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Health Professions Council of South Africa

SYLLABUS

HISTOPATHOLOGICAL TECHNIQUES

MEDICAL TECHNOLOGIST and MEDICAL LABORATORY SCIENTIST

PBMT approved in November 2022 for training implementation in 2023 for students who write from March 2024 onwards

CONTENTS

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1. INTRODUCTION

Evidence of ''*Evaluation''* criteria as detailed within this syllabus [write ups and slides, etc] must be available prior to the Board Examination and be available for five (5) years after the candidate has passed the Board Examination for inspection / audit by the HPCSA. [These records / material cannot be held at the SMLTSA office, but must be retained by the candidate in the laboratory in which they are working in the event of an audit].

HPCSA regulations require that accredited training laboratories perform a minimum of 80% of the tests identified / listed in this syllabus. Laboratories are required to ensure that intern / students receive appropriate training in the tests contained within the syllabus but which are not routinely performed on site.

Plagiarism, of any sort, is unacceptable.

''For the purposes of this syllabus ''processing'' includes where applicable and appropriate, the macroscopic examination and description of small tissue specimens; annotated using terminology and ontology in compliance with standards used in pathology, which have been submitted in their entirety and not requiring dissection; for microscopic examination. Individuals performing this task require a level of knowledge of Anatomy / Anatomy and Physiology as successfully obtained from an accredited University or University of Technology. High complexity testing such as ''Grossing''; defined in this context as macroscopic examination of more complex specimens requiring dissection or small specimens requiring dissection; should be undertaken by a pathologist, pathology resident, or an individual ''qualified'' to perform high complexity testing under the supervision of a pathologist and meeting the requirements above.

While some laboratories may delegate the dissection of specimens to non-pathologist individuals such as Medical Technologists and Medical Laboratory Scientists [BHSc:MLS]; these individuals must, in addition to knowledge of Anatomy and Physiology [see above requirements], have successfully completed an approved and accredited clinical laboratory training program based on accepted pathology standards and guidelines; at the facility in which they practice with a minimum of at least 3 months documented laboratory training [and deemed competent by a pathologist] in each specialty {e.g. GIT specimens; breast (mastectomies / reductions); lymph nodes} etc.; in which the individual performs high in this complexity testing.''

In addition, it is expected that the intern / student will have, where applicable, knowledge and understanding of the following:

2. STATUTORY REGULATIONS AND ETHICS

Objective:

Provide the intern / student with information on the regulations and ethical principles which apply to the practice of medical technology.

Specified outcomes:

The intern / student should be able to:

- Demonstrate knowledge of the structure and function of the Health Professions Council of South Africa.
- Demonstrate knowledge of the structure and function of the Professional Board for Medical Technology.
- Discuss the regulations relating to the scope of practice for Medical Laboratory Scientists (BHSc:MLS / Medical Technologists / Technicians / Laboratory Assistants.
- Describe the legal and ethical standards related to the professional practice of medical technology.
- Demonstrate knowledge of the requirements for the acquisition of Continual Education Units (CEUs).

3. TOTAL QUALITY MANAGEMENT SYSTEM

3.1 Laboratory Safety

Objective:

Provide knowledge of all safety procedures that must be applied in the workplace and an understanding of the relevant legislation.

Specified outcomes:

On completion of the syllabus the intern / student should be able to:

- Explain and apply the fundamental concepts of the relevant legislation pertaining to laboratory safety.
- Range: Occupational Health and Safety Act; Compensation for Occupational Injuries and Diseases Act; Hazardous Substances Act; Transport of Hazardous goods, IATA guidelines and other as detailed in this syllabus.
- Demonstrate knowledge of the protocols to follow in the event of injuries on duty, including needle-stick injury.
- Demonstrate knowledge of the procedures to follow in the event of laboratory accident or emergency.
- Range: Chemical or bio-hazardous spill; fire; flood; bomb threat.
- Describe procedures to follow for the prevention, control and management of laboratory acquired infections including the prevention and control of infection by blood borne viruses.

