

GENERAL OUTLINE

HISTOTECHNOLOGY KNOWLEDGE AREAS

Histotechnician HT (ASCP)

Histotechnologist HTL (ASCP)



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CERTIFICATION OF TECHNICIAN

HT(ASCP) *Histologic Technician*

The HT(ASCP) application is valid for five (5) years from the date it is received. Both the practical and multiple-choice components of the examination must be successfully completed within this five year period for certification to be granted.

To be eligible for this examination category, an applicant must satisfy the requirements of at least one of the following qualification routes:

1. Successful completion of a NAACLS accredited Histologic Technician program.
2. Associate degree or at least 60 semester hours (90 quarter hours) of academic credit from a regionally accredited college/university with combination of 12 semester hours (18 quarter hours) of biology and chemistry, **and** one year full time acceptable experience in histopathology within the last ten years under the supervision of a pathologist (*certified by the American Board of Pathology in Anatomic Pathology, or eligible*), or an appropriately certified medical scientist.

CERTIFICATION OF HISTOTECHNOLOGIST

HTL(ASCP) *Histotechnologist*

The HTL (ASCP) application is valid for five (5) years from the date it is received. Both the practical and multiple-choice components of the examination must be successfully completed with this five year period for certification to be granted.

To be eligible for this examination category, an applicant must satisfy the requirement of at least one of the following qualification routes:

1. Baccalaureate degree from a regionally accredited college/university with a combination of 30 semester hours (45 quarter hours) of biology and chemistry, **and** one year full time acceptable experience in a histopathology laboratory within the last ten years, under the supervision of a pathologist (*certified by the American Board of Pathology in Anatomic Pathology, or eligible*), or an appropriately certified medical scientist.
2. Baccalaureate degree from a regionally accredited college/university including the above course requirements, **and** successful completion of a NAACLS accredited Histology Technician or Histo0technology program.

Acceptable Experience:

Full-time experience is defined as a minimum of thirty-five (35) hours per week. Individuals who have part-time experience may be permitted to utilize prorated part-time experience to meet the work experience requirements. For example, if you are employed 20 hours per week for one year, experience would be computed as 20 divided by 35 multiplied by 52 weeks which is the equivalent of 29.7 weeks of full-time employment. Contact the Board of Registry prior to applying if you have questions concerning part-time experience.

The Board of Registry does not accept clinical laboratory experience obtained in a foreign country outside the United States or Canada.

To fulfill the experience requirements for the examinations, you must have experience in the following areas within the last ten years.

- | | |
|------------|---|
| -fixation | -specimen processing, including embedding |
| -microtomy | -staining, including reagent preparation |

Applications can be obtained from: ASCP, Board of Registry
P.O. Box 12277
Chicago, IL 60612
1-800-621-4142

SUGGESTED READING IN PERPARATION FOR TAKING THE HT/HTL EXAMS

Material will provide a broad spectrum of knowledge in histotechnology.

(*These references are more appropriate for HTL level.)

HISTOCHEMISTRY – THEORETICAL & APPLIED, 4th ed., Vol 2, 1985, A.G.E. Pearse, Churchill-Livingstone.
(ISBN #0-443-02997-0)

HISTOLOGICAL & HISTOCHEMICAL METHODS – THEORY & PRACTICE, 1999, J.A. Kiernan,
(Order #0-7506-4936-4) Elsevier Science Publishing Company.

FIXATION FOR ELECTRON MICROSCOPY, 1982, M.A. Hayat, (ISBN # 0-12-333920-0),
Elsevier Science Publishing Co.

THEORY & PRACTICE OF HISTOTECHNOLOGY, 1980, 2nd ed., D. Sheehan & B. Hrapchak,
Battelle Press.

HISTOTECHNOLOGY: A SELF INSTRUCTIONAL TEXT, 2nd ed., 1997, F. Carson, ASCP Press.
Text: (#0-89189-411-X) Workbook (#0-89189-412-8)

HISTOPATHOLOGIC METHODS AND COLOR ATLAS OF SPECIAL STAINS AND TISSUE ARTIFACTS,
Lee G. Luna, 1993. American HistoLabs, Inc.

***IMMUNOHISTOPATHOLOGY: A PRACTICAL APPROACH TO DIAGNOSIS**, 2nd ed., 2003, J.M. Elias,
(Order #0-891502-7) ASCP Press

***THEORY & PRACTICE OF HISTOLOGICAL TECHNIQUES**, 5TH ED., 2001, J. Bancroft & M. Gamble,
(ISBN # 0443-0644350) Churchill-Livingston.

ADDITIONAL STUDY AIDS

THE NSH/ASCP Board of Registry Study Guide for Histotechnology Examinations, 2nd ed., 2001, F. Carson (Ed.),
(Order #089189473X) ASCP Press

JOURNAL OF HISTOTECHNOLOGY, by membership or subscription, National Society for Histotechnology,
4201 Northview Drive, Suite 502, Bowie, MD 20716-2604; Phone: 310-262-6221; Fax: 301-262-9188

SELF-ASSESSMENT EXAMINATION BOOKLETS, NSH (See last page for order form)

HT/HTL STUDY GUIDE, 2nd ed., Michigan Society for Histotechnologists, c/o Dick Dapson, 1020 Harts Lake Road, Battle
Creek, MI 49015 or contact: Micki Conrad, W: 269-668-3336 x353; H: 269-668-2726; email: mickiconrad@mickiconrad.com.

