Course Title: Theory and Practice of Histotechniques

Course Number: HT4412

Course Description: This course consists of the principles of routine histologic techniques and the basic principles, components and use of instruments in the histopathology laboratory. The students will acquire basic knowledge in the theory of fixation and processing/embedding for routine and electron microscopy. Students will also acquire basic knowledge in the theory and practical applications of microtomy, and routine staining. Students will learn to use various chemicals and equipment. Preventive maintenance, troubleshooting and comparison of types of equipment are also discussed.

Principles and establishment of quality control methods and maintenance of records are presented. Current federal regulations dealing with accreditation of laboratories and safety procedures are also discussed.

Semester Hours Four (4)

Course Approved: June, 2001 (Revised July, 2007)

Faculty: Mark Bailey, MA, HT, (ASCP), HTL
        Samuel Agbe, Ph.D., HT(ASCP)
        Toysha Mayer, MBA, HT(ASCP)

Methods of Instruction: Lecture
                        Demonstration of equipment

Goal: To provide students with basic theoretical knowledge and practical application of processing and embedding tissue, microtomy, routine and special staining techniques.

Objectives: Specific course objectives are included for individual unit of study in Fixation, Processing and Embedding, Microtomy, Routine Staining and the Microscope.
In addition to specific course objectives, the student will be evaluated on professional attitudes in didactics based on the following affective domain objectives:

**Affective Domain Objectives**

While attending didactics in HT4412, the student will:

1. Demonstrate reliability in attendance by:
   - A. Being prepared to start class on time.
   - B. Reporting for class at the scheduled time.
   - C. Remaining in class until sessions are completed.
   - D. Following procedure for reporting absences.

2. Display self motivation and initiative by:
   - A. Preparing for daily instructions.
   - B. Remaining alert during lectures
   - C. Participating in group class projects and demonstrations.
   - D. Displaying willingness and enthusiasm to learn.
   - E. Occupying time productively when instructor is unavailable.
   - F. Participating in continuing education lectures.

3. Display maturity and integrity by:
   - A. Accepting and applying constructive criticism toward improving performance.
   - B. Accepting responsibility for errors made.
   - C. Maintaining composure.

4. Demonstrate interpersonal skills by:
   - A. Showing respect when communicating with instructors and fellow students.
   - B. Listening to details of lectures and asking relevant questions.
   - C. Following verbal and written instructions.

5. Value the quality of work by:
   - A. Completing all assignments before and during lectures on time.
   - B. Performing required class assignments accurately and precisely.
UTMDACC Program in Histotechnology
Affective Domain Objectives
Student Evaluation Checklist

Student ___________________________ Course ___________________________

Student ___________________________ Date Reviewed ______________________
(signature)

Instructor _________________________ Date Reviewed ______________________
(signature)

The following evaluation of affective domain objectives, consist of twenty-five (25) items valued at up to three (3) points each for a total of seventy five (75) points. This affective domain evaluation checklist is worth 50% of the total course grade.

Select and check (√) the number that best describes the student’s attitude/behavior at the clinical site listed:

1 = Never or Poor (0 points)  Student displays consistently unacceptable, difficult attitude.

2 = Sometimes or Good (1 point) Student displays inconsistent attitude, sometimes acceptable, sometimes unacceptable.

3 = Always or Excellent (2 points) Student displays consistently acceptable attitude in every way.

<table>
<thead>
<tr>
<th>Attendance and Reliability</th>
<th>Always 3</th>
<th>Sometimes 2</th>
<th>Never 1</th>
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</thead>
<tbody>
<tr>
<td>Reports to laboratory and begins work on time</td>
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<tr>
<td>Reports to laboratory on time after breaks</td>
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<tr>
<td>Use proper protocol to report absences</td>
<td></td>
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<tr>
<td>Remain until work is complete or arrange to complete work later</td>
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Total Points: _____________

Comments on attendance and reliability:
### Cooperation and Interpersonal Skills

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<thead>
<tr>
<th></th>
<th>Always</th>
<th>Sometimes</th>
<th>Never</th>
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<tbody>
<tr>
<td>Shows respect when communicating with preceptors and others in the laboratory</td>
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<tr>
<td>Follows written instructions</td>
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<tr>
<td>Follows verbal instructions</td>
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<tr>
<td>Cooperates and works with others as a team</td>
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<tr>
<td>Adjusts to changes in schedule</td>
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Total Points: ___________

Comments on cooperation and interpersonal skills:

