

Dear New Graduate Student:

Welcome to the Department of Veterinary Pathology. This Orientation Manual has been written by our staff and faculty to aid you in rapidly becoming acquainted with routine departmental procedures as well as serving as a reference. Please read the material and ask appropriate people if you have questions. We would appreciate constructive suggestions on the format, contents and needed additions.

The manual also briefly lists some of the materials and resources available to aid you in your studies.

Our faculty and staff are here to facilitate your education. We hope your time in our department will help you reach your career goals and gain an unsatisfiable appetite for knowledge. We also hope the experience will be enjoyable and fulfilling in a personal sense.

Good Luck.

Sincerely,

Joseph Haynes  
Chair

Jesse Hostetter  
Director of Graduate Education

**VETERINARY PATHOLOGY  
IOWA STATE UNIVERSITY**

**ORIENTATION MANUAL**

(Revised March 2017)

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# I. INTRODUCTION

## *The Department of Veterinary Pathology*

The Department of Veterinary Pathology at Iowa State University has a rich tradition in graduate education. We have conferred over 200 MS and PhD degrees dating back to the 1930's. We offer graduate study toward either the Ph.D. or M.S. degree in several areas. The Department has faculty with specialty training in anatomic and clinical pathology, parasitology and molecular biology.

Areas of emphasis include:

- Anatomic pathology
- Clinical pathology
- Parasitology
- Genetics (Interdepartmental)
- Immunobiology (Interdepartmental)
- Molecular, Cellular and Developmental Biology (Interdepartmental)
- Toxicology (Interdepartmental)

General information about the Department is available on our web site at <http://vetmed.iastate.edu/vpath/>. This site also provides links to a directory of Departmental faculty and their research interests.

### **Administration and Contact Information**

#### **Department Chair:**

Dr. Joseph S. Haynes  
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## II. WEB SITE RESOURCES

### **University web site**

The Iowa State University homepage is at

<http://www.iastate.edu/>

### **International Student Orientation**

<https://www.isso.iastate.edu/new-student-orientation>

### **Center for Communication Excellence**

<http://cce.grad-college.iastate.edu/>

### **Graduate College Handbook**

<http://www.grad-college.iastate.edu/handbook/>

### **Electronic Theses/Dissertations (ETDs) at ISU**

<http://www.grad-college.iastate.edu/current/thesis/>

### **Iowa State Graduate College forms**

<http://www.grad-college.iastate.edu/common/forms/index.php>

### **Additional Iowa State University forms**

<http://www.policy.iastate.edu/forms.php>

### **General Catalog**

<http://catalog.iastate.edu/>

### **Schedule of Classes**

<http://classes.iastate.edu/>

### **AccessPlus** and class registration

<https://accessplus.iastate.edu/frontdoor/login.jsp>

Iowa State University's AccessPlus is a personalized secure university information resource that provides on-demand accessibility to your confidential information. It is also used for registration for classes. This source provides confidential access to:

- Address Change
- Current Student Information
- Financial Aid Information
- Grade Report
- Job Board
- Long Distance Codes
- Registration for Classes
- Tax Information
- Residence Hall information
- Unofficial Transcript
- View Class Schedule

### **Iowa State University Directory Information**

<http://www.info.iastate.edu/>

(2009/2010 is the final edition of the printed directory)

### III. GENERAL

**KEYS** - Office personnel will issue you the necessary keys for your work. If one is lost, there is a \$25 replacement fee.

**ISU Card** (dual technology) – Office personnel will add building and room access as needed.

**LAB COATS** - Different sizes and styles of lab coats may be obtained in the histology laboratory. Soiled coats may be left in the hamper in the histology lab or in either one of the locker rooms. Clean lab coats are put in the histology lab unless your name is in your lab coat; then it will show up in your mailbox. If you have special requirements see Toni Christofferson in the histology laboratory and she will order for you.

**SUPPLIES** - Office supplies, batteries, microscope bulbs, etc., are distributed to employees from the office, room 2764.

**LIBRARY** - There is a departmental library located in room 2768 (Conference Room). These books are available only to Veterinary Pathology faculty and graduate students. The books are not to be removed from the library unless checked out through the office, room 2764.

**COPY MACHINES** - There are copy machines available in the Vet Med Library (requires a copy card or cash) and in Room 2760 Vet Path (requires a code).

**COMPUTERS** - Computers are available in the Vet Med personal computer lab (Rm 2272). These computers are available for use outside of class hours. There are also computers available in the Vet Med Library (Rm 2280-2).

**TELEPHONES/FAX** - Telephone service is provided for conducting university business. Iowa Code, Section 721.2, prohibits use for any private purpose or personal gain.

The University recognizes that employees may have a need to use University telephones for personal calls (voice and fax), as long as calls are of reasonable duration and frequency.

Employee options for placing long distance personal calls from University phones:

- Purchase prepaid calling card.
- Use calling card associated with personal long distance carrier.
- Place collect call.
- Use pay phones located on campus.
- Avoid using a University phone by using a personal cell phone.