DERMATOPATHOLOGY: A GUIDE FOR THE HISTOLOGIST, 2004, C. chapman. Orders may be placed at:
www.medi-sci.com, or Medi-Sci Consultants, 22 Pitman Avenue, Wakefield, MA 01880, Tel: 781-246-3280.

ORDER TEXTS DIRECTLY FROM PUBLISHER:

Academic Press
6277 Sea Harbor Dr.
Orlando, FL 32887
800-321-5068

ASCP Press
2100 W. Harrison St.
Chicago, IL 60612-0075
800-621-4142

Churchill-Livingstone
6277 Sea Harbor Dr
Orlando, FL 32887
800-553-5426

Lippincott-Raven
P.O. Box 1600
Hagerstown, MD 21741-1600
800-777-2295
email: l1orders@ph1.l1rpub.com

American HistoLabs
7605-F Airpark Road
Gaithersburg, MD 20879

Battelle Press
505 King Avenue
Columbus, OH 43201-2693

Elsevier Science
P.O. Box 945
New York, NY 10159-0945
888-437-4636
email: usinfo-f@elsevier.com

NOTICE TO USERS OF GENERAL OUTLINE HISTOTECHNOLOGY KNOWLEDGE AREAS

The General Outline was developed by the National Society for Histotechnology (NSH) Education Committee using the American Society of Clinical Pathologists (ASCP) Board of Registry Examination Content Guidelines with their permission. The General Outline has not been approved by the ASCP Board of Registry.

The General Outline is intended for use as a guide by NSH members when studying for the ASCP Board of Registry Histologic Technician (HT) and Histotechnologist (HTL) exams. These exams are periodically changed to reflect new procedures, techniques, and concerns in the field. While every effort has been made to be current, the following outline may not contain all areas to be tested.

Please refer to the “Histologic Technician HT (ASCP) and Histotechnologist HTL (ASCP) Examination Content Guideline”, published by the American Society of Clinical Pathologists (ASCP) Board of Registry for more complete information, descriptions and explanations of:

- Career Entry competency statements for both HT and HTL

- Taxonomy levels for exam questions

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GENERAL OUTLINE – HISTOTECHNOLOGY KNOWLEDGE AREA
Histologic Technician HT (ASCP) and Histotechnologist HTL (ASCP)
(knowledge areas specific to the HTL)**

- I. **FIXATION (10-25%)**
 - A. **Tissues**
 1. **Morphology/Anatomy** (see **STAINING**)
 2. **Cell/Component preservation**
 - a. Indications for use
 - (1) Required by what stain
 - (2) Required for preservation of what cell/component
 - b. Contra-indications for use
 - (1) Should not be used, as destroys/injures what component/cell
 3. **Pathology****
 - a. Required for which diseases
 4. **Biochemistry principles/theories****
 - a. Binds with what amino acid/nucleic acid, etc.
 - B. **Procedures**
 1. **Light microscopy**
 - a. Which fixative
 - b. Primary and Secondary
 2. **Electron microscopy**
 - s. Which fixative required (see **STAINING**)
 3. **Special stains**
 4. **Frozen sections/tissues**
 - a. Fixed or unfixed
 - b. Which fixatives
 5. **Enzyme histochemistry**
 - a. Fixed or unfixed
 - b. Which fixative
 - c. Best temperature
 6. **Immunohistochemistry**
 - a. Fixed or unfixed
 - b. Advantages/Disadvantages of various fixatives
 7. **Artifacts/Precipitates/Pigments**
 - a. Endogenous/Exogenous
 - b. Cause of formation
 - c. Methods to prevent
 - d. Identification of pigments through microscope
 - (1) Size/shape
 - (2) Location
 - (3) Polarization
 3. Methods of removal
 8. **Quality Control**
 - a. Shelf-life
- C. **Parameters** (To be applied to all fixatives listed below)
 1. **Size of specimen**
 - a. Maximum section thickness
 - b. Effects of too thick specimen
 2. **Volume of specimen/fixative**
 - a. Minimum ratio required
 - b. Effects of inadequate volume
 3. **Time in fixation**
 - a. Minimum time
 - b. Maximum time
 - c. Effects of underfixation
 - d. Effects of overfixation
 4. **Temperature**
 - a. Effects of adding heat
 - b. Effects of too high temperature
 - c. Effect of too long time in heat
 - d. Effects of freezing
 5. **Other**
 - a. Autolysis vs. Putrefaction
 - b. Chemical vs. Physical effects
 - c. Effects of pH
 - d. Primary vs. Secondary vs. Post-fixation
 - e. Coagulative vs. non-coagulative
 - f. Additive vs. non-additive
 - g. Effects of delay in fixation
- D. **Reagents**
 1. **Types/Components**
 - a. The following information is needed on the fixatives listed below:
 - (1) Reagents used
 - (2) Alternate reagents that could be used
 - (3) Direction for preparation
 - (4) Direction for storage
 - (5) Hazards and handling precautions
 - (6) Disposal
 - b. Aldehydes
 - (1) 10% unbuffered formalin
 - (2) higher % formalin
 - (3) 10% neutral buffered formalin
 - (4) alcoholic formalin
 - (5) formalin ammonium bromide (FAB)
 - (6) glutaraldehyde
 - (7) paraformaldehyde
 - c. Picric acid
 - (1) Bouin
 - (2) Duboscq Brasil (DB)
 - (3) Zamboni