### Maturity and Integrity

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<tr>
<th></th>
<th>Always</th>
<th>Sometimes</th>
<th>Never</th>
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</thead>
<tbody>
<tr>
<td>Accepts responsibility for work produced</td>
<td></td>
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<tr>
<td>Admits errors when they occur</td>
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<tr>
<td>Corrects errors</td>
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<tr>
<td>Maintains composure</td>
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<tr>
<td>Accepts constructive criticism as a means of improving performance</td>
<td></td>
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<tr>
<td>Respects the confidentiality of patient information and laboratory data</td>
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Total Points: ___________

Comments on maturity and integrity:

### Self Motivation and Initiative

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<tr>
<th></th>
<th>Always</th>
<th>Sometimes</th>
<th>Never</th>
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<tbody>
<tr>
<td>Seeks additional tasks</td>
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<td></td>
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<tr>
<td>Works independently but follows established laboratory protocol</td>
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<td>Volunteers to help staff members when appropriate.</td>
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<tr>
<td>Participates in continuing education and/or opportunities to learn new information when available</td>
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</table>

Total Points: ___________

Comments on self-motivation and initiative:
<table>
<thead>
<tr>
<th>Values Professionalism and Quality of Work</th>
<th>Always 3</th>
<th>Sometimes 2</th>
<th>Never 1</th>
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</thead>
<tbody>
<tr>
<td>Follows appropriate dress code for assigned area</td>
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<td></td>
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<tr>
<td>Follows established laboratory policies and procedures</td>
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<tr>
<td>Completes assigned work in the time expected</td>
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<tr>
<td>Pays attention to details of work assigned</td>
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<tr>
<td>Keeps the work area clean and orderly.</td>
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<tr>
<td>Provides neat and accurate written documentation where applicable.</td>
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Total Points: __________
Comments on professionalism and quality of work:

Total Combined Points: __________

Please provide comments on the overall attitude and conduct of the student while in your area:
Grades: The grade for this course will be determined by the student’s performance on 5 unit exams and 1 final exam consisting of multiple choice questions, and evaluation of affective domain objectives which will be weighted as follows:

<table>
<thead>
<tr>
<th></th>
<th># Questions</th>
<th>Points</th>
<th>Total</th>
<th>% of Total Grade</th>
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<tbody>
<tr>
<td>4 Unit Exams</td>
<td>100</td>
<td>1</td>
<td>400</td>
<td>(10% each) 40%</td>
</tr>
<tr>
<td>1 Unit Exam</td>
<td>50</td>
<td>2</td>
<td>200</td>
<td>10%</td>
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<tr>
<td>1 Mid-Term</td>
<td>100</td>
<td>1</td>
<td>100</td>
<td>20%</td>
</tr>
<tr>
<td>1 Final Exam</td>
<td>100</td>
<td>1</td>
<td>100</td>
<td>20%</td>
</tr>
<tr>
<td>1 Evaluation checklist</td>
<td>25</td>
<td>2</td>
<td>50</td>
<td>10%</td>
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</table>

(Affective Domain)

Required Reading:


Competencies:

The student will be considered competent after successfully completing 5 units in the course with an overall score of 75% (C) or better.
Upon completion of the study of fixation, the student will:

1. Define:  (Cognitive)
   a. Autolysis
   b. Artifact
   c. Fixation
   d. Putrefaction

2. State and discuss the general principles, purposes and functions of fixation.  (Cognitive)

3. Describe the actions of fixation and fixatives on tissue. (Cognitive & Psychomotor)

4. Differentiate between chemical fixation and physical fixation to include: (Cognitive & Psychomotor)
   a. physical fixation
   b. chemical fixation

5. Compare and state the differences between:  (Cognitive)
   a. additive and non-additive fixatives
   b. coagulant and non-coagulant fixatives
   c. aqueous and non-aqueous fixatives

6. List and discuss the type and composition of chemical fixatives reagents to include indications for use, advantages, disadvantages, safety hazards and special considerations for:  (Cognitive)

   a. Simple aqueous
      1. acetic acid
      2. formaldehyde
      3. glutaraldehyde
      4. paraformaldehyde
      5. mercuric chloride
      6. osmium tetroxide
      7. picric acid
      8. potassium dichromate
      9. zinc
      10. chromium trioxide
   b. Compound aqueous
      1. Bouin
      2. Flemming
      3. formalin combinations
      4. formalin substitutes
      5. Helly/Zenker
      6. Orth’s
      7. B-5
      8. Herman
      9. Zamboni’s
      10. Zinc salts
c. simple non-aqueous  
d. compound non-aqueous