- Describe and apply good general housekeeping procedures including the decontamination of equipment.
- Describe the application of laboratory safety procedures to the collection, transport, storage and analysis of biological specimens.
- Describe the purpose and basic content of the safety date sheet (SDS), [previously known as material safety data sheet (MSDS), or product safety data sheet (PSDS)].
- Describe the basic principles for the storage, handling and disposal of chemicals; poisons; flammable substances; gases and infectious and non-infectious material.
- Describe the correct procedures for the storage, handling and disposal of laboratory waste.
- Range: biological specimens; human tissue; solid and liquid bio-hazardous waste; radioactive waste and sharps.
- Define the role of the designated safety personnel.
- Range: First aid officer; fire marshal; safety representative.
- Recognise the situational safety symbols used in the laboratory environment.

3.2 Specimens

Objective:

Provide an understanding of the optimal specimen requirements for the maintenance of the integrity and suitability for ***all types*** of laboratory analysis with particular reference to the tests specified throughout this syllabus.

Specified Outcomes:

On completion of the syllabus the intern / student should be able to:

- Collect specimens as defined within current statutory requirements and limitations.
- Describe the optimal specimen requirements and or fixative / transport medium for the individual tests required such as frozen sections / Molecular techniques / renal biopsy / muscle biopsy, parathyroid and lymph node collection.
- Have an understanding of the conditions under which the specimens must be transported to the laboratory including the use of appropriate transport media for micro-organisms and other non-histology specimens.
- Display knowledge of the optimal storage conditions should testing be delayed and the stability of the specimen for the individual testing process / processes.
- Capture the data and patient demographics that are required for the registration of the specimens at the laboratory accurately.
- Explain the principle of continuous identification of the specimen, raw data and documentation.
- Have an understanding of the process for the rejection of unsuitable specimens.
- Conduct the pre-analytical processes required for specimen type and test requested as included in this syllabus.

3.3 Laboratory Equipment

Objective:

Explain the correct use, principle of operation, maintenance of laboratory equipment and the appropriate troubleshooting procedures to apply when and where relevant as indicated in this syllabus.

Range: All glassware – volumetric and graduated; pipettes – glass, automated and disposable; balances – top pan and fine chemical; stirrers; hotplates; pH meters; rotators; shakers; roller, flat bed and vortex mixers; rubber teats; microscopes – light, EM, IF etc; fume cupboards and bio-safety cabinets; temperature controlled, ultra-water-baths; water-baths and floatation baths; stopwatches / timers; thermometers; incubators; knives – disposable and other; knife sharpeners; microtomes – sliding, rotary, sledge, ultratome, cryostat and CO_2 freezing.

Specified outcomes: – applicable to all equipment / instruments and analysers where relevant and appropriate as contained in this syllabus.

The intern / student should be able to:

- Describe the principle of operation where applicable.
- Operate all equipment optimally in accordance with the recommended procedures.
- Apply the correct safety precautions during the operation and maintenance of equipment.
- Demonstrate full knowledge of, and apply, the correct maintenance, service and calibration requirements.
- Conduct applicable decontamination procedures.
- Apply the appropriate functional checks to ensure optimal operation.
- Demonstrate knowledge of, and maintain, all equipment records and documentation required for Good Laboratory Practice (GLP).

3.4 Laboratory Reagents

Objective:

Provide details of the correct preparation, use, storage and disposal of laboratory reagents where applicable to this syllabus.

Range: Stock solutions; working solutions; working reagents; controls; calibrators; reagent kits.

Specified outcomes:

On completion of the syllabus the intern / student should be able to:

- Prepare, store, and safely dispose of laboratory reagents.
- Demonstrate knowledge of the objective, use and retention of package inserts.

3.5 Stock Control

Objective

Outline the processes involved in good stock management**.**

Specified outcomes:

On completion of this section the student should be able to:

- Demonstrate an understanding of the receipt of stock including the required records regarding condition of goods, expiry dates and lot numbers.
- Demonstrate an understanding of stock rotation with particular reference to expiry dates.
- Describe the correct storage conditions of stock.
- Demonstrate knowledge of company policy with regard to the use of expired reagents, controls and calibrators.