- d. Mercuric/Chromate/Zinc/Copper
 - (1) Zenker
 - (2) Helly
 - (3) B-5
 - (4) Heidenhain (Susa)
 - (5) Muller
 - (6) Orth
 - (7) Zinc-formalin
 - (8) Hoallnde
- e. Osmium
 - (1) 1% osmium tetroxide
 - (2) Marchi
- f. Nonaqueous
 - (1) Carnoy
 - (2) Clark
 - (3) Acetone
 - (4) Menthanol
 - (5) Ethanol
 - (6) Acetic acid
- g. Other
 - (1) Proprietary
 - (2) Formalin substitutes
 - (a) Glyoxal
 - (3) Dual purpose (light and EM)

2. Properties/Function/Actions

- a. Covering the fixatives listed in above list:
- b. Purpose of each reagent/effect on tissue
 - (1) nucleic acid
 - (2) cytoplasm
 - (3) lipids
 - (4) all other components (connective tissue, muscle, etc.)
- c. Directions for use
- d. Coagulative or non-coagulative
- e. Additive or non-additive

3. Chemistry principles/theories**

E. Instrumentation (e.g. microwave)

- 1. See **LABORATORY OPERATIONS**

II. PROCESSING/EMBEDDING (10-14%)

A. Tissues

- 1. **Morphology/Anatomy**
 - a. Embedding
 - (1) Specimen orientation
 - (2) Cross-contamination
 - (3) Specimen identification
 - b. See **STAINING**
- 2. **Cell component preservation**
 - a. Reagent required
 - b. Reagent to be avoided

B. Procedures

- 1. **Light microscopy**
 - a. Purpose of steps involved
- 2. **Electron microscopy**
 - a. Purpose of steps involved

3. Frozen sections/tissues

- a. Methods of freezing
 - (1) freezing bar
 - (2) isopentane
 - (3) liquid nitrogen
 - (4) liquid nitrogen/isopentane
 - (5) carbon dioxide
 - (6) cryogenic spray
- b. Advantages/Disadvantages/each method
- c. Hazards/each method
- d. When to use frozen section/each method

4. Enzyme histochemistry

- a. Frozen section of fixation/processing
- b. When to use each method
- c. Effects of each method on enzymes

5. Decalcification

- a. The following may be asked for each procedure:
 - (1) Purpose of reagent(s)
 - (2) Directions of use
 - (3) Effects of:
 - (a) Temperature
 - (b) Section thickness
 - (c) Volume tissue/decalcification solution
 - (d) Time
 - i) Maximum/minimum
 - ii) Effects of over decalcification
 - a) Cell/Component
 - b) Staining
 - (e) pH/Buffers
 - (f) Concentration
 - (g) Agitation
 - (4) Indications for use
 - (a) Required for which procedures/stains
 - (b) Which procedure would be best for each procedure/stain
 - (5) Contra-indicated for use
 - (a) When would decalcification NOT be needed
 - i) Undecalcified bone
 - ii) Preserve microcalcification
 - (6) Damages caused by each procedure
 - (7) Hazards and handling/disposal precautions
- b. Method
 - (1) Strong acid
 - (a) hydrochloric acid
 - (b) nitric acid
 - (c) sulfuric acid
 - (2) Formic acid
 - (a) Formic acid (%)
 - (b) Formic acid-sodium citrate (FASC)
 - i) Effects of buffering
 - (3) Weak acids
 - (a) Picric acid in fixatives
 - (b) Acetic acid in fixatives
 - (4) Chelating agents (e.g. EDTA)
 - (5) Ion Exchange Resin
 - (6) Miscellaneous

- (a) Electrolytic
- (b) Ultrasonics
- c. End-point determination
 - (1) For each of the following, may be asked:
 - (a) How to perform method
 - (b) Advantages/disadvantages
 - (2) Method
 - (a) Physical
 - i) Bending, probing, etc.
 - (b) X-ray
 - (c) Weighing
 - (d) Chemical
 - i) Calcium oxalate

6. **Immunohistochemistry**

- a. Frozen section or fixation/processing

7. **Quality Control**

C. **Instrumentation**

- 1. See **LABORATORY OPERATIONS**

D. **Reagents/Media**

1. **Types/Components**

- a. The following information is needed on each reagent:
 - (1) Reagent(s) used
 - (2) Alternate name(s)
 - (3) Purpose of reagent
 - (4) Hazards and handling precautions/disposal
- b. Reagents
 - (1) Dehydrants
 - (a) Ethanol
 - i) pure
 - ii) denatured (reagent)
 - (b) Mehtanol
 - (c) Isopropanol
 - (d) Butanol
 - (e) Acetone
 - (f) Cellosolve (ethylene glycol monoethyl ether)
 - (g) propylene oxide
 - (h) proprietary
 - (i) solid
 - i) anhydrous copper sulfate
 - ii) anhydrous calcium sulfate
 - (2) Universal solvents
 - (a) Tetrahydrofuran (THF)
 - (b) Tertiary butyl alcohol
 - (c) Diozane
 - (3) Clearants
 - (a) Xylene
 - (b) Toluene
 - (c) Benzene
 - (d) Chloroform
 - (e) Carbon tetrachloride
 - (f) Methyl salicylate
 - (g) Xylene substitutes
 - i) Limonene derivatives
 - ii) Aliphatic hydrocarbons
 - (h) Essential oils
 - (4) Infiltrating/Embedding media