There is a Fax machine in the Vet Path office to be used for business purposes only.

**REGISTRATION** - Registration for classes is done through the web. The reference numbers for classes are found in the Schedule of Classes (<http://classes.iastate.edu/>)

**GRADUATE STUDENT HANDBOOK** - You should obtain the Graduate Student Handbook from the ISU web site <http://www.grad-college.iastate.edu/handbook/>

**PURCHASING** - ALL purchases you make must first be authorized. Check with the Vet Path office BEFORE you purchase anything that you expect the department to pay for.

## **MISCELLANEOUS**

**INVENTORY** - Notify the Vet Path office if you want to move equipment from one office or lab to another. Departmental inventory is done biennially, and is easier to perform if all equipment is where it is listed.

**TRAVEL** - Forms for travel (authorization forms and expense reimbursement forms) are available in the Vet Path office.

**DEPARTMENTAL EVENTS** - From time to time there will be departmental get-togethers such as a soup or salad luncheon, holiday party, farewell party, etc. Notices and sign-up sheets for these events will be posted on the bulletin board by the break room (room 2708).

## IV. SEMINARS

604 HISTOPATHOLOGY SEMINAR - Each semester a Thursday noon seminar is held in the Conference Room, 2768. Interesting cases are selected by an anatomic and a clinical pathologist. Slides to be discussed are available the previous week in the histopathology lab (Room 2709). The seminar may be taken as a 1-credit or 2-credit course fall and spring. You may also attend on a non-credit basis. *All Vet Path majors are expected to attend, whether or not you are taking it for credit.*

Following the seminar, the slides are to be returned to the histopathology laboratory. The slides are made into study sets and given to graduate students.

GRAND ROUNDS - Grand rounds are held at 8:00 a.m. each Friday morning. Rounds begin in Room 1226.

JPC SEMINAR – JPC (Joint Pathology Center) seminar is held one hour each week. The schedule varies each semester.

GROSS SEMINAR – This biweekly seminar (Friday, 1:10) is held in room 1694.

605 TOPIC SEMINAR - This is traditionally a one hour presentation of the PhD candidate's research given prior to defense of the dissertation. 1 credit.

DERMATOPATHOLOGY ROUNDS - Dermatopathology rounds is a monthly meeting of dermatologists, pathologists, residents, clinicians, and other interested individuals. The purpose of the rounds is to discuss diagnostic, prognostic and other key aspects of dermatology cases and exchange ideas and experiences between participants. There is an emphasis on cases that pose to be a challenge in diagnostic terms; however, routine conditions are also encouraged.



## V. STUDY SETS AVAILABLE

Any of these study sets can be checked out through the Histopathology Laboratory.

General Pathology  
Systemic Pathology  
Cellular Pathology  
Ophthalmic Pathology  
Neuropathology  
Dermatopathology  
Musculoskeletal Pathology  
Cardiovascular Pathology  
Gastrointestinal Pathology  
Urinary Pathology  
Endocrine Pathology  
Lymphoreticular Pathology  
Hepatic and Pancreatic Pathology  
Granuloma Diseases  
Avian Pathology  
Laboratory Animal Medicine  
Parasitology  
Parasitic Protozoan  
Histochemistry

Neoplasm Sets  
Lymphatic  
Urinary  
Gastrointestinal  
Respiratory  
Musculoskeletal  
Ophthalmic  
Soft tissue  
Neural  
Endocrine  
Reproductive (male and female)  
Mammary

Normal Sets  
Cat  
Dog  
Pig  
Equine  
Avian  
Rat  
Elephant

Also available:

ACVP Gross Video Disk  
C.L. Davis Foundation Study Sets  
AFIP Study Sets  
Study sets from past seminars

## VI. DEPARTMENT OF VETERINARY PATHOLOGY

For course and degree requirements, see the following web site.

<http://vetmed.iastate.edu/vpath/academics/graduate-program>

### MS & PHD EXAMINATIONS

#### Introduction

As stated in the ISU Graduate College Handbook: “A doctoral dissertation must demonstrate conclusively the ability of the author to conceive, design, conduct, and interpret independent, original, and creative research. It must attempt to describe significant original contributions to the advancement of knowledge and must demonstrate the ability to organize, analyze, and interpret data.”

As stated in the Graduate College Handbook, “the PhD degree preliminary oral examination rigorously tests a graduate student’s knowledge of major, minor, and supporting subject areas as well as the student’s ability to analyze, organize, and present subject matter relevant to the field.” This philosophy is implemented throughout all examinations in conjunction with the PhD preliminary oral examination. The purpose of the preliminary oral examination is to admit the student to PhD candidacy. Therefore, the PhD preliminary oral examination should take place before the student has advanced too far into the PhD program, and after a majority, but not necessarily all, of the course work is completed. The following guidelines also apply to the MS degree examination.

