.genetics.utah.edu/content/labs/extraction/howto/> The extraction of DNA from anything living. A kitchen level actual lab, which shows the principles of DNA extraction.

Virtual Lab: <http://learn.genetics.utah.edu/> DNA Extraction

Readings: MD, Chapter 4

11. Separation and Detection Part I: *Presentation 11, Marilee Means*

This presentation discusses electrophoresis, nucleic acid hybridization, and Southern, Northern, and Western blotting. (39 slides)

Objectives:

- Discuss the principles of electrophoresis as it applies to nucleic acids.
- List the similarities and differences between agarose and polyacrylamide gels and uses of each.
- Explain the use of buffers and additives in electrophoresis.
- Discuss capillary electrophoresis.
- Describe the types of equipment used for electrophoresis and how samples are handled for the procedure.
- Discuss differences and similarities between pulse field gel electrophoresis and regular electrophoresis regarding method and applications.
- Discuss detection systems used in nucleic acid applications.

<http://www.dnalc.org/ddnalc/resources/electrophoresis.html> - gel electrophoresis

[http://highered.mcgraw-](http://highered.mcgraw-hill.com/olcweb/cgi/pluginpop.cgi?it=swf::535::535::/sites/dl/free/0072437316/120078/bio_g.swf::Southern%20Blot)

[hill.com/olcweb/cgi/pluginpop.cgi?it=swf::535::535::/sites/dl/free/0072437316/120078/bio_g.swf::](http://highered.mcgraw-hill.com/olcweb/cgi/pluginpop.cgi?it=swf::535::535::/sites/dl/free/0072437316/120078/bio_g.swf::Southern%20Blot)

[Southern%20Blot](http://highered.mcgraw-hill.com/olcweb/cgi/pluginpop.cgi?it=swf::535::535::/sites/dl/free/0072437316/120078/bio_g.swf::Southern%20Blot) – Southern blot

<http://www.gene-quantification.de/movoie.html> Northern blot

Virtual Lab: Gel Electrophoresis – <http://learn.genetics.utah.edu/>

Actual Labs: Colorful Electrophoresis, Build a Gel Electrophoresis Chamber.
<http://teach.genetics.utah.edu/content/begin/dna/electrophoresis/index.html>

Resource: Roche CD, Molecular Technology: Microarrays, in situ Hybridization, Agarose Gel Electrophoreses, SDS-PAGE, Southern, Northern, and Western Blots, Troubleshooting

Readings: MD, Chapter 5 and Chapter 6: 94-102

12. Separation and Detection Part II: *Presentation 12, Marilee Means*

This section discusses ISH, FISH, Dot Blot, and Microarrays. (19 slides)

Objectives:

- Discuss the theory of *in situ* hybridization (ISH)
- Define how FISH differs from ISH
- Explain the methodology of detecting chromosomal mutations by FISH.
- Describe the methodology used in Dot blot technique
- Describe the methodology used in microarrays and its advantages

<http://www.cytologystuff.com/indexmolecular.htm> - Molecular Methods of Separation and Detection

1. Nucleic Acid Purification
2. Electrophoresis
3. FISH
4. Blotting

<http://www.cytologystuff.com> Instrumentation – Electrophoresis Lab, ISH Lab, Amplification Lab.

Virtual Lab: www.learn-genetics@utah.edu – DNA Microarray

<http://www.dnalc.org/resources/3d/index.html>

Cutting and Pasting – Restriction enzymes 2-D and Recombining DNA 3-D animation

Transferring and Storing DNA – Transformation – 2-D animation, Storing DNA 2-D animation

Large Scale Analysis - GeneChips® 2-D animation, Making GeneChips® Slideshow, DNA arrays 2-D

13. FISH: *Presentation 13, Keisha Brooks*

This presentation discusses in more detail the principles of in situ hybridization and its use in FISH and CISH. (16 slides)

Objectives:

- Define FISH and its purpose for use in the clinical laboratory
- Distinguish between FISH and CISH
- Explain the basic steps in an ISH procedure
- Identify the cause of common problems in the procedure
- Describe amplification techniques used to detect small targets

Readings: MD, Chapter 6: 103 - 120

14. Nucleic Acid Amplification: *Presentation 14, Keisha Brooks and Sandra Giroux*

This presentation discusses the principles and basic steps of PCR. The advantages and disadvantages of PCR are discussed, as well as the importance of contamination control when using these techniques. Applications which utilize PCR are also discussed. (58 slides)