- (a) Aliphatic
 - i) Paraffin
- (b) Water soluble waxes
 - i) Carbowax
- (c) Double embedding
 - i) Agar
 - ii) Gelatin
 - iii) Method
 - a) Frozen sectioning
 - b) Paraffin
- (d) Celloidin
- (e) Resins
 - i) Electron microscopy
 - a) Hardeners
 - b) Accelerators
 - c) Plasticisors
 - d) Resins (Epon, Spurr, etc.)
 - ii) Glycolmethacrylate

Properties/Function/Action – The following is needed for above reagents:

- a. Directions for use
- b. Definition of dehydrating, clearing, infiltrating
- c. Temperature
 - (1) Maximum/minimum
 - (2) Effects of too high/too long
- d. Maximum section thickness
- e. Volume tissue: reagent
- f. Time
 - (1) Minimum/Maximum
 - (2) Causes and effects of over and/or under dehydration/clearing/infiltrating
- g. Vacuum
- h. Advantages/Disadvantages
- i. Refractive index (clearing)
- j. How to solidify (infiltration)
 - (1) crystallization
 - (2) evaporation
 - (3) polymerization
- k. How to store
 - (1) reagents
 - (2) blocks

3. **Quality Control**

- a. Cause and effect of
 - (1) using wrong reagent
 - (2) following directions wrong
 - (3) contamination
- b. Methods to correct errors

4. **Chemistry principles/theories****

III. **MICROTOMY (10-14%)**

A. **Tissues (see STAINING)**

- 1. **Morphology**
- 2. **Cell/Component Demonstration**
 - a. What thickness
 - b. Frozen vs. processed/embedded

B. **Procedures**

- 1. **Paraffin**

- a. Knife
 - (1) Profile types
 - (a) bevel
 - (b) wedge
 - (c) clearance
 - (2) Angles
 - (a) bevel
 - (b) wedge
 - (c) clearance
 - (3) Material
 - (4) Sharpening
 - (a) Hone
 - (b) Strop
 - (c) Abrasive material
 - b. Microtome
 - (1) Type
 - (a) Rotary
 - (b) Sliding
 - (c) Base sledge
 - (d) Rocking
 - (2) Sections
 - (a) thickness
 - (b) temperature
 - (3) Troubleshoot
 - (a) thick/thin
 - (b) Venetian blind/micro chatter
 - (c) holes/tears/knife lines
 - (d) folds/wavy sections
 - (e) etc.
 - c. Floatation bath
 - (1) Solutions/Adhesives
 - (2) Cleaning
 - (3) Contamination
 - d. Drying oven
 - (1) temperature
2. **Frozen Section**
- a. Type of microtome
 - (1) Cryostat
 - (2) Clinical freezing
 - b. Temperature
 - (1) majority tissue
 - (2) fatty tissue
 - (3) tissue with high water content
 - (4) fixed tissue
 - c. Frozen sections
 - (1) Cutting techniques
 - (2) Troubleshooting
 - (a) Ice crystal artifact
 - (b) Poor freezing
 - (c) Sectioning artifacts
 - (d) Anti-roll plate
3. **Agar/gelatin**
- a. Double embedding
 - (1) paraffin
 - (2) frozen sectioning
 - b. Orientation of specimen
 - (1) during grossing
 - (2) on edge/friable
4. **Quality Control**
5. **Epoxy resin****
- a. Type of knives
 - (1) Glass
 - (2) Diamond
 - (3) How to make
 - (4) How to determine good knife edge
 - b. Thickness
 - (1) Colors seen during sectioning
 - (2) Cause of colors
 - (3) Need for thickness
 - (a) 0.5-1.0 μm
 - (b) 600-800 \AA (60-80 nm)
 - c. Troubleshooting
6. **Glycol methacrylate****
- a. Type of knife
 - b. Thickness
 - c. Troubleshooting
- C. **Instrumentation** – See above (IIIB)
1. See **LABORATORY OPERATIONS**
- IV. **STAINING (40-50%)**
- A. **Tissues**
1. **Morphology/Anatomy**
 - a. Be able to identify tissues/organs
 2. **Cell/Component demonstration**
 - a. Be able to identify cell/component
 - b. Select appropriate stain
 3. **Function**
 - a. Identify function of the tissue/cell/component
 4. **Pathology****
 - a. Identify diseases as to the stain required
 5. **Biochemistry principles/theories****
- B. **Procedures**
1. **Nucleus/Cytoplasmic**
 - a. Nuclear stains
 - (1) Hematoxylin
 - (a) Alum
 - (b) Iron
 - (c) Tungsten
 - (d) Other
 - (2) Methylene blue
 - (3) Celestine blue
 - (4) Carmine
 - b. Cytoplasm stains
 - (1) Eosin(s)
 - (2) Phloxine
 2. **Blood/Bone Marrow**
 - a. Giemsa and all formulations
 3. **Carbohydrates**
 - a. periodic acid-Schiff (PAS) with and without digestion
 - b. Mucicarmine
 - c. Alcian blue with and without digestion

