University Hospital/UT Health

Histotechnology Training Program Course Description

I. Description of Histotechnology Course

The Histotechnology curriculum is taught over a twelve-month period. The trainees rotate clinical learning experiences at University Hospital Surgical Pathology Laboratory (including Cytology), UT Health San Antonio (including STRL Histology & Electron Microscopy Laboratories). During the clinical rotations, the trainees are given additional technical instruction so that they continue to expand their knowledge and practical skills. In the STRL Histology laboratory, they follow a prescribed weekly schedule for completion of specific special staining procedures along with introductions to muscle enzyme histochemistry, and immunofluorescence on frozen tissue biopsies.

The didactic portion of the Histotechnology course is coordinated with the practical portion to provide the trainees a better understanding of the correlation of technical skills and diagnostic importance. Lectures begin the first week of training and proceed on a weekly basis into the third quarter. Numerous technical and professional faculty participate in the lectures and instruction of the trainees. Written examinations are scheduled weekly and evaluated by the program faculty.

II. Objectives

The Histotechnology Training Program will provide the trainees with an understanding and working knowledge of histopathology, histology, and those techniques necessary to produce quality slides for diagnosis. Upon completion of the curriculum, the trainee will be able to demonstrate a working knowledge of those techniques necessary to produce quality slides. This knowledge will prepare the trainees for immediate employment in a histology laboratory.

University Hospital/UT Health
Histotechnology Training Program
HISTOLOGY COURSE OUTLINE

I. Introduction & Orientation
- A. Medical Ethics
- B. Role of each in modern medicine
  1. Biopsy
  2. Autopsy
  3. Research
- C. Relation to other laboratories
- D. Record Keeping
- E. Medical Terminology
  1. Prefixes
  2. Suffixes

II. Basic Principles of Laboratory Work
- A. Laboratory Safety
  1. Fire and explosion
  2. Poisonous, corrosive, and caustic reagents
     a. Strong mineral acids
     b. Strong bases
     c. Strong oxidants
     d. Poisonous compounds
     e. Toxic vapors
  3. Burns and Scalds
     a. Electrical burn
     b. Electrical shock
  4. Glass handling accidents
  5. Bacterial, viral, or parasitic infection
  6. Animal handling
  7. Radiation hazards
  8. Biological hazards
- B. Laboratory glassware
  1. Use and care
  2. Cleaning

III. Instrumentation
- A. Principles
- B. Components
- C. Use of instruments
- D. Preventive maintenance
- E. Trouble shooting
- F. Comparison of types of equipment

These topics will be covered as they relate to the following instruments.
1. Automatic tissue processors
2. Microtomes
   a. Freezing
      (1) Compressed gas
      (2) Cryostat
   b. Rotary
   c. Sliding
3. Microscopes

a. Light
b. Electron
c. Fluorescent
d. Polarizing

4. Knife sharpening equipment
    a. Knives
    b. Electrically powered instruments
    c. Hones and strops
5. Paraffin related equipment
    a. Dispensers and baths
    b. Embedding stations and centers
    c. Water baths
    d. Slide drying equipment
    e. Incubators and ovens
6. Automated staining equipment
7. Balances and pH meters

IV. Fixation
A. General principles
1. Purposes and function
2. Actions
    a. Chemical effects on tissue
    b. Mordanting
    c. Prevention of autolysis and putrefaction
3. Quality Control
    a. Selection of appropriate fixative
    b. Size and thickness
    c. Temperature
    d. Time
    e. Volume

B. Types and composition of fixatives
1. Aqueous
    a. Bouin's
    b. Fleming's
    c. Formalin
    d. Helly's
    e. Orth's
    f. Zenker's
    g. Others
2. Non-aqueous
    a. Acetone
    b. Alcohol
    c. Alcoholic formalin
    d. Carnoy's
    e. Others

C. Advantages and disadvantages
1. Aqueous/non-aqueous
2. Coagulant/non-coagulant

D. Specific use
1. Aqueous/non-aqueous
2. Coagulant/non-coagulant

E. Artifacts

1. Causes
2. Prevention and Removal

V. Processing

A. Dehydration

1. General principles
   a. Action
   b Purpose and function
   c. Problem solving
   d. Quality control
2. Types, advantages, and disadvantages
   a. Acetone
   b. Alcohol
   c. Ethylene glycol monomethyl ether (Cellosolve)
   d. Universal solvents
3. Clearing
   General principles
   a. Action
   b Purpose and function
   c. Problem solving
   d. Quality control
      (1) Contamination
      (2) Time
      (3) Volume
4. Types, advantages, and disadvantages
   a. Benzene
   b. Xylene
   c. Toluene
   d. Cedar wood Oil
   e. Chloroform
   f. Universal solvents
   g. Purified food grade oils