1. acetone  
2. alcohol  
3. methanol  
1. Carnoy  
2. methacarn

5. List the recommended fixatives used to preserve specific tissue elements and/or disease states.  (Cognitive)

6. Differentiate between formalin and formaldehyde.  (Cognitive & Psychomotor)

7. Compare Zenker and Helly fixatives.  (Cognitive)

8. Describe and discuss quality control in fixation to include the following factors:  (Cognitive & Psychomotor)
   a. selection of appropriate fixative
   b. size and thickness of tissue
   c. temperature, time, volume and concentration of fixative
   d. storage
   e. pH and osmolarity

9. Microscopically recognize artifact caused by fixation.  (Cognitive)

10. Identify and differentiate between mercury and formalin pigment formed in tissue.  (Cognitive & Psychomotor)

11. Describe how fixation pigment may be prevented and/or removed.  (Cognitive & Psychomotor)

Student’s Reading Assignment:


References:


Hopwood, D. et. al.: *Microwave Fixation: Its Potential for Routine Techniques*,


Competencies

The student will be considered competent and have met the objectives upon completion of a written test consisting of 100 questions, with a score of 75% or better. This score is worth 10% of the total course grade.
Upon completion of the study of processing techniques, the student will be able to:

1. Discuss and the various types of laboratory instruments associated with processing to include: 
   *(Cognitive)*
   a. Open systems:
      (1) Autotechnicon – Mono, Duo and Ultra
   b. Closed systems:
      (1) VIP
      (2) Shandon Path Center
   c. The role of agitation, vacuum and pressure in processing tissue
   d. Embedding modules
   e. Accessories
      (1) paraffin dispensers
      (2) vacuum apparatus
      (3) forcep warmers
      (4) decalcifier
      (5) gross area
      (6) hydrometer
      (7) automatic cassette labelers
      (8) solvent recycler
      (9) exhaust hood
   f. Temperature control and proper temperature for each instrument

2. Define and state the principles, and/or actions of dehydration. *(Cognitive)*

3. State the purpose and function of dehydration. *(Cognitive)*

4. State and discuss advantages of different dehydrating agents. *(Cognitive)*

5. Differentiate between universal solvents and other non-universal solvents and comparative dehydrating agents. *(Cognitive & Psychomotor)*

6. Describe quality control methods as related to dehydration. *(Cognitive & Psychomotor)*
7. Define, list and relate to, the principles and/or actions of clearing.  (Cognitive)

8. State the purpose and function of clearing.  (Cognitive)

9. State the advantages and disadvantages of different clearing agents; discuss differences between universal and non-universal solvents as related to clearing agents. (Cognitive)

10. Discuss quality control methods as related to clearing.  (Cognitive)

11. Discuss and evaluate the effectiveness of various problem solving techniques used in dehydration and clearing relating to:  (Cognitive)
    a. time
    b. volume
    c. contamination

12. Define and state the principles and/or actions of infiltration and embedding.  (Cognitive)

13. State the purpose and function of infiltration and embedding.  (Cognitive)

14. State and discuss advantages and disadvantages of various infiltrations and embedding media used in histological preparations.  (Cognitive)
    a. paraffin
    b. carbowax
    c. celloidin
    d. plastic
    e. agar and gelatin
    f. freezing
    g. double embedding

15. Describe quality control methods as related to infiltration and embedding.  (Cognitive & Psychomotor)

16. Discuss and evaluate the effectiveness of problem solving techniques used in infiltration and embedding relating to:  (Cognitive)
    a. time
    b. volume
    c. temperature
    d. contamination

17. Demonstrate proper orientation of specimens embedded in paraffin for routine histology when embedding.  (Cognitive & Psychomotor)
    a. skin
    b. tubular structures
    c. structures with wall
    d. bone
Students’ Reading Assignment

Carson, Freida L. & Christa Hladik: *Histotechnology: A Self Instructional Text. 3rd Edition*  
ASCP Press, 2009, Chap. 2, pgs. 31-52; Chap. 3, pgs. 65-67; 74.

Hrapchak, Barbara B. and Dezna C. Sheehan: Theory and Practice of Histotechnology,  

Chap 1, pgs. 27-30.