#### 1. Diagnostic/Slide Examination

The department will administer a diagnostic/slide examination, when appropriate, to students training with an ultimate goal of board certification in pathology. The diagnostic/slide examination is separate from other examinations pertaining to the preliminary PhD examination and is not part of the written portion of the PhD preliminary oral examination. The time at which this examination is administered during the training program is determined by the POS committee. It is preferable to administer the diagnostic examination close to the time of the preliminary PhD examination to assure qualifications in both diagnostic disciplines and related disciplines culminating in the PhD. The examination will be composed of appropriate material for either anatomic pathology or clinical pathology.

A student who fails the diagnostic examination may or may not be allowed to retake the examination. Only 1 retake is allowed. Students may be allowed to again retake the examination after a specified period of time. Permission to retake the examination and the time allowed between examinations will be determined by the POS committee.

#### 2. Written Preliminary PhD Examinations

The written portion of the preliminary PhD examination will be given prior to the preliminary oral PhD examination and used to determine if the student is allowed to progress to the preliminary oral PhD examination. The written and the oral examination are 2 different portions of the PhD preliminary examination. The POS committee will determine if the written examination is a pass by a similar mechanism used for the preliminary oral examination.

- a. Each POS committee member will administer a portion of the written examination and grade the examination.
- b. The POS committee in conjunction with the major professor will determine if any open book questions are allowed. Closed book examinations are the usual examination given.
- c. If more than one member of the committee votes not to pass the student, the candidate does not pass the examination and will not proceed to the preliminary oral examination.

- d. The POS committee is responsible for determining if the student will be allowed to retake the written examination and determining the time period between the first attempt and retaking the written examination. Only 1 retake of the examination is allowed during the PhD program.
- e. Factors that may be used, but are not limited to, to determine if the student is allowed to retake the examination are: 1) a plan of study by the student that outlines correction of deficiencies, 2) prior progress in the PhD program, 3) prior progress in course work, and 4) demonstrated ability to meet the standards of a PhD program as outlined in the introduction.

### 3. Preliminary Oral Examination

The Graduate College Handbook is specific about procedures for the PhD preliminary oral examination. One retake of the examination is allowed. The Pathology department follows these guidelines.

#### Diagnostic Examination (discussed in the Graduate College Handbook)

A diagnostic examination has not traditionally been used in the Department of Veterinary Pathology. This examination, as used by other departments, is separate from the preliminary examination and occurs very early in the training program to determine the background knowledge of a student. For example, if there is a concern that the student received appropriate background training to obtain the DVM or similar degree, a diagnostic examination may be administered prior to admission in the PhD program. The department and/or POS committee may determine if this is necessary and the guidelines may be followed in the Graduate College Handbook.

#### Qualifying Examination (discussed in the Graduate College Handbook)

The Department of Veterinary Pathology does not have a defined qualifying examination. The written examination for the preliminary PhD examination is sometimes considered a qualifying examination, but guidelines are vague. Therefore, the written preliminary PhD examination procedure is defined in this document.

## VII. CRITERIA FOR SUCCESS IN THE PROGRAM OF STUDY

### Successful advancement toward the graduate degree

The Department of Veterinary Pathology has certain benchmarks that define successful advancement toward the graduate degree. A student must:

1. Comply with the general guidelines outlined in the University *Graduate College Handbook*.
2. Identify a qualified faculty member willing to serve as major professor.
3. Actively pursue, define, and engage in a research project suitable for the thesis or dissertation. This includes a brief outline of a research project within 12 months after the beginning of graduate studies.
4. Establish a program of study (POS) committee within 2.5 years after the beginning of graduate studies.
5. Successfully complete the preliminary examination as defined in the section "Degree examinations," in this manual.
6. Successfully defend the thesis or dissertation as determined by their POS committee and major professor.
7. In addition to those requirements above, to fulfill assigned teaching and service duties to the satisfaction of the Department Chair when applicable.
8. For graduate students funded by K08 or other training grants. In addition to numbers 1-6 above, maintain compliance with the duties and responsibilities outlined in the funded grant proposal and comply with departmental guidelines on K08 training programs.

### Unsatisfactory performance

Unsatisfactory performance is defined as:

1. Consistent non-compliance with the University *Graduate College Handbook* including, but not limited to, lack of research progress (determined by the major professor and POS committee; see below), inappropriate behavior, and academic dishonesty (in the classroom, in research, or other).
2. Inability to identify a qualified faculty member to serve as major professor. A faculty member who wishes to terminate service as major professor may do so by notifying the student and Chair in writing. If the student is in good-standing, but the major professor is simply leaving the university, then the student has 6 months to find another major professor. An extension can be requested by the student, but the extension must be approved by the Chair.
3. Unsuitable advancement in a research project as determined by the major professor and POS committee (if formed). It is expected that the student develop a simple outline of a research project and begin research activity within 12 months of the beginning of the graduate studies. An extension can be requested by the student, but the extension must be approved by the major professor. It is also expected that the student diligently works on research projects, writing results, attempting to publish, and attending scientific seminars. Inactivity or continued failure in one or all of these areas can result in termination by the major professor and POS committee as outlined below.
4. Inability to establish a POS committee, including a research plan within 2.5 years of the beginning of the graduate studies. An extension can be requested by the student, but the extension must be approved by the major professor.
5. Unsatisfactory performance in the preliminary examination as defined in "Degree examinations," of this manual and is determined by the POS committee.
6. Unsuccessful defense of the thesis or dissertation as determined by the POS committee and major professor.
7. For students with assignments in service and teaching duties, failure to fulfill assigned duties. It is expected that students: attend assigned courses, laboratories and service rotations unless prior permission is requested and received from the faculty member in charge. It is also expected that the students are prepared, competent, and communicate clearly in spoken and written forms.
8. For graduate students funded by K08 or other training grants. Maintain compliance with the duties and responsibilities outlined in the funded grant proposal.