- (1) pH 2.5 and/or pH 1.0
- d. Colloidal iron with/without digestion (Mowry)
- e. Amyloid
 - (1) Congo red
 - (2) Sirius red
 - (3) Thioflavin T/Thioflavin S
 - (4) Crystal violet/Methyl violet

4. **Connective/Supporting Tissue**

- a. Collagen
 - (1) Masson trichrome
 - (2) Gomori trichrome
 - (3) Other trichromes (Goldner, Mallory, etc.)
 - (4) Movat pentachrome
- b. Elastin
 - (1) Verhoeff-van Gieson
 - (2) Aldehyde fuchsin
 - (3) Orcein (Pinkus)
 - (4) Resorcin-Fuchsin
- c. Muscle/Fibrin
 - (1) PTAH
 - (2) Masson or Gomori trichrome
 - (3) Lendrum (MSB)
- d. Reticulin
 - (1) all silver procedures
- e. Basement membrane
 - (1) PAS
 - (2) Periodic acid-Methenamine silver (PASM, PAMS, Jones)

5. **Lipids**

- a. Oil red O
- b. Sudan black B
- c. Osmium tetroxide

6. **Microorganism**

- a. bacteria
 - (1) Gram
 - (2) Brown & Hopps
 - (3) Brown & Brenn
 - (4) Giemsa
 - (5) Spirochete stains
- b. Mycobacteria
 - (1) Kinyoun
 - (2) Ziehl-Neelsen
 - (3) Fites
 - (4) Auramine-Rhodamine
- c. Spirochetes
 - (1) Dieterle
 - (2) Warthin-Starry
 - (3) Steiner & Steiner
- d. Helicobacter
 - (1) Bacteria stains
 - (2) Spirochete stains
 - (3) Toluidine blue
 - (4) Diff-Quik
 - (5) with mucin counterstains
- e. Fungus/Yeast/Cryptococcus
 - (1) PASH
 - (2) Gridley
 - (3) Grocott Methenamine Silver (GMS)

- (4) Mucicarmine
- f. Pneumocystis
 - (1) Grocott Methenamine Silver (GMS)
- g. Amoeba
 - (1) Iron hematoxylin
 - (2) PASH
- h. Virus
 - (1) H&E
 - (2) Giemsa
 - (3) Hepatitis B
 - (a) Orcein
 - (b) Aldehyde fuchsin
 - (c) Victoria blue

7. **Nerve**

- a. Fibers
 - (1) Bodian
 - (2) Bielchowsky
 - (3) Sevier-Munger
 - (4) Holmes
- b. Myelin
 - (1) Luxol fast blue (LFB) (Kluver)
 - (2) Weil
 - (3) Trichrome stains
 - (4) Marchi
- c. Nissl
 - (1) Cresyl echt-violet (cresyl violet acetate)
 - (2) Thionin
- d. Glial cells
 - (1) Holzer
 - (2) Cajal
 - (3) PTAH

8. **Pigments/mineral Granules**

- a. Bile
 - (1) Fouchet (Hall)
 - (2) Stein
- b. Calcium
 - (1) von Kossa
 - (2) Alizarin red S (Dahl)
- c. Iron
 - (1) Prussian blue
- d. Copper
 - (1) Rhodanine (Lindquist)
 - (2) Rubeanic acid
- e. Melanin/Argentaffin
 - (1) Fontana-Masson
 - (2) Schmorl
- f. Argyrophil
 - (1) Grimelius
 - (2) Churukian-Schenk
 - (3) Sevier-Munger
 - (4) Pascual
- g. Urates
 - (1) Methenamine silver stains

9. **Quality Control**

- a. See **QC Reagents/Dyes**

C. **Miscellaneous procedures**

1. **Nucleic acids**

- a. Feulgen
- b. Methyl green-pyronin (MGP)
- c. Acridine orange
- d. Extraction of nucleic acids

2. **Tissues/Cells/Components**

- a. Fibrin
 - (1) See above (Connective Tissue)
- b. Mast cells
 - (1) Giemsa
 - (2) Toluidine blue
 - (3) Alcian blue pH 1.0
- c. Beta cells pancreas
 - (1) Aldehyde fuchsin
- d. Lipofuchsin
 - (1) PAS
 - (2) Lipid stains
 - (3) Carbol-fuchsin stains

3. **Quality Control**

- a. see QC Reagents/Dyes

4. **Electron Microscopy****

- a. Thick sections
 - (1) Polychromatic dyes
- b. Thin sections
 - (1) Uranyl acetate
 - (2) Lead citrate
 - (3) Negative staining

5. **Enzymes****

- a. Chloroacetate esterase (Leder)
- b. Muscle
 - (1) ATPase
 - (2) NADH
 - (3) SDH
 - (4) alpha-naphthyl acetate esterase
 - (5) Alkaline phosphatase
 - (6) Acid phosphatase
 - (7) Cytochrome oxidase
 - (8) Phosphorylase
- c. Techniques
 - (1) Metal salt
 - (2) Azo dye
 - (3) Indoxyl
 - (4) Oxidation-Reduction