References:

Bancroft, John D. and Alan Stevens: *Theory and Practice of Histological Techniques*,  

Hopwood, D. et. al.: *Microwave Fixation: Its Potential for Routine Techniques*,


Competencies

The student will be considered competent and have met the objectives upon completion of a  
written test consisting of 100 questions relating to processing and embedding, with a score of  
75% or better. This score is worth 10% of the total course grade.
OBJECTIVES

Upon completion of the processing and embedding lab exercise, the student will be able to:

1. Choose the appropriate size of tissue that can be processed and embedded. (Cognitive & Psychomotor)
2. Correlate the appropriate size and type of tissue to the appropriate processing schedule. (Cognitive)
3. Prepare processing reagents. (Cognitive & Psychomotor)
4. Recognize the change in appearance or refractive index of the tissue when the tissue is submerged in the clearing reagent (xylene). (Cognitive)
5. Produce and illustrate the method in which tissue is processed with processing reagents. (Cognitive & Psychomotor)
6. Produce and illustrate the method in which tissue is oriented and embedded after the tissue is processed. (Cognitive & Psychomotor)

Processing & Embedding Laboratory Exercise

Processing Schedule – After Fixation

Follow the prescribed tissue processing schedule

1. 95% ETOH 15 min. RT
2. 100% ETOH 15 min. RT
3. 100% ETOH 15 min. RT
4. 100% ETOH 15 min. RT
5. Xylene 15 min. RT
6. Xylene 15 min. RT
7. Xylene 15 min. RT
8. Paraffin 20 min. W/O Vacuum – 58°-60° C
11. Embed the tissue.
Follow the instructions of each step below. (Write the purpose of each step next to each instruction.)

1. Prepare appropriate volume of 95% ETOH and dispense the 95% ETOH into a processing container.

2. Label biopsy tissue cassette. (E.g. ST09 – 01) (Place the pre dissected and fixed biopsy tissue into labeled tissue cassette and place in 95% ETOH.)

3. Discard 95% ETOH and dispense 100% ETOH into processing container.

4. Discard 100% ETOH and dispense 100% ETOH into processing container.

5. Repeat step 4.

From this point through to step 9 perform under the hood.

6. Discard 100% ETOH and dispense appropriate volume of Xylene into processing container.

7. Discard Xylene and dispense appropriate volume of Xylene into processing container.

8. Repeat step 7.

9. Discard Xylene and place tissue cassettes into paraffin container located in the 60º oven.

10. Remove tissue cassettes form paraffin container #1 and move cassettes to paraffin container # 2.

11. Remove tissue cassettes form paraffin container #2 and move cassettes to paraffin container # 3.

12. Embed tissue. Remove tissue cassettes from paraffin container #3 and place the tissue cassettes in the heated module of the embedding unit. Dispense molten paraffin into a small pre warmed tissue mold. Place the mold on the heated portion of the embedding unit and with forcep transfer processed tissue from your tissue cassette into the mold. Hold the tissue with tamper and place the mold onto the cold plate of the embedding unit. After you see the paraffin solidify in the bottom of the mold place the tissue cassette on top of the mold and dispense molten paraffin until it fills to the top of the cassette. Place the mold with the embedded tissue onto the cold plate of the embedding unit.
References:

Carson, Freida L. & Christa Hladik: Histotechnology: A Self Instructional Text. 3rd Edition

Hrapchak, Barbara B. and Sheehan, Dezna C., Theory and Practice of Histotechnology, 2nd ed.,

Competencies

The student will be considered competent and have met the objectives upon completion of the lab
exercise with a score of 75% or better. The score from this unit is worth 10% of the total course
grade.
HT4412
Theory and Practice of Histotechniques
Unit III: Microtomy

Objectives:

Upon completion of lectures in the theory and practice of Histotechniques in microtomy, the student will:

1. Define: (Cognitive)
   a. Microtome
   b. Microtomy
   c. Recuts
   d. Levels (steps)
   e. Serial sections

2. Discuss and state the use of various types of microtomes utilized in sectioning tissue. (Cognitive)

3. Name the parts of the rotary microtome. (Cognitive)

4. Identify the different types of knives utilized in sectioning and list the recommended use for each. (Cognitive & Psychomotor)

5. Describe the various rotary microtome knife angles and state the significance of each. (Cognitive & Psychomotor)

6. List and discuss various knife sharpeners. (Cognitive)

7. Explain the critical factors in knife sharpening. (Cognitive)

8. Identify microtomy sectioning accessories. (Cognitive & Psychomotor)

9. Relate the proper temperature of floatation bath and drying apparatus to the type of paraffin used in paraffin embedded techniques. (Cognitive)