### **Unsatisfactory behavior**

The Department of Veterinary Pathology follows the guidelines set forth in the University *Graduate College Handbook*. Those students not in compliance will be dismissed.

### **Dismissal**

Dismissal will occur for unsatisfactory performance or behavior as outlined above. The procedures are as described in the *Graduate College Handbook*. The Department Chair will confer with the major professor and/or POS committee. Before the dismissal is decided, the Chair must give the student a written notice explaining why dismissal is being considered. The Chair must discuss the situation with the student, the POS committee and Major professor in an attempt for resolution. This discussion constitutes the “informal conference” as described in the *Graduate College Handbook*. Dismissal is decided by the Chair in conjunction with the major professor and POS committee. If there is disagreement between the Chair and major professor, the POS committee, if formed, may vote on a majority-rule basis. If there has not been a POS committee formed, then either the Departmental Graduate Curriculum Committee or an ad hoc committee formed by the Dean of the College will vote on a majority-rule basis after receiving written and/or oral information from the student, major professor and Chair. The student may appeal the decision to the Associate Dean, as stated in the *Graduate College Handbook*. Dismissed students will not be able to begin another project with a different faculty member within the Department of Veterinary Pathology.

In the case of academic dishonesty, procedures outlined in the *University Catalog* and the *Faculty Handbook* will be followed. Punishments can include dismissal from the Department and expulsion from the University, depending on the severity of the offense.

## VIII. POST MORTEM

PERSONNEL – Dr. Yaeger is in charge of the post mortem service. Ms. Deb Moore is the technician in charge of the area.

A departmental technician and/or hourly Vet Med students are available to take care of the post mortem room, prepare fixatives, help set up before and clean up after laboratories, photograph gross specimens, and run errands.

SUPPLIES – Provided on necropsy floor.

Coveralls are available in the locker rooms. If you are unable to find ones that fit, see the histopathology laboratory supervisor about ordering some for you. You may select any available locker, but you will have to supply your own padlock. White disposable aprons are available in the post mortem room to help protect your coveralls.

Towels are available in the locker rooms. Dirty laundry goes into the hampers and is collected weekly.

TEACHING AND SERVICE ROTATION - Post Mortem is taught as a class to senior students and occasionally graduate students. The typical class size is 6 students. Class is scheduled 1:10 – 5:00 p.m. Monday through Friday, and 10:00 a.m. to noon on Saturday.

A senior and a junior pathologist will be assigned. The senior pathologist will assign duties to the junior pathologist dependent upon his/her training and experience.

Pathologists assigned to a case are expected to cut-in the tissues in a timely manner. (See “Cutting-in” under the Biopsy and Post Mortem Service Rotations).

Students in the clinics will enter cases into CVIS. Cases should **not be necropsied** if they have not been entered or if a necropsy form **signed by the submitting VTH clinician** has not been received.

CLEAN UP - Please dampen the floors before starting a post mortem examination (it will be easier to flush away blood and wastes later). A departmental technician should be available for cleanup; if hourly students are not available, the cleanup should be done by pathologists and/or graduate students.

If the technician is not available, the senior or junior pathologist should see that the camera room, and window between the post mortem room, tissue room, and Gross Path Seminar room are locked. The lights should be turned off.

Anyone discovering broken or malfunctioning equipment should report it to Dr. Yaeger or the departmental technician.

PHOTOGRAPHY - There is digital photographic equipment in the small room off the post mortem room. The technician can be called to take gross photographs. If the technician is not available, consult with the senior pathologist.

Digital images will be placed in each block's folder on the T drive and attached to the case by the departmental technician.

The department has Olympus microscopes available for taking photomicrographs.

## **PROCEDURES FOR SUBMITTING TISSUES FOR MICROBIOLOGIC TESTS**

### **A. Bacteriology**

Clin Micro Lab – for bacteriology

Enter test on Vetstar.

Alert Linda Nelson that there will be an extra charge to VCS.

### **B. Virology and Toxicology**

VDPAM – for most viral tests

Complete submission form

Submit sample in labeled container that is clean on the outside.

Alert Linda Nelson that there will be an extra charge to VCS.

### **C. Rabies Samples**

The pathologist will remove appropriate CNS tissue (brain, spinal cord).