C. Paraffin infiltration and embedding - general principles

1. Actions, including vacuum
2. Problem solving
3. Purpose and function
4. Types, advantages, and disadvantages
   a. Paraffin
      (1) Resin (natural and commercial)
      (2) Paraplast
      (3) Tissue Prep
      (4) Others
   b. Plastics
      (I) Methacrylate (glycol & methyl)
      (2) Epon
      (3) Others
   c. Celloidin
   d. Agar
   e. Gelatin
5. Quality Control

a. Contamination
b. Temperature
c. Time
e. Volume

IV. Microtomy - General Principles

A. Paraffin

1. Proper technique
   a. Adhesives
   b. Knife angles
   c. Placement of sections on slides
   d. Temperature of water bath
   e. Quality of section
2. Problem solving

B. Cryostat

1. Proper techniques
   a. Embedding matrix
   b. Knife angles
   c. Temperature of cryostat
   d. Quality of section
2. Problem solving

VII. Staining

A. Classification of dyes

1. Natural dyes
2. Synthetic dyes
   a. Acid dyes
   b. Basic dyes
   c. Leuco compounds

B. Theory of staining and impregnation

1. Chemical
2. Metallic impregnation
3. Mordants, oxidizers, accentuates
4. Physical
5. Progressive staining
6. Regressive staining

C. Routine nuclear and cytoplasmic staining

1. Nuclear stains
   a. Carmine
   b. Celestin Blue
   c. Hematoxylin
      (1) Advantages and disadvantages
      (2) Sources of error and corrective action
      (3) Various formulas
   d. Methylene Blue
2. Cytoplasmic stains
   a. Eosin
      (1) Advantages and disadvantages
      (2) Sources of error and corrective action
      (3) Various formulas
   b. Phloxine

3. Routine staining methods
    a. Celestine blue and eosin
    b. Hematoxylin and eosin
    c. Phloxine and methylene blue
4. Quality control
5. Trouble shooting

VIII. Mounting
A. Media
1. Aqueous
    a. Advantages and disadvantages
    b. Indication for use
    c. Sealers
2. Resins - natural - Advantages and disadvantages
3. Resins - synthetic
    a. Advantages and disadvantages
    b. Refractive indices
    c. Solvents
B. Techniques
1. Quality control
2. Slides and cover glasses
    a. Types and thickness
    b. Refractive indices

IX. Decalcification
A. Definition
B. Methods including advantages and disadvantages
1. Buffer mixtures
2. Chelating agents
3. Commercial preparations
4. Electrolytic
5. Ion exchange
6. Simple acid solutions

X. Basic chemistry
A. Fundamental concepts
1. Definitions
    a. Acids and bases
    b. Compounds and mixtures
    c. Element
    d. Molecule
    e. Salts
2. pH including buffers
B. Metric system
1. Conversion of metric & apothecary system
2. Conversion of C° and F° temperatures

C. Solution preparation
1. Calculations
2. Pipetting
3. Dilution
4. Molar

5. Normal
6. Percent

XI. Microscopic Anatomy

A. Cell structure and function
   1. As a whole (cell)
   2. Nucleus and nucleoli
   3. Mitochondria
   4. Endoplasmic reticulin
   5. Lysosomes
   6. Filaments
   7. Glycogen
   8. Cell membrane
   9. Pinocytosis
   10. Lipid

B. Classification of Tissue
   1. Connective tissue - function and identification
      a. Cells
      b. Fibers
   2. Epithelium - function and identification
   3. Muscle - function and identification
   4. Nerve - function and identification
      a. Neurons
      b. Glia
      c. Myelin sheath

C. Gross Anatomy
   1. Skin
   2. Connective tissue and adipose tissue
   3. Bone and joints
   4. Skeletal muscle
   5. Coelomic cavities
   6. Arteries, veins and peripheral nerves
   7. Lungs (thoracic cage and diaphragm)
   8. Heart
   9. Liver
   10. Spleen
   1I. Pancreas
   12. Adrenal, thyroid, parathyroid, pituitary
   13. Kidneys (ureter & bladder)
   14. Esophagus & stomach
   15. Duodenum, jejunum, ileum
   16. Colon, rectum and anus
   17. Male and female reproductive
   18 Brain and spinal cord
   19. Eye