6. **Immunohistochemistry****

- a. Immunofluorescence
 - (1) Fluorochromes (eg. FITC)
 - (2) When used
 - (3) Methods
 - (a) Direct
 - (b) Indirect
 - (c) Sandwich
- b. Immunoenzyme
 - (1) Types
 - (a) Immunoperoxidase
 - (b) Avidin-biotin complex (ABC)
 - (c) Labeled avidin biotin (LAB)
 - (d) Alkaline phosphatase

- (e) Immunogold
- (f) Other enzymes

(2) **Dyes**

- (a) DAB
- (b) AEC
- (c) etc.

c. **Staining components**

- (1) **Unmasking**
 - (a) digestion (trypsin, pronase, etc.)
 - (b) retrieval methods
 - i) Citrate, EDTA, etc.
 - ii) steamer, microwave, etc.
- (2) **Buffer washes**
- (3) **Removal of endogenous peroxidases**
- (4) **Protein blocking**
- (5) **Antibodies (primary, secondary, etc.)**
 - (a) dilution
 - (b) animal antibody raised in
 - (c) monoclonal vs. polyclonal
- (6) **Conjugated enzyme**
- (7) **Dye/development of color**
- (8) **Counterstains**
- (9) **Mounting media**

D. **Instrumentation**

1. See **LABORATORY OPERATIONS**

E. **Reagents/Dyes – Apply to all stains listed above**

1. **Types/Components**

- a. What dyes/reagents are used?
- b. Alternate for the dye/reagent (if possible)

2. **Properties/Functions/Actions**

- a. **Chemistry**
 - (1) atom/molecule/element
 - (2) compound/mixture
 - (3) salts/buffer
 - (4) acid/base/pH
 - (5) isoelectric point/zwitterion
 - (6) protein
 - (7) carbohydrate
 - (8) enzyme
 - (9) lipid
 - (10) etc.

b. **Used to:**

- (1) Stain
- (2) Sensitize
- (3) Oxidize
- (4) Differentiate
- (5) Reduce/Develop
- (6) Mordant
- (7) Impregnate
- (8) Adjust pH
- (9) etc.

c. **Staining mechanism**

- (1) Progressive/Regressive
- (2) Impregnation
- (3) Argentaffin/Argyrophil
- (4) Absorption/Adsorption
- (5) Metachromatic/Polychromatic
- (6) Physical/Chemical

- (7) etc.
- d. Color results
 - (1) Expected
 - (2) Troubleshooting

3. **Quality Control**

- a. Fixative to use/avoid
- b. Processing to use/avoid
- c. Special procedure required
 - (1) Frozen section
 - (2) Avoid water
 - (3) Decalcification
 - (4) etc.
- d. Control
 - (1) Best tissue to use
 - (2) Alternative control tissue
 - (3) Component/Cell that stains
- e. Recommended section thickness
- f. Sources of error and corrective action

4. **Chemistry principle/theories****

F. **Mounting Procedures**

1. **Media**

- a. Aqueous
 - (1) Indications for use
 - (2) Advantages/Disadvantages
 - (3) Sealers
- b. Resinous
 - (1) natural/Synthetic
 - (2) Advantages/Disadvantages
 - (3) Solvents

2. **Coverglass**

a. **Thickness**

3. **Refractive index ****

- a. Definition

V. **LABORATORY OPERATIONS (10-15%)**

A. **Safety**

1. **Storage**

- a. Reagents/Solutions
- b. Blocks/Slides
- c. Equipment

2. **Disposal**

- a. **Heavy metals**
- b. Organic Solvents
- c. Acids/Bases
- d. Formaldehyde/other fixatives

3. **Hazards**

- a. Reagents
 - (1) Health Hazard
 - (a) biohazardous
 - (b) carcinogenic
 - (c) toxic
 - (d) irritant
 - (e) corrosive
 - (f) sensitizer

- (2) Physical hazard
 - (a) Flammable/Combustible
 - (b) Reactivity (explosive)
 - (c) Radioactive
 - (d) Oxidizer
 - (e) Compressed gas

- (3) Route of entry
- (4) Target organs and effects

- b. Electrical
- c. Ergonomics (carpal tunnel, etc.)
- d. Identification – labeling
 - (1) Target organs
 - (2) NFPA diamond

4. **Regulations**

- a. Federal
 - (1) OSHA
 - (2) EPA
- b. State

5. **Procedures**

- a. Storage
- b. Recycling
- c. Disposal
- d. Training

6. **Quality control**

B. **Laboratory Mathematics**

1. **Metric system**

2. **Percent solutions/Dilutions**

3. **Normal/Molar solution****

C. **Equipment**

1. **Components** (identification)

2. **Use**

- a. Time schedules
- b. Vacuum
- c. Temperature
- d. Reagents

3. **Maintenance**

- a. Routine/Preventative
- b. Repair

4. **Troubleshooting**

- a. Equipment
- b. Failures
- c. Ergonomics
 - (1) Cause
 - (2) Prevention

5. **Quality Control**

- 6. The above information should be known on the following histology equipment
 - a. Tissue processors
 - b. Embedding Centers and equipment
 - c. Microtomes (See **MICROTOMY**)