Submit specimens of brain to VDL as per CDC standards. Retain unused tissue for histopathology.

### **D. West Nile Virus – See USDA recommendations at [www.aphis.usda.gov/oa/wnv/wnvguide.html](http://www.aphis.usda.gov/oa/wnv/wnvguide.html)**

## IX. BIOPSY AND POST MORTEM SERVICE ROTATIONS

### Biopsy Service

VTH surgicals (H)  
Mail-in specimens (M)

### Post Mortem Service

VTH cases (H)  
Ambulatory, Prairie Meadows, and other cases (M)

**ASSIGNMENTS** - There is one senior pathologist assigned to each service. One graduate student may be assigned to each service. Schedules are prepared before the academic year begins. Duty assignments start on Monday and are for a week at a time. It is your responsibility to resolve any scheduling conflicts. Please notify the Vet Path Laboratory Services office and the Histopathology Lab of any changes in the schedule. The biopsy and necropsy rotations may be taken for credit (VPTH 550 and 551). Days missed due to illness/meetings/courses will be made up at the discretion of the senior pathologist.

**“CUTTING-IN”** - *Post Mortem* Cases are generally cut-in downstairs in room 1690 by the pathologist or graduate student. Cut-in tissues are to be brought to the Histopathology Lab or left in small containers of formalin on the tray on the counter by the door. (Histopathology technicians will take them upstairs for processing.) If you have an especially large case requiring extensive identification of individual specimens, you may ask the histology technicians for assistance as you cut-in. Remaining tissue on the case is placed in the area designated on the counter. The laboratory technician will file the tissue. Tissues are saved until one month after the case is completed unless a request to save the tissues is made.

*Biopsies* are cut-in by graduate students in room 2703 (Histopathology Laboratory) with the assistance of a technician. If you are on duty please make arrangements with the Histopathology Laboratory personnel for doing this at a convenient time. Scheduling is flexible, but please try to complete by 4:00 p.m.

**OBTAINING COMPLETED SLIDES** - Completed slides are placed in folders with the corresponding case report and placed near the phone in room 2709 (Histopathology Laboratory). Typically, cases from one day will be completed by 10:00 a.m. (biopsies), or 11:00 a.m. (necropsies) the next day.

**SPECIAL STAINS** - The finished slides will be placed on the counter where you pick up the rest of your slides. Special stains are done daily and in most cases you can expect the special stain (especially if requested early in the day) to be done before the end of the day. A list of special stains and their usage is included in this manual.

**FROZEN SECTIONS** - The pathologist on biopsy duty is responsible for reading the infrequent frozen sections. If you are on duty, please keep the Histopathology Laboratory personnel informed of your whereabouts so the technician will be able to find you if a specimen for frozen sections arrives. They will try to keep you informed if they know ahead of time, but often frozen sections arrive with no warning.

**COMPLETED CASES** – Paperwork for completed cases should be placed in the tray on the desk of Linda Nelson, Laboratory Services (Room 2706). (See section entitled “Case Reports – VADDS”.) Slides of completed cases should be turned in to the Histopathology Laboratory by placing them on the counter by the bookcase. If you wish to retain the slides and report for further study, photos, publications, etc., please turn in the original report. We will make a copy of the report for you and check the slides out to you. The original can then be coded and filed in the permanent records.



## FIXATION

**PRINCIPLE** - Protoplasm is mainly composed of proteins, lipids, carbohydrates and inorganic salts. The main purpose of fixation is the coagulation or precipitation of these protoplasmic substances which renders the cells and tissue elements resistant to further changes from the reagents to which they are subjected before microscopic sections can be prepared. Fixation, the stabilization of protein, is the singularly most important step in producing good histologic slides. The choice of fixative will depend on the nature of the pathologic lesion present in the tissue.

Small blocks of tissue, ideally not more than 2 cm square and not more than 3 to 4 mm thick, are placed in 10 to 20 times their volume of fixative. Surgical and necropsy specimens should be fixed as soon as possible after removal. The length of time required for fixation will depend on the size and density of the tissue, the rate of penetration of the fixing fluid, and the temperature. Small biopsy specimens and loose-textured tissues will fix far more rapidly than masses of fibrous tissue or whole organs.

**FIXATIVES AVAILABLE** - Neutral Buffered Formalin - 10% neutral buffered formalin is considered to preserve the structure of the living cells better than any other fixative. It is comparatively rapid acting and permits the use of a variety of staining procedures. Formalin is also cheap and does not over harden tissue even during long periods of immersion.

The first station of the tissue processor contains NBF and is the holding station for tissue until the processing cycle starts at 5:30 p.m. Partially fixed tissue may be put into the processor and fixation completed during this time. On weekends the processor does not start the processing cycle until 5:30 p.m. on Sunday. Until the processing cycle begins, the processor will say DELAY at the bottom of the screen - the processor lid may be opened and cassettes added during this time. Please do not add cassettes after the processing cycle has begun!!!