Objectives:

- Describe the principle and basic steps of amplification by polymerase chain reaction (PCR)
- List the advantages and limitations of PCR
- Describe the importance of contamination control in labs performing PCR
- List PCR applications

<http://www.cytologystuff.com/indexmolecular.htm>

Molecular Methods - Amplification

1. PCR

Virtual Lab – Website: <http://learn.genetics.utah.edu/>

Click on "Virtual Labs" and then "PCR"

Resources:

Roche CD, 2001, Polymerase Chain Reaction (**This CD may no longer be available.**)

http://www.roche.com/home/science/sci_gengen_cdrom.htm

(Click on order form)

PCSEC1.ppt – DNA to PCR (30 slides)

PCSEC2.ppt – In the test tube (12 slides)

PCSEC3.ppt – Minimal requirements (2 slides)

PCSEC4.ppt – Machines used in the process (31 slides)

(PCSEC1 and 2 above are best to learn PCR basics.)

Glossary – List of important terms.

www.dnalc.org/ddnalc/resources/pcr.html - animation of PCR cycles

<http://www.dnalc.org/resources/3d/index.html> Dolan DNA Learning Center – DNA Interactive – Code. Manipulation, Techniques:

Amplifying – Making many copies 2-D and Amplifying – PCR 3-D

Readings: MD, Chapter 7, 121-143

15. Alternatives to PCR – I: *Presentation 15, Stephanie Hamilton*

This presentation discusses amplification techniques other than PCR such as TAS, TMA, NASBA, RCA, LCR, SDA, and Q β . (20 slides)

Objectives:

- Discuss the principles and specific components involved with the various techniques for the amplification of target nucleic acids, probes or signals

<http://www.cytologystuff.com/indexmolecular.htm>

Molecular Methods - Amplification

2. Invader

3. Hybrid Capture

4. TMA

5. SDA

6. LCR

Readings: MD, Chapter 7, 144-149

16. Alternative to PCR – II: *Presentation 16, Stephanie Hamilton*

This PowerPoint describes bDNA, hybrid capture, cleavase invader and cycling probe technologies. (16 slides)

Objectives:

(See Alternatives..., Part 1 above)

Readings: same as above for Part 1

17. DNA Sequence Analysis: *Presentation 17, Marilee Means*

This presentation describes pyrosequencing, restriction fragment length polymorphism, chain terminators, ASO, manual sequence analysis, and automated sequence analysis methods to sequence DNA. (29 slides)

Objectives:

Describe the principles and uses for the following:

- Pyrosequencing
- Restriction Fragment Length Polymorphism (RFLP)
- Chain Terminators (Sanger Method)
- Allele Specific Oligonucleotide (ASO)
- Manual Sequence Analysis
- Automated Sequence Analysis

<http://www.dnalc.org/resources/3d/index.html> - Sanger Sequencing

<http://www.dnalc.org/resources/3d/index.html> In Dolan DNA Learning Center, see **Flyover** – a close look at chromosome 11, and **Chromosome Close Up** which contains a 3-D animation of chromosome coiling from DNA to nucleosome to mitosis. It also includes information on X chromosome banding areas, a 2-D animation including explanations of exons and introns.

Genome mining includes information about transcription, 3-D animation of promoters, TATA boxes, etc. **Genome FISHing** has a video regarding variability in the genome such as SNPs and RFLPs. **Genome Spots** compares various areas of chromosomes as to their variability, genes encoded, etc.

<http://www.dnalc.org/resources/3d/index.html> - **Sorting and Sequencing** – Gel 2-D, Early DNA Sequencing 2-D, Cycle Sequencing 2-D

18. Other Techniques to Detect Mutations: *Presentation 18, Marilee Means*

This section discusses types of mutations, denaturing gradient gel, heteroduplex analysis, SSCP, denaturing HPLC, and melting curve analysis. (34 slides)

Objectives:

- Discuss the principles and uses for denaturing gradient gel, heteroduplex analysis, SSCP, denaturing HPLC, and melting curve analysis.

Readings: MD, Chapter 9

CHAPTER 4 – LABORATORY OPERATIONS

19. Designing the Molecular Laboratory to Decrease Contamination: *Presentation 19, Keisha Brooks*

This section discusses ways in which the molecular laboratory can be designed as well as ways in which the workflow can be scheduled in order to eliminate inadvertent cross contamination of samples. Physical barriers, chemical barriers, and even timing of runs between pre-PCR and post-PCR are also discussed. (22 slides)

Objectives:

- Discuss how the different types of PCR (real-time vs. conventional) vary in their susceptibility to cross contamination.