- (1) Paraffin
- (2) Resin **
- (3) Cryostat
- d. Knife Sharpening Equipment (See **MICROTOMY**)
- e. Floatation baths (See **MICROTOMY**)
- f. Slide dryers
- g. Incubators
 - (1) Water baths
 - (2) Incubators/ovens
- h. Balances
- i. pH meters
- j. Staining equipment (automated)
- k. Coverslippers (automated)
- l. Microwave ovens, use in:
 - (1) fixation/processing
 - (2) slide drying
 - (3) staining
- m. Computers and Laboratory Information Systems
 - (1) data processing
 - (2) spread sheet
 - (3) presentations
 - (4) hardware vs. software
- n. Solvent recovery/distillation
- o. Freezers

D. Management**

- 1. **Theories****
- 2. **Procedures****
- 3. **Examples****
 - a. statistical methods
 - b. cost accounting
 - (1) procedures
 - (2) personnel/budget
 - c. test/equipment selection
 - d. quality control/assurance
 - e. personnel practices
 - f. workload recording

E. Education**

- 1. **Theories****
- 2. **Procedures****
- 3. **Examples****
 - a. Taxonomy levels
 - (1) recall
 - (2) analysis
 - (3) troubleshooting
 - b. Domains
 - (1) cognitive
 - (2) psychomotor
 - (3) affective
 - c. Competency/Proficiency

F. Regulations**

- 1. Federal Government**
 - a. OSHA
 - b. EPA
 - c. CDC
 - d. etc.
- 2. **Accrediting agencies****
 - a. JCAHO
 - b. CAP
 - c. NCCLS
 - d. NAACLS (training programs)
 - e. etc.
- 3. NOTE: Both HT and HTL are expected to know those regulations required of all people working in a hospital
 - a. Blood-borne pathogens
 - (1) universal precautions
 - b. Right-to-know
 - (1) Hazard communication
 - (2) MSDS
 - c. Laboratory Chemical Standard
 - (1) Chemical Hygiene Plan
 - d. Formaldehyde Standard
 - (1) levels
 - (2) monitoring

****HTL EXAM ONLY**

**LIST OF TISSUES AND STAINS FOR
ASCP BOARD OF REGISTRY HT AND HTL
PRACTICAL EXAMINATIONS**

A list of tissues and stains from which the HT practical examination will be selected:

HISTOTECHNICIAN (HT)

TISSUE	MINIMUM SIZE	STAIN
AFB -ant tissue -do NOT use embedded culture material -block is NOT submitted		Carbol fuchsin
AORTA	2.0 cm in length	H&E Verhoeff-Van Gieson (VVG)
ARTERY -complete cross-section	0.5 cm (outside diameter)	H&E Trichrome Verhoeff-Van Gieson (VVG)
BLADDER -not autolyzed -to include all layers -epithelium to cover one entire surface	1.5 cm in length	H&E
BONE -to include cortex and hematopoietic marrow	1.0 cm in length	H&E
BREAST -to include ductal epithelium	1.0 cm x 1.0 cm (square)	H&E
CERVIX -to include ectocervix -epithelium to cover one entire surface	1.5 cm in length	H&E Periodic acid Schiff/hematoxylin (PASH)
COLON -not autolyzed -to include all layers -epithelium to cover one entire surface	2.0 cm in length	H&E Alcian blue (pH 2.5) Fontana-Masson Trichrome
ESOPHAGUS -not autolyzed -to include all layers -epithelium to cover one entire surface	1.5 cm in length	H&E
FALLOPIAN TUBE -complete cross-section		H&E Trichrome
FUNGI -ant tissue -do NOT use embedded culture material -block is NOT submitted		Grocott Methenamine Silver (GMS) Periodic acid-Schiff/light green (PAS/LG)
KIDNEY -to include cortex and medulla	1.0 cm in length	H&E Periodic acid-Schiff/hematoxylin (PASH)

HISTOTECHNICIAN (HT)

TISSUE	MINIMUM SIZE *	STAIN
LIVER	1.0 x 1.0 cm (square)	H&E Reticulin (silver impregnation) Trichrome
LUNG	1.0 x 1.0 cm (square)	H&E
OVARY -to include cortical surface along one entire side	1.0 cm in one dimension	H&E Trichrome
PANCREAS -not autolyzed -to include islets	1.0 x 1.0 cm (square)	H&E
PLACENTA	1.0 x 1.0 cm (square)	H&E
SKIN -epithelium to cover one entire surface	1.0 cm in length	H&E Fontana-Masson Trichrome
SMALL INTESTINE -not autolyzed -to include all layers -epithelium to cover one entire surface	2.0 cm in length	H&E Alcian blue (pH 2.5) Fontana-Masson Mucicarmione Trichrome
SPINAL CORD -complete cross-section -cut sections at 8-10 micrometers		H&E Myelin
SPLEEN	1.0 x 1.0 cm (square)	H&E Reticulin (silver impregnation)
STOMACH -not autolyzed -to include all layers -epithelium to cover one entire surface	2.0 cm in length	H&E Periodic acid-Schiff/Hematoxylin (PASH)
THYROID	1.0 x 1.0 cm (square)	H&E
TONSIL -Cut section at 2-3 micrometers	1.0 x 1.0 cm (square)	H&E
UTERUS -to include endometrium and myometrium -endometrium to cover one entire surface	1.5 x 1.5 cm (square)	H&E

*Please note that the minimum measurements indicated for the blocks are after processing and the stained sections are after coverslipping. When square measurements are indicated, the section must cover a square of the size indicated. All tissues must exhibit positive results with the technique utilized.