Carboys of ready to use NBF are available in the necropsy room, the tissue storage room and the histopathology laboratory (2703).

**BOUIN'S** - Bouin's is used for soft, delicate tissues. Specimens should be cut small enough to fix in 4-6 hours. After fixation the specimens are placed in alkaline alcohol (to remove the yellow picric acid). After tissues have been trimmed they should be placed in 70% alcohol until processed. Wet tissue should be stored in 70% alcohol. Specimens fixed in Bouin's may be processed starting in 70% alcohol or NBF.

Eyes are fixed in Bouin's for 24 hours, then washed for at least an hour in running tap water (do not wash other types of tissue fixed in Bouin's in running water), and placed in alkaline alcohol for several hours before trimming. Wet tissue should be stored in 70% alcohol.

Prepared Bouin's is available in the histopathology laboratory in the cabinet under the Technicon (old processor), and in the necropsy lab.

**B-5 FIXATIVE** - B-5 Fixative has been used on some research projects. Again, specimens should be cut small enough to fix in only 4-24 hours. Minimal times are optimal if there is a desire to do immunohistochemistry procedures. After fixation is complete, the fixative should be discarded in the mercury waste bottle in the fume hood in room 2703 using a plastic strainer - do not use metal, it reacts with B-5 Fixative to form a hazardous mercuric precipitate. Tissues should be placed in 70% alcohol and not NBF.

B-4 Fixative is available in the lower cupboard behind the door in room 2703. The small vial of formaldehyde attached to the container should be added just before use.

Because B-4 contains mercuric chloride, special treatment of slides is needed to eliminate the precipitate that forms. Please indicate on the side of the processing cassette that the specimen was fixed in B-5 Fixative.

**JORE'S FIXATIVE FOR GROSS SPECIMENS** - A carboy of prepared Jore's is available in the necropsy room for the preservation of large specimens for the museum and Gross Path Seminar.

Special fixatives can be prepared. Talk with the Histopathology personnel.

## ACCESSIONS

### Diagnostic Cases

1. All diagnostic cases are logged in chronologically in a single accession book kept in the Histopathology Laboratory (2703).
2. Accession numbers are assigned in the following manner:
  - Year (03)
  - Source (H-post, H-surgical biopsy, M-mail-in)
  - Sequential number (ie: 03-H-1234; 03-H-1235; 03-M-1236, etc)
3. Individual blocks within each case are lettered alphabetically (ie: 03-H-1234-A; 03-H-1234-B).

### Research Cases

1. Research, both funded and unfunded, may be processed through the Histopathology Laboratory.
2. Research cases are logged into a separate book (drawer of desk in 2703). The numbering system is similar but with an "R" in them (i.e.: 03-R-345; 03-R-346).
3. Be sure to put complete information in the book when entering research cases (there may not be other good records).
4. Fill out a blue NCR research form (available in the Histopathology Laboratory) completely. Include special instructions for processing, cutting, or staining.
5. Research tissues will be processed on a space-available basis according to order of arrival in the laboratory unless there are extenuating circumstances.
6. There is a charge for procedures done on funded research projects. A sheet containing current prices is available from the Histopathology laboratory supervisor.

### Veterinary Diagnostic Laboratory Cases

1. Occasionally special stains or procedures are requested on specimens from the Veterinary Diagnostic Laboratory.
2. Diagnostic Laboratory specimens are logged into a separate log book (also located in the drawer of the desk in 2703).
3. The numbering system used by the Diagnostic Laboratory is retained.

## **DEMINERALIZATION**

**PRINCIPLE** - Demineralization methods include use of acids, ion-exchange resins and electrical ionization. Acid solutions are most widely used for routine decalcification of bone and calcified tissue. The principle underlying the action of acid decalcifying agents involves the solubilities of metallic salts. Calcium occurs in bones chiefly as carbonate and phosphate salts, and these salts are only slightly soluble in water. An acid will act to release the calcium from its combination with the anions and effect an ion exchange to give a soluble calcium salt which is released into the decalcifying fluid.

**SPECIMEN** - Tissues containing calcium deposits in sufficient quantity to hinder cutting of sections for microscopic examination should be decalcified. If you have questions about whether a tissue should be decalcified, consult with Histopathology Laboratory personnel. Tissue should be well-fixed before being subjected to the decalcifying chemicals. The fixative of choice is 10% formalin, although other fixatives may be used. Formalin fixed tissue should be washed in running water before decalcification.

**DEMINERALIZATION PROCEDURE** - The routine decal solution used in the Histopathology Laboratory is a mixture of sodium formate and formic acid, or 25% formic acid alone. Prepared solutions are located on the counter next to the sink in room 2703. Histopathology technicians will assist with the procedure.

## PROCESSING

**PRINCIPLE** - The most commonly used method of examining tissue microscopically is by sectioning. Since fixed tissues are not firm enough and cohesive enough to permit perfect thin sections to be cut on a microtome at 4 to 6 microns, it is necessary that they be completely impregnated with some supporting medium to furnish stability and to hold the cells and intercellular structures in proper relationship to each other. Histopathology technicians operate this procedure.