- Indicate how the need for physical, space, and timing barriers influence the design of the molecular laboratory.
- Discuss common chemical and physical barriers used to reduce contamination.
- Explain the differences in the design of pre-PCR labs and post-PCR labs.
- Compare DNA amplicon vs. RNA amplicon as to the degree of potential contamination risk.

Readings: MD, p. 76-77, 131-133, 298, Chapter 16

20. Quality Assurance: *Presentation 20*

This presentation discusses all of the facets of quality assurance in the molecular laboratory. This includes CLIA '88 regulations, specimen handling, collection, holding and storage, test validation, QA of reagents, probes, ASR, controls, reporting, procedures, and the quality monitoring of various types of instruments which are used in a molecular lab. Decontamination, sterilization, contamination of nucleic acid, quality control and proficiency testing are also discussed. (44 slides)

Objectives:

- Students will be able to discuss the regulations involved in the quality control, quality assurance, and quality monitoring of the various aspects of a molecular laboratory including specimen issues, quality monitoring of chemicals and instruments, and other contamination and proficiency testing issues encountered in this type of laboratory.

21. Guidelines and Regulations, Personnel, and Billing and Coding: *Presentation 21, Stephanie Hamilton*

This presentation discusses regulatory information, means of performing quality control and quality improvement, HIPAA, regulatory agencies, CLIA '88, ASR requirements, reporting, interpretation of results, personnel, proficiency testing, and billing and coding issues for the molecular laboratory. (45 slides)

Objectives:

- Identify specific quality control measures in the molecular and ISH testing, including interpretation and reporting of test results
- Describe HIPAA, CLIA and regulatory agencies affecting molecular laboratories
- Differentiate the approval designations given by the FDA
- Define ASR and its appropriate quality control measures
- Discuss requirements for personnel training
- Identify qualifications for personnel in various job titles
- Describe CPT coding and regulations related to billing

Resource: Roche CD, Regulatory -

This CD reviews the basics of in vitro diagnostics, discusses premarket approval, premarket notification, investigational new drug regulations, investigational use only and research use only regulations, analyte specific reagent regulations, and CLIA. Important factors for molecular laboratories are also addressed.

22. Safety: *Presentation 22, Stephanie Hamilton*

This presentation discusses various aspects of safety for laboratory workers. (29 slides)

Objectives:

- Define OSHA and discuss its requirements and its role in the laboratory
- Discuss the proper handling, labeling and storage of specimens, chemicals, reagents, glassware and laboratory equipment
- Identify various warning labels and NFPA codes
- Define MSDS and their required information

- Describe the procedure for cleaning a chemical spill, for operating a fire extinguisher and fire evacuation
- Differentiate between the various types of fire extinguishers

<http://www.cytologystuff.com/indexmolecular.htm> Regulations and Licensure – Links to state regulations and definitions of terms.

Readings: MD, Chapter 16

CHAPTER 5- APPLICATIONS OF MOLECULAR TESTING

23. Molecular Pathology Detection and Diagnosis of Microorganisms: *Presentation 23, Marilee Means*

The use of molecular pathology in the detection and diagnosis of microorganisms is discussed in this section. (46 slides)

Objectives:

- Discuss parameters for specimen collection, preparation, and quality control
- Discuss molecular methods to detect bacterial organisms and resistance to antibiotics
- Describe the use of molecular methods in epidemic epidemiology
- Describe the use of molecular methods in detection of viral diseases
- Discuss molecular methods to detect fungi and parasites

Readings: MD. Chapter 12

24. Hematology and Oncology: *Presentation 24, Marilee Means*

This presentation discusses the use of molecular pathology in oncology for diagnosis and treatment. The types of cancer, the cell cycle and its checkpoints, cancer mutations, therapy based on treating specific mutations, HER2/neu, comparison of IHC vs. FISH for diagnosis, EGFR, K-ras and its mutations, EWS translocation, BRCA1 and BRCA2, RET proto-oncogene, microsatellite instability, lymphoma and leukemia, and detection of clonality. (50 slides)

Objectives:

- Describe the uses of molecular pathology in the treatment and diagnosis of cancer.