10. Differentiate between some of the common microtomy sectioning difficulties encountered in paraffin sectioning and list the appropriate corrective actions for each. (Cognitive & Psychomotor)

11. Identify and describe some common sectioning difficulties encountered in frozen sectioning and list the appropriate corrective actions. (Cognitive & Psychomotor)

12. Demonstrate proper operation and maintenance of the rotary microtome. (Cognitive & Psychomotor)

13. Demonstrate proper operation and maintenance of the open top cryostat. (Cognitive & Psychomotor)

14. Visually identify sectioning artifact that occurs from floatation bath in slides, colorplates and using the microscope. (Cognitive & Psychomotor)

15. List section adhesives that may be used in sectioning. (Cognitive)

16. Discuss safety procedures for handling blades and knives. (Cognitive)

17. Relate significance of section thickness in frozen sections and paraffin embedded specimens. (Cognitive)
Reading Assignments:


Competencies:

The student will be considered competent and have met the objectives upon successful completion of a written exam consisting of 100 questions relating to microtomy with a score of 75% (C) or better. The score on this exam is worth 10% of the total course grade.
Upon completion of the study of routine staining and coverslipping, the student will be able to:

1. State and explain the purpose of each of the following steps used in routine Hematoxylin and eosin (H&E) staining techniques:  
   a. Deparaffinization  
   b. Hydration  
   c. Staining  
   d. Dehydration  
   e. Clearing  
   f. Mounting  

2. Differentiate between hematein and hematin.  

3. Identify the ingredients and their purpose in the following hematoxylin formulas:  
   a. Harris  
   b. Mayer  
   c. Gill  
   d. Ehrlich  
   e. Delafield  
   f. Weigert iron  
   g. Janssen iron  

4. List the advantages and disadvantages of the hematoxylin formulas listed above.  

5. Compare and differentiate between hematoxylin formulas that are used in progressive and regressing staining.  

6. List two (2) frequently used methods of differentiation in routine H&E staining.  

7. List two (3) bluing “agents” frequently used in H&E staining.
8. List and describe (3) dyes frequently used for cytoplasmic stains in routine H&E:  
   (Cognitive & Psychomotor)

9. Discuss factors affecting routine staining with special emphasis on:  (Cognitive)
   a. nuclear staining
   b. cytoplasmic staining

10. Describe and compare the use of various types of mounting media to include advantages and disadvantages of each. (Cognitive & Psychomotor)

11. Discuss size and thickness of slides and coverslips used in the staining of microscopic slides.  (Cognitive)

12. State and relate the approximate refractive index of mounting media and coverslips.  (Cognitive)

13. Troubleshoot and exercise quality control in routine H&E staining to determine possible sources of errors and the appropriate corrective action as indicated in the NSH Guidelines for Hematoxylin & Eosin Staining.  (Cognitive & Psychomotor)

Reading Assignments:


Competencies

The student will be considered competent and have met the objectives upon completion of a written test consisting of 100 questions with a score of 75% or better. The score from this unit is worth 10% of the total course grade.
OBJECTIVES

Upon completion of the routine staining exercise lab exercise, the student will be able to:

1. List the reagents needed to perform a regressive H&E procedure. (Cognitive)

2. Prepare a staining set-up and reagents for a regressive H&E procedure. (Cognitive & Psychomotor)

3. Explain the principles of a regressive stain to include: decolorizing, bluing, and differentiation. (Cognitive)

4. Recognize the change in appearance or refractive index of the tissue when the tissue is submerged in the clearing reagent (xylene). (Cognitive)

5. Differentiate, compare and contrast a tissue slide that has been treated with both acid alcohol and a bluing reagent to a tissue slide that has only been treated with acid alcohol and to a tissue slide that has been only treated with a bluing reagent. (Cognitive & Psychomotor)

6. Validate the results of the procedure by visualizing the glass slides utilizing a microscope. (Cognitive & Psychomotor)

Use the following laboratory materials to perform the student exercise:

- Coplin Jars or staining containers
- Assorted Cylinders
- Funnel
- Whatman’s Filter paper
- Eye Protection
- 95% ETOH
- Acid Alcohol
- Permount
- Eosin Y
- Erlenmeyer flask
- Forceps
- Gauze & Kimwipes
- Gloves
- 100% ETOH
- Clearing Reagent
- Bluing Reagent
- Harris Hematoxylin
- Coverslips
Please follow the instructions of each step below. (Write the purpose of each step next to each instruction.)