3.6 Quality Assurance / Accreditation

Objective:

Expose the intern / student to all aspects of Quality Assurance / Accreditation and be able to outline the process of running an accredited laboratory system.

Specified outcomes:

On completion of the syllabus the intern / student should be able to:

- Display, discuss and demonstrate an understanding of Quality assurance and Quality control in the correct context.
- Define and apply the appropriate processes of quality assurance in the pre-analytical, analytical and post analytical areas relevant to the syllabus.
- Apply the appropriate quality control processes which must be performed in the analysis of all tissues, organisms, equipment and analyser operation, reagent and stains / dye preparation and advanced techniques as contained within this syllabus.
- Display an understanding of the principles of internal and external quality control procedures in the context of the tests performed.
- Apply the appropriate quality control for all testing procedures included in this syllabus.
- Explain the principles of internal and external quality control procedures in the context of the tests performed.
- Apply a sound knowledge of all the principles, procedures, calculations and interpretation of all related internal and external, ***quantitative*** quality control data.
- Apply a sound knowledge of all the procedures, principles and interpretation of internal and external ***qualitative*** quality control data.
- Describe the potential causes and apply appropriate troubleshooting procedures in the event of failed Internal and external, quantitative and qualitative quality control.
- Define all terminology used in the assessment of quality control results.
    - **Range**: positive and negative; specificity / sensitivity; interference in the PCR process; enumeration of SISH/CISH/FISH signals wrt Advanced staining techniques; staining localisation; noise to signal ration [IHC]; Core: halo ratios [EM]
- Describe and apply the appropriate quality control for all testing procedures included in this syllabus.
- Demonstrate an understanding of the accreditation process as laid down by SANAS.
- Demonstrate knowledge of principles applied to meeting the requirements of an accredited facility.
- Demonstrate knowledge of the company's policies with regards to accreditation.
- Have a working understanding of auditing, vertical audit, internal audit, horizontal audit.
- Have an understanding of performing a root cause analysis and non-conformance management.

Note: In addition refer to section **4.0** Laboratory Related mathematics.

3.7 Quality Control

Objective:

Expose the intern / student to all aspects of quality control.

Specified outcomes:

On completion of the syllabus the intern / student should be able to:

- Display, discuss and demonstrate an understanding of Quality assurance and Quality control in the correct context.
- Define and apply the appropriate processes of quality assurance in the pre-analytical, analytical and post analytical areas relevant to the syllabus.
- Apply the appropriate quality control processes which must be performed in the analysis of all tissues, organisms, equipment and analyser operation, reagent and stains / dye preparation and advanced techniques as contained within this syllabus.
- Display an understanding of the principles of internal and external quality control procedures in the context of the tests performed.
- Apply the appropriate quality control for all testing procedures included in this syllabus.

3.8 Method Evaluation

Objective

The student should have a working knowledge of the validation process of kits and reagents

Specific outcomes

- Know the process of validating a kit.
- Validation of all Histology / Immunocytochemistry instrumentation as detailed in this syllabus.

3.9 Personnel

Objective:

Demonstrate an understanding of basic requirements for personnel in terms of Good Laboratory Practice (GLP).

Specified outcomes:

On completion of the syllabus the intern / student should be able to:

- Describe the personal documents and records which are required for all personnel.
- Demonstrate an understanding of the terms ‘competency’ and ‘ongoing competency’ in terms of the training of all laboratory personnel.

3.10 Documentation

Objective:

Provide knowledge of basic requirements of documentation in terms of GLP.

Range: Policies; SOPs; equipment records; quality control records; personnel records; package inserts [SDS] and archiving.

Laboratory Policies: Issue of new documents, review process, process for obsolete documentation, document retention and disposal.

Specified outcomes:

On completion of the syllabus the intern / student should be able to:

- Demonstrate and understanding of the management of laboratory documentation in terms of GLP and in terms of International Organization for Standardization (ISO) and ISO/IEC standards.
- Range: Issue of new documents; review process; process for obsolete documentation; document retention and disposal.
- Demonstrate knowledge of the required content of SOPs.