Before an embedding medium such as paraffin (or plastic) can enter tissue, fixed tissue that contains a high water content must be dehydrated. Dehydration of tissue is usually carried out with ethyl alcohol. This process is done in graded strengths of ethanol to gradually displace the water in the tissue. The time of immersion in the various strength alcohols varies with the size and permeability of the tissue.

Alcohols and paraffin are not miscible. Clearing agents, therefore, which are miscible with both are used between the alcohol and the paraffin. The phrase "clearing agent" is used because the high refractive index of these substances renders the tissue more or less transparent.

After tissue specimens have been completely dehydrated and cleared, they are immersed in melted paraffin for 2 to 4 hours. Usually two or more changes of paraffin are required to eliminate the traces of the clearing agent which would prevent the paraffin from hardening properly. The liquid paraffin infiltrates the tissue, and when cold and solidified it provides the support necessary for cutting thin sections.

Cassettes Color Key:

White - Used for routinely handled specimens (cut at 5 microns, 1 H&E)

Orange - Used for lymph nodes or tissue to be cut at 3 microns

Pink - Teaching

Green - Controls

Gray - Used for small biopsies that might go through the slots in the routine white cassettes

Biopsy bags and sponges are also available. Use the sponges in the drawer next to the cut-in hood. Wet sponges before using - dry sponges cause a drying artifact on the tissue.

Large cassettes are available for eyes.

To ensure best results of processed specimens:

1. Please cut specimens 3-4 mm maximum thickness.
2. Allow room in the cassette for processing fluids to flow around tissue. Don't squeeze tissue into cassettes.
3. Submit properly fixed tissue for processing. Don't put fresh tissue in the processor at 5 pm - Let Histopathology Lab personnel know if it has to be out the next day, and we will fix with microwave procedures.

**LOADING PROCESSOR** - The processor is set up to run on a preprogrammed cycle. Specimens are held in the first (NBF) station until the processing cycle begins at 5:30 p.m. (routine). *Fixed* specimens may be added to the processor before the processing program has begun - unlatch and raise the lid of the process chamber, lift the weighted metal plate, place cassettes in the rack, replace the weighted metal plate, close the lid and latch snugly. On weekends the processor is programmed to begin the processing cycle at 5:30 Sunday night. Do not add specimens to the processor after the processing cycle has started!!! Opening the processing chamber after the processing cycle has begun will set off alarms and automatically telephone a member of the histology staff at home. If it will be necessary for you to add specimens after the processing cycle has started, contact the Histopathology Laboratory supervisor and she will assign an access code to you. Remember, there is the option of a rush processing run the next morning.

**PLASTICS** - Glycolmethacrylate processing is available for research projects. Talk with the histopathology laboratory supervisor.

## **CASE REPORTS – VADDS**

Please refer to the VADDS Standard Operating Procedures notebook in the Histopathology Lab (Rm 2703)

# SPECIAL STAINS

## BACTERIA -

- Modified Steiner for Spirochetes
- PVK (Pierce-Vanderkamp Modification of Gimenez for Chlamydia and Rickettsia)
- Himes and Moriber Triple Stain for coccidia
- Ziehl-Neelsen AFB (Acid Fast Bacteria)
- Macchiavello's - Rickettsia
- Warthin-Starry pH 4.0 - Spirochetes
- Acid Fast Method for Nocardia

## CARBOHYDRATES -

- Diastase digestion of Glycogen
- PAS (Periodic Acid-Schiff)
- PASM (Periodic Acid-Schiff-Methenamine)
- Alcian Blue pH 2.5
- PAS-Alcian Blue for Mucopolysaccharides
- HID-AM (High Iron Diamine-Alcian Blue)
- Alcian Blue pH 1.0

## FUNGI -

- GMS (Grocott's Methenamine Silver)
- Gridley
- Mayer's Mucicarmine
- PASH (Periodic Acid-Schiff's-Hematoxylin)
- GMS/H&E

## MUSCLE/CONNECTIVE TISSUE -

- Gomori's Reticulum Stain
- Gomori's Trichrome Stain
- Verhoeff's-van Gieson's Elastic Stain
- Heidenhain's Iron Hematoxylin
- HBFP (Hematoxylin-Basic Fuchsin-Picric Acid)
- Masson Trichrome
- Mallory's PTAH (Phosphotungstic Acid-Hematoxylin)

## PIGMENTS AND MINERALS -

- Warthin-Starry pH 3.2 - pre-melanin
- Rhodamine Method for Copper
- Perl's Method for Iron
- Hall's - Bilirubin
- von Kossa's - Calcium
- Melanin Bleach
- Schmorl's Ferric-Ferricyanide Reduction Test
- Fontana-Masson Silver Method
- AFB for Lead Inclusion
- Alizarin Red S for Calcium
- Rubeanic Acid Method for Copper