1. Remove 3 slides from the basket and write your initials. Also label slides (1-H&E) (1-No Acid) (1-No Bluing)
2. Xylene I, II, III (3) min. each
3. 100% ETOH I & II (2) min. each
4. 95% ETOH (1) min.
5. Tap Water (TW) – (Dip slide until ETOH residue is removed.) 1 slide at a time
6. Harris Hematoxylin (5) min.
7. Running Tap Water (RTW) (5) min.

(The remainder of the procedure will be performed 1 slide at a time.)

8. 1% Acidic ETOH (Dip slide x 2) (Skip one slide)
9. TW (Dip slide until Acetic ETOH residue is removed.)
10. Bluing Reagent (Dip slide until the section blues) (Skip 1 slide.)
11. TW (Dip slides until residue is removed.)
12. Eosin (1) min.
13. 95% ETOH (Dip slides x 3)
14. 100% ETOH III (Dip slide x 3-5)
15. 100% ETOH IV, V (1) min

(The remainder of the procedure must be performed under the hood.)

16. Xylene IV, V, (3) min ea.
17. Xylene VII (Hold until coverslipped.)
18. Permount and coverslip.

References


Competencies

The student will be considered competent and have met the objectives upon completion of the lab exercise with a score of 75% or better. The score from this unit is worth 10% of the total course grade.
Upon completion of the study of the microscope, the student will be able to:

1. Describe the following types of microscopy and identify indications for their use: (Cognitive & Psychomotor)
   a. light (compound microscope)
   b. polarizing
   c. phase-contrast
   d. dark-field
   e. fluorescence
   f. electron (scanning and transmission)

2. Identify the components of the light microscope and their function: (Cognitive & Psychomotor)
   a. Lens system
      (1) Ocular (monocular, binocular)
      (2) Objective: most common objectives used and approximate magnification of each
   b. Substage
      (1) condenser
      (2) diaphragm

3. Describe the method of determining total magnification. (Cognitive & Psychomotor)

4. Define: (Cognitive)
   a. resolution
   b. achromatic
   c. apochromatic
   d. aberration
   e. parfocal

5. Describe the Koehler Illumination Method: (Cognitive & Psychomotor)

6. Identify accessories for use with microscope: (Cognitive & Psychomotor)
   a. Immersion oil
   b. Lens paper
7. Adjust and focus the microscope by: (Psychomotor)
   
   a. positioning a slide on the stage  
   b. adjusting the eye pieces  
   c. adjusting the light source  
   d. using the low power objective  
   e. adjusting the diaphragm and substage condenser  
   f. using the high power objectives

READING ASSIGNMENT AND REFERENCES:


Competencies

The student will be considered competent and have met the objectives upon completion of a written test consisting of 100 questions with a score of 75% or better. The score from this unit is worth 10% of the total course grade.
**Performance Evaluation Checklist for Adjusting and Focusing the Microscope**

Each student will be expected to use the microscope according to established protocol. The evaluation checklist consists of 8 items worth 3 points each for a total of 24 points and item 9 is worth 1 point for a total of 25 points. *(Psychomotor)*

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<tbody>
<tr>
<td>1. Positioned slide on the microscope stage</td>
<td>0</td>
<td>1</td>
<td>2</td>
<td>3</td>
</tr>
<tr>
<td>2. Adjusted the eyepieces</td>
<td>0</td>
<td>1</td>
<td>2</td>
<td>3</td>
</tr>
<tr>
<td>3. Adjusted the light source</td>
<td>0</td>
<td>1</td>
<td>2</td>
<td>3</td>
</tr>
<tr>
<td>4. Used low power objective to focus slide</td>
<td>0</td>
<td>1</td>
<td>2</td>
<td>3</td>
</tr>
<tr>
<td>5. Adjusted the diaphragm and substage condenser</td>
<td>0</td>
<td>1</td>
<td>2</td>
<td>3</td>
</tr>
<tr>
<td>6. Used high power objective to focus slide</td>
<td>0</td>
<td>1</td>
<td>2</td>
<td>3</td>
</tr>
<tr>
<td>7. Adjusted the diaphragm and substage condenser</td>
<td>0</td>
<td>1</td>
<td>2</td>
<td>3</td>
</tr>
<tr>
<td>8. Turned off microscope</td>
<td>0</td>
<td>1</td>
<td>2</td>
<td>3</td>
</tr>
</tbody>
</table>

9. The type of microscope that I have used is: _________________________________

**Comments:**