4. Laboratory related Mathematics

Objective:

Provide the student with instruction on the application of the correct mathematical formulae to relevant calculations.

Specified outcomes:

On completion of the syllabus the intern / student should be able to:

- Demonstrate proficiency in the calculations required for the preparation of solutions or patients samples. Normal solutions, percentage solutions, molar solutions, titrations/dilutions, serial and doubling dilutions.

MODULE 1

Laboratory Administration

A Outcomes

On completion of this module the intern / student must have:

a a thorough knowledge of the administrative structure of the laboratory that he / she is working in. This structure must be compared with another such facility and a critical comparison documented

b a sound knowledge of the collection and handling, logging, distribution, data recording, reporting, accession and retrieval of data of specimens / cases received in the laboratory

B Objectives

1.1 The intern / student must have a sound knowledge of:

1.1.1 The hierarchical structure of a histopathology laboratory (organogram). In the interest of completeness, the intern / student must be exposed to another such laboratory to broaden their outlook on this discipline

1.1.2 Collection / fixation / receipt / data recording / cut up / significance for diagnosis / precautions necessary for handling of specimens for the following:

- Light microscopy
- Electron microscopy (TEM and SEM)
- Immunohistochemistry / immunocytochemistry
- Immunofluorescence
- FISH; DISH [(B-DISH (Bright-field Double in-situ hybridisation)]; CISH; PCR
- Frozen sections
- Enzyme histochemistry
- Renal biopsies
- Bladder tissue / ureter / urethra
- Liver biopsies
- Gallbladder
- Pancreas

- Skin biopsies – naevi; solar keratosis; melanoma, basal cell carcinoma (BCC) – lines of excision
- Bone marrow trephine biopsies (dry tap and tumours) – lymphoma / leukaemia / anaemia
- Lymph nodes / tonsil / spleen- lymphoma / leukaemia / anaemia / tumours
- Tumours
- Gastro intestinal tract (GIT) – whole stomach; intestines; biopsies; polyps
- Rectal biopsies
- Endocrine system – hypothalamus; pituitary gland; thyroid gland; parathyroid gland; adrenal gland; ovaries; testis
- Diffuse neuroendocrine system specimens
- Total hysterectomy – uterus / cervix, fallopian tubes and ovaries
- Ligation – pieces of fallopian tube (legal implications)
- Total mastectomy / breast only / lumpectomy (lines of excision)
- Ectopic pregnancy
- D&C (dilatation and curettage) – endometrial scrapings
- Prostate chippings (weight / amount in cassette / embedding / planes of sectioning / levels)
- Testis
- Penis / penile warts
- Vasectomy specimen – pieces of vas deferens (legal implications)
- Whole brain / brain biopsies / brain smears
- Spinal cord
- Whole eyes
- Muscle biopsies
- Nerves / ganglia
- Heart
- Blood vessels / atheroma / arteriosclerosis
- Lung specimens (biopsies and whole lungs)
- Whole or part of limbs

- Museum specimens
- Post mortem specimens
- Bone and other calcium containing tissues
- Specimens with orientation markings – stitches and indelible ink
- Specific cytology specimens

1.1.3 Data capturing, logging and distribution of specimens to the different laboratories / subsections within histopathology

1.1.4 Data capturing, reporting of and archiving results into the central computerised programme

1.1.5 Retrieval of previous electronic pathology diagnoses on patients with repeat specimens

1.1.6 Retrieval of diagnostic data for retrospective research and writing of articles for presentations and publication

1.1.7 Block and slide filing systems

1.1.8 Gross specimen disposal

1.2 *be familiar with the:*

1.2.1 National Health Act No. 61 of 2003 and subsequent amendments

1.2.2 Human Tissue Act (65, 1983)

1.2.3 Human Tissue Amendment Act (51, 1989)

1.2.4 Patient Rights Charter (108, 1996); and all subsequent updates

1.2.5 Births and Deaths Registration Amendment Act 67, of 1997

1.2.6 HPSCA regulations relevant to the Profession; including the Health Professions Act 56 of 1974 and subsequent updates