Readings: MD, Chapter 14

25. Genetic Diseases: *Presentation 25, Marilee Means*

This section discusses the molecular testing used to help detect genetic diseases. The main patterns of mendelian inheritance and non-mendelian inheritance are discussed. The PowerPoint also describes chromosomal abnormalities and their detection by karyotyping, flow cytometry, and FISH as well as detection by molecular techniques.. Explanation of terminology used in cytogenetics and examples of various diseases, including single gene, trinucleotide expansion disorders, and genomic imprinting are included. (44 slides)

Objectives:

- Describe the three main patterns of mendelian inheritance
- Discuss laboratory methods to detect common single-gene disorders
- Discuss non-mendelian inheritance
- Discuss how genomic imprinting can affect disease phenotype

Readings: MD, Chapter 13

26. Hybrid Capture II Triage: *Presentation 26, Tim St. John and Sandra Giroux*

This presentation deals with the association of HPV and cervical cancer, describes how the testing for HPV can help to stratify risk for those with abnormal paps, ALTS trial, Hybrid Capture technology as used in this application, and uses of the test. (37 slides)

Objectives:

- Describe the association of HPV and cervical cancer
- List the common high risk subtypes of HPV.
- Understand the role of persistent HPV infection in the development of cervical cancer.
- Define when it is appropriate to utilize HPV testing.
- List the basic steps in the HPV Digene Hybrid Capture test.
- Define when it is not appropriate to test for HPV DNA.

27. Histocompatibility: *Presentation 27, Marilee Means*

This section deals with the need for the testing of organs in order to assure the best possible match between donor and recipient. GVHD, rejection, MHC, and HLAs are defined and discussed. HLA polymorphisms and various methods of detecting them are discussed as well. Various methods of molecular pathology and how they are used in tissue typing are also discussed. (44 slides)

Objectives:

- Discuss why matching is so important in organ transplant.
- Discuss how the MHC controls the genetic matching of patients.
- Describe how the molecular analysis of the MHC is done.
- Describe the additional factors that may need to be taken into account outside of the MHC.

Readings: MD, Chapter 15

28. Molecular Tests for Identity: *Presentation 28, Stephanie Hamilton*

This section describes the various uses for identity testing using molecular methods. These include forensics, parentage testing, immigration, specimen identification, drug screening, bone marrow typing and monitoring for acceptance. (38 slides)

Objectives:

- Define mutation and polymorphism
- Discuss types of polymorphisms and structure and detection methods for each
- Explain the process for parentage testing and analyze the test results
- Define paternity index (PI)
- Discuss molecular testing for gender identification and mitochondrial DNA
- Discuss the various applications for molecular testing and identity

<http://www.dnalc.org/resources/3d/index.html> Dolan DNA Learning Center – Molecular Applications: Human Identification, Family Identification, Murder (forensics), Innocence (forensics).

Readings: MD, Chapter 11

29. Pharmacogenomics: *Presentation 29, Marilee Means*

This section discusses the attempt to optimize drug therapies based on the patient's DNA. Examples such as HER2/neu and Warfarin are discussed. (22 slides)

Objectives:

- Explain how pharmaceutical companies may develop drugs using molecular techniques to tailor the drug to each patient.
- Describe why a patient's Her-2 status changes their response to Herceptin.
- Explain how 2 different patients may have varying responses to the same drug based on their DNA.
- Describe how Warfarin (Coumadin) is tolerated differently depending on the patient's genetics.

<http://www.dnalc.org/resources/3d/index.html> - 3-D: How Gleevec® Works, Cell Signals,

<http://learn.genetics.utah.edu/content/health/pharma/> Genetics and Health, Personalized Medicine: a. Your Doctor's New Genetic Tools, b. Making SNPs Make Sense, c. Drug Development Today and Tomorrow. 3 modules, which talk about genetics and drug development. The interactive frog module is kind of fun also but may be too "young" for our students

Resource: Roche CD, Genetics, Pharmacogenetics – The Gene Scene; Ethical, Legal, and Social Issues – Genetics and Medicine

Readings: Roche CDs on Genetics - Pharmacogenetics; Genetics and Medicine "Learn genetics" site above

CHAPTER 6 – FISH

30. Fluorescence in Situ Hybridization (FISH) - Principles and Preparatory Techniques: *Presentation 30, Amy Wendel Spiczka*

This section describes the use of FISH in the detection of neoplasia. The use of FISH for the analysis of urothelial carcinoma, the development and validation of UroVysion testing, and how to prepare the specimen for testing are also discussed. Probes, both centromeric and locus specific, are discussed, as well as other applications in conditions such as biliary tract cancer, Barrett's esophagus, cervical cancer, and lung cancer. The usefulness of cytologic urine specimens vs. other methods of detection are compared to the FISH method of screening for the presence or absence of certain molecular targets. Methodologies to prepare the slides for FISH are examined. (51 slides)