## PITUITARY -

- Wilson-Ezrin

## PROTEINS/FIBRIN/AMYLOID -

- Crystal Violet for Amyloid
- Eastwood Congo Red
- Lieb's Crystal Violet Method for Amyloid
- Benhold's Congo Red

CNS -

- LFB (Luxol Fast Blue)
- LFB-PASH (Luxol Fast Blue-Periodic Acid-Schiff's Hematoxylin)
- Bielschowsky's - Axis cylinders and dendrites
- Mallory's PTAH for CNS (Phosphotungstic Acid-Hematoxylin)

INCLUSION BODIES -

- Schorr's
- Lendrum's
- Pierce Modification of M-M for Negri Bodies

CELL GRANULES -

- Differentiated Eosin for Eosinophils
- Astra Blue - Mucosal Mast Cells
- Pascual's for Argrophil
- CSABA's mast Cell Stain
- Toluidine Blue for Mucosal Mast Cells
- Azure II/Methylene Blue/Basic Fuchsin (Plastic sections)
- Gomori's Chromaffin Stain
- LFB for Eosinophils

FATS AND LIPIDS -

- Oil Red O
- Acid Fast for Ceroid
- Sudan Black B

BLOOD SMEARS -

- Sudan Black B
- Giemsa Blood Smear
- Peroxidase Procedure
- Gomori's Method for Iron

RNA/DNA -

- Feulgen Reaction
- MGPY (Methyl Green-Pyronin Y)

If you need stains other than these, talk with the histopathology laboratory supervisor.

## PRIMARY ANTIBODIES AVAILABLE

### PRIMARY ANTIBODIES -

ACTH (Adrenocorticotrophic Hormone)  
Actin (Muscle specific)  
Actin (Smooth muscle specific)  
B-cell (CD79)  
Calcitonin  
Canine CD18  
Chromogranin-A  
c-kit  
Desmin  
Factor VIII  
Feline CD18  
Gastrin  
GFA (Glial Fib. Acid)  
Glucagon  
Insulin  
Keratin  
Melan A  
Myoglobin  
Neurofilaments  
NSE (Neuron Specific Enolase)  
Pancreatic Polypeptide  
PTH (Parathyroid Hormone)  
S-100  
T-Cell (CD3)  
Thyroglobulin  
Vimentin  
Rabies is available by special request

### IMMUNOGLOBULINS - Kappa/Lambda (any species)

<b>Canine</b>	<b>Feline</b>
IgG	IgG
IgA	IgA
IgM	IgM
Canine All Ig's (IgG, A, M)	Feline All Ig's (IgG, A, M)

Additional antibodies are used in research projects. Discuss purchasing of antibodies with the histopathology laboratory supervisor.



## **IMMUNOHISTOCHEMICAL STAINING**

FIXATION: Neutral buffered formalin, B-t, paraformaldehyde or Bouin's

IMMUNOHISTOCHEMICAL STAINING

Each Ab – our established protocols (antibodies)

## X. CLINICAL PATHOLOGY LABORATORY

The staff of the clinical pathology laboratory is anxious to assist you in your research efforts. Listed below is information you will find helpful in coordinating your research project with our laboratory operations.

1. Please schedule all research projects with the clinical pathology supervisor as soon as possible. Generally, at least 2 week's notice is required, however, longer is appreciated. We may need to order extra reagents and we must schedule your project so it does not conflict with other, ongoing research activities.
2. A completed copy of the Research Agreement form must be submitted at the time you are scheduling your project. We will also need to know the frequency of sampling and a list of your test requests.
3. Normal values will be established by the researchers using the results from their control animals.
4. Current lists of available tests and their charges can be obtained by calling the Clinical Pathology Lab at 294-0957.
5. Charges for tests do not include EDTA or serum vacutainers or needles. These may be purchased from the Veterinary Medicine storeroom.
6. Each sample submission must be accompanied by a specific test request form identified by your project title and tests requested. The form must be left with the samples on the desk in the receiving area of our laboratory.
7. Completed results will be sent to you via email or fax.

## **SAMPLE SUBMISSION PROTOCOLS**

**SAMPLE LABELING** - Animal identification should be established and defined by the researcher; this identification will appear on each report.

A laboratory sample identification number (SID) will be assigned to each sample tube submitted to the laboratory.

- Urine Samples - Collect 5cc of urine into a clean glass or plastic container which can be sealed. Refrigerate sample at 2-8° C until delivered to the laboratory.
  
- EDTA - Purple top vacutainer tube. Fill to the level predetermined by the vacuum within the tube. Gently rotate the tube immediately upon filling to prevent clot formation. Samples will be processed within the submission day or stored at 2-10° C until time of processing.
  
- Serum - Red top vacutainer tube. Do not rotate or mix, allowing sample to clot in tube (to avoid hemolysis). Submit samples to the laboratory within 60 minutes after collection. Samples will be centrifuged and the serum separated from the clot, for storage at 2-10° C until samples are analyzed. If you are unable to submit specimens immediately, contact the laboratory for possible alternative methods.