**LIST OF TISSUES AND STAINS FOR
ASCP BOARD OF REGISTRY HT AND HTL
PRACTICAL EXAMINATIONS**

A list of tissues and stains from which the HTL practical examination will be selected:

HISTOTECHNOLOGIST (HTL)

TISSUE	MINIMUM SIZE	STAIN
ADRENAL -complete cross-section		H&E Oil red O Reticulin (silver impregnation)
AMYLOID -any tissue containing amyloid -block is NOT submitted -cut sections at 8-10 micrometers		Congo Red
APPENDIX -complete cross-section		H&E Fontana -Masson
ARTERY	0.5 cm (outside diameter)	H&E Verhoeff-Van Gieson (VVG)
BONE -to include cortex and hematopoietic marrow	1.5 x 1.5 cm (square) 1.0 x 1.0 cm (square)	H&E Giemsa Iron (Prussian blue)
CEREBELLUM -to include grey and white matter -cut sections at 8-1- micrometers	1.0 x 1.0 cm (square)	H&E Bielschowsky Luxol fast blue-Cresyl echt violet (LFB-CEV)
CERVIX -to include endocervix and ectocervix -epithelium to cover one entire surface	1.5 cm in length	H&E Alcian blue (pH 2.5) Alcian blue-Periodic acid Schiff (AB-PAS) Colloidal iron Immunoenzyme Pan-Keratin
ESOPHAGUS -not autolyzed -to include all layers -epithelium to cover one entire surface	2.0 cm in length	H&E
GALLBLADDER -not autolyzed -to include all layers -epithelium to cover one entire surface	2.0 cm in length	H&E

HISTOTECHNOLOGIST (HTL)

TISSUE	MINIMUM SIZE*	STAIN
HEART -to include either epicardium or endocardium	1.5 x 1.5 cm (square)	H&E
HELICOBACTER PYLORI -any tissue containing <i>H-pylori</i> -block is NOT submitted -do NOT use embedded culture material		Silver Technique
KIDNEY -to include cortex and medulla	1.0 x 1.0 cm (square)	H&E Periodic acid-methenamine silver (PAM)
LIVER	1.0 x 1.0 cm (square)	H&E Reticulin (silver impregnation)
LUNG -to include bronchus with cartilage	1.5 x 1.5 cm (square)	H&E Colloidal iron without digestion Trichrome
MEDULLA OBLONGATA -complete cross-section -to include inferior olivary nucleus -cut sections at 8-10 micrometers		H&E Bielchowsky Luxol fast blue-Cresyl echt violet (LFB-CEV)
OVARY -to include cortical surface along one entire side	1.0 x 1.0 cm (square)	H&E
PANCREAS -not autolyzed -to include islets	1.0 x 1.0 cm (square)	H&E Immunoenzyme Chromogranin
PROSTATE -to include glands and stroma	1.0 x 1.0 cm (square)	H&E Immunoenzyme Prostate specific antigen (PSA) OR Prostate acid phosphatase (PAP)
SKELETAL MUSCLE -longitudinal section	1.0 x 1.0 cm (square)	H&E Phosphotungstic acid-hematoxylin (PTAH)
SKIN -epithelium to cover one entire surface	2.0 cm in length	H&E Verhoeff-Van Gieson (VVG)

HISTOTECHNOLOGIST (HTL)

TISSUE	MINIMUM SIZE*	STAIN
SMALL INTESTINE -not autolyzed -to include all layers -epithelium to cover one entire surface	2.0 cm in length	H&E Alcian blue-periodic acid-Schiff (AB-PAS) Colloidal iron Methyl green-pyronin Mucicarmine Immunoenzyme
SPIROCHETE -any tissue -do NOT use embedded culture material -block is NOT submitted		Silver technique
SPLEEN	1.0 x 1.0 cm (square)	Naphthol AS-D Chloracetate esterase (Leder) Reticulin (silver impregnation)
STOMACH -not autolyzed -to include all layers -epithelium to cover one entire tissue	2.0 cm in length	H&E Periodic acid-Schiff/hematoxylin (PASH)
TESTIS -to include tunica albuginea and seminiferous tubules	1.0 x 1.0 cm (square)	H&E
THYROID	1.0 x 1.0 cm (square)	H&E
TONSIL -cut section at 2-3 micrometers	1.0 x 1.0 cm (square)	H&E Immunoenzyme Leukocyte common antigen (LCA) Feulgen
UTERUS -to include endometrium and myometrium -endometrium to cover one entire surface	1.5 x 1.5 cm (square)	H&E Trichrome

*Please note that the minimum measurements for the blocks are after processing and the stained sections are after coverslipping. When square measurements are indicated, the section must cover a square of the size indicated. All tissues submitted must exhibit positive results with the technique utilized.