31. Fluorescence in Situ Hybridization (FISH) – Analysis: *Presentation 31, Amy Wendel Spiczka*

This presentation discusses the characteristics of benign, equivocal, and malignant cell populations by FISH. These characteristics include the pattern of DAPI staining, nuclear features, and chromosomal signals. Appropriate filters on the fluorescent microscope are explained, as well as methods used in screening to detect malignant cells. Common artifacts and normal appearances of cells and nondiagnostic debris as well as characteristic appearances of tumor cells are covered. Types of chromosomal abnormalities and how they appear on FISH are also explained. Caveats such as split signals, cell overlap, and background staining are also discussed. (65 slides)

Objectives:

- Describe use of FISH in the detection of neoplasia
- Define the following:

| | |
|-------------------------------|----------|
| Amplification | Aneusomy |
| Chromosome enumeration probes | Disomy |

| | |
|-----------------------------|---------------------------------|
| Filters | Fluorophore |
| Hemi- & homozygous deletion | Hybridization |
| <i>In situ</i> | Locus specific indicator probes |
| Ploidy | Polysomy |
| Tetrasomy | Trisomy |

- Characterize the stepwise progression through the FISH preparation process, detailing what is happening at the molecular level for each step
- Identify characteristics of a benign, equivocal or malignant cell population by FISH (to include DAPI pattern, nuclear features & chromosomal signal pattern)

Readings:

Halling, K.C. & Wendel, A.J. ***In situ hybridization: Principles and Applications.*** In P.T. Cagle & T.C. Allen (Eds). *Basic Concepts of Molecular Pathology.* New York: Springer, 2009. pp. 109-118

Leonard, D.G.B. *Diagnostic Molecular Pathology.* Philadelphia: Saunders, 2003. pp. 46-8.

Roulston, *Molecular Diagnosis of Cancer – Methods and Protocols.* Totowa, N.J. : Humana Press, 2004. pp. 77-85

CHAPTER 7 – CIRCULATING TUMOR CELLS

32. Circulating Tumor Cells: Presentation 32, Jill Caudill and Kara Hansing

The search for the presence of metastatic disease is an important one for clinicians and patients. This system uses biomarkers and computers to present cells of interest to the technologist for evaluation as potential metastatic tumor cells. This approach may be very beneficial to direct future therapy for the patient. This section discusses the process of metastasis, the detection of circulating tumor cells in blood, various previous methods of detection, the CellSearch® technique, advantages and disadvantages, and method of operation. (18 slides)

Objectives:

- Outline basic steps in the process of cancer cell metastasizing via the bloodstream
- Discuss the clinical utility of identifying circulating tumor cells in the blood
- Explain basic concepts in the CellSearch® System.

33. CellSearch Cell Interpretation: Presentation 33, Jill Caudill and Kara Hansing

This presentation discusses the method of operation and evaluation of cells in the CellSearch® system. The various biomarkers (CK-PE, DAPI, CD45-APC) are discussed as well as their appearance in tumor cells and non-epithelial cells. Suspicious objects, computer noise, and other distractor objects are also evaluated. The criteria for counting tumor cells, evaluating slide quality, and analyzing control samples are given as well. (35 slides)

Objectives:

- Explain the process used for identification of circulating tumor cells as used by the CellSearch® System.
- Discuss the criteria for tumor cell identification

- Compare/contrast the criteria of circulating tumor cells and non-circulating tumor cells
- Discuss the common pitfalls in cell analysis
- Explain the criteria for evaluating scan quality
- List other techniques for evaluating circulating tumor cells

Resources:

1. Molecular Biology of the Cell, 4th ed. – Alberts et al. *Garland Science* 2002. pp1324 – 1326.
2. Essential Pathology, 3rd ed – Rubin. Lippincott Williams and Williams 2001. pp 96-100.
3. Addlar WJ, Matera J, Miller MC, et al. Tumor cells circulate in the peripheral blood of all major carcinomas but not in healthy subjects or patients with nonmalignant diseases. *Clinical Cancer Research* (2004) vol 10, 6897-6904.
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* MD = Molecular Diagnostics: Fundamentals, Methods, and Clinical Applications, Buckingham L, Flaws M, Davis, 2007