XII. Special Stains

A. Most desirable fixative
B. Mode of action (if known)
C. Purpose of reagents and dyes

D. Quality control including control material
E. Color results
F. Sources of error and trouble shooting
G. Rational for use

These topics will be covered as they relate to these special stains:

A. Connective Tissue
    1. Collagen
        a. Masson Trichrome
        b. Van Gieson

Special Stains
Connective Tissue

    2. Reticulum
        a. Lillie
        b. Snook
        c. Gomori
        d. Gordon & Sweet
    3. Elastic
        a. Verhoeff - Van Gieson
        b. Gomori Aldehyde fuchsin
    4. Muscle
        a. Masson's trichrome
        b. PTAH
        c. Gomori trichrome
    5. Basement Membrane
        a. Periodic acid-Methenamine silver (PAMS)
        b. Periodic acid - Schiff (PAS)
    6. Fibrin
        a. PTAH
        b. Wiegert’s stain for fibrin
    7. Bones, cartilage, and bone marrow
        a. Giemsa

B. Organisms, fungi, and parasites
    1. Acid fast
        a. Kinyoun's
        b. Ziehl-Neilson
        c. Fite-Faraco
    2. Ordinary bacteria gram positive and negative
        a. Brown & Brenn
        b. Brown & Hopps
    3. Spirochetes
        a. Warthin-Starry
        b. Steiner & Steiner
        c. Dieterle

    4. Fungi
        a. Gridley's
        b. Grocott's Methenamine silver (GMS)
        c. Periodic-acid Schiff (PAS)
    5. Amoeba

a. Best's carmine
b. Periodic acid Schiff (PAS)
c. Iron Hematoxylin

Organisms, fungi, and parasites

6. Inclusion bodies
   a. Lendrum's phloxine tartrazine method
7. Camphylobacter Pylori
   a. Wright-Giemsa
   b. Steiner Modification

C. Fats and Lipids
1. Neutral fats
   a. Sudan IV
   b. Oil red O
   c. Osmium tetroxide
   d. Sudan black B

D. Cell products - Carbohydrate stains
1. Glycogen
   a. Best's carmine
   b. Periodic Acid-Schiff (PAS) with and without digestion
2. Neutral Mucins
   a. Mucicarmine
   b. Periodic acid Schiff (PAS)
3. Amyloid
   a. Benhold's Congo red
   b. Crystal violet
   c. Puchtler's Congo red
   d. Thioflavin T

4. Acid Mucopolysaccharide
   a. Colloidal iron w/wo digestive enzymes (hyaluronidase)
   b. Alcian blue w/wo digestive enzymes (hyaluronidase)

E. Pigments and minerals (including classification)
1. Iron
   a. Prussian blue reaction
   b. Schmorl's ferric ferricyanide technique
   c. Turnbull's blue reaction
2. Calcium
   a. Alizarin red S
   b. Von Kossa
3. Bile pigment
   a. Stain's bile pigment stain
   b. Fouchet's method for bile

Pigments and minerals (including classification)

4. Melanin
    a. Fontana - Masson
    b. Warthin-Starry Modification
    c. Schmorl's
5. Urate crystals
    a. Gomori's method for urate crystals
6. Copper
    a. Rhodamine

F. Cytoplasmic granules
1. Argentaffin
    a. Schmorl's Method
    b. Fontana – Masson
2. Chromaffin
    a. Periodic acid Schiff (PAS)
    b. Mallory's aniline blue collagen stain
3. Pituitary
    a. Periodic acid Schiff (PAS)
    b. Gomori's chrome alum hematoxylin phloxine
4. Pancreas
    a. Scott's
    b. Gomori
5. Mast Cells
    a. Toluidine blue
    b. Giemsa
6. DNA & RNA
    a. Methyl green pyronin
    b. Feulgen
7. Argyrophilic Cells
    a. Churukian silver stain

G. Nervous System
1. Nerve fibers, endings & axis cylinders
    a. Bodian's
2. Myelin
    a. Weil's
    b. Luxol fast blue
    c. Phosphotungstic acid hematoxylin (PTAH)
3. Nissl substance
    a. Thionin
    b. Cresyl echt violet

Special Stains

H. Enzyme Stains
    1. Calcium ATPase
    2. NADH
    3. Phosphorylase
    4. Esterase

I. Immunohistochemical Techniques
    1. Immunofluorescence
        a. direct
        b. indirect