1.2.7 Data Protection within the framework of the South African Constitution

1.2.8 Protection of Personal Information Act, No 4 of 2013 (PoPI); Government Gazette Notice 37067

C Evaluation

The intern / student will be competent when he / she is able to:

a Draw an annotated organogram of the hierarchical structure of two histopathology departments / laboratories.

b Compare and evaluate the two laboratories and assess whether there is balance in their structure.

c Draw an annotated diagram, following receipt of a specimen in the laboratory until results are reported to the wards / clinicians. This diagram must include all the sections of a histopathology department as mentioned in Point 1.1.2.

d Critically evaluate and compare how data / results / archived data can be recorded and accessed from the electronic systems of the two laboratories. If the laboratory in which the intern / student is trained is not computerised, the intern / student must be sent to such a facility for training. In this instance the two laboratory systems must be critically compared.

e Evaluate if the systems in 'd' are secure from unauthorised access.

D Recommended Textbooks and Resources for Module 1

- Theory and Practice of Histological Techniques, John D Bancroft and Marilyn Gamble. 5th or 6th Edition.
- Manual of Histological techniques and their diagnostic applications, John D Bancroft and Harry C Cook, Churchill and Livingstone. 1994
- Laboratory Biosafety Manual, WHO, 1993.

E Other

Websites

- http://www.hpcsa.co.za
- http://www.smltsa.org.za
- Government Gazettes – various
- Government Acts - various

MODULE 2

Safety in the Histopathology Laboratory

A Outcomes

On completion of this module the intern / student must be:

a able to recognise the health hazards involved in handling fresh and unfixed tissue and body fluids

b conversant with all regulatory and laboratory safety rules especially with regard to storage / transport and safe usage of:

- Chemicals – liquids and solids; concentrated and diluted
- Reagents – documentation rules regarding: date of receipt; date reagent was made up; expiry date; who made up the solution; safety hazards to be documented on bottle
- Stains / dyes

c able to critically design an **ergonomically** sound histopathology facility that is functional, free of hazards, allows easy workflow and access to storage and disposal of specimens and material

B Objectives

The intern / student must be able to define and explain the following terms:

2.1 Hazard groups 1, 2, 3, and 4 relating to the classification of microorganisms

2.2 *have a basic knowledge of the following groups of microorganisms and / or the diseases they cause: (See Module 9: Section 9.9.1 for detailed requirements)*

2.2.1 Bacteria – various gram positive / negative cocci and bacilli

2.2.2 Rikettsia

2.2.3 Spirochaetes – leptospira (Weil's disease); *Treponema pallidum* (Syphillis)

2.2.4 Mycobacteria - TB; leprosy

2.2.5 Bacillus anthracis – anthrax

2.2.6 Enterobacteria - Salmonella species

2.2.7 Brucella – gram negative coccobacilli

2.2.8 Viruses – Human Immune Deficiency Virus (HIV), viral hepatitis, spongiform encephalopathy (Creuzfeldt-Jacob disease) and haemorrhagic fevers (Congo-Crimea Fever); Ebola, EBV, CMV, HPV, HSVI and HSVII Ref: Module 9 (9.9.1)

2.2.9 Fungi – spores and hyphae

2.2.10 Protozoa - Amoeba

2.2.11 Parasites – intestinal worms; schistosoma ova

2.3 *ensure that he / she:*

2.3.1 Undertakes correct procedures regarding the hazards of receipt and handling of fixed and unfixed tissue and body fluids in the laboratory

2.3.2 Know where in the laboratory to handle the above mentioned tissues and fluids

2.3.3 Takes precautions regarding the danger posed during frozen sectioning of fresh tissue – vapourisation of fluid and wearing protective apparatus

2.4 *be familiar with the use, hazards and safe disposal of hospital / laboratory antiseptics and disinfectants such as:*

2.4.1 Methylated spirit

2.4.2 Chlorhexidine

2.4.3 Phenolic disinfectants

2.4.4 Hypochlorite's

2.4.5 Alcohols

2.4.6 Aldehydes - formaldehyde and glutaraldehyde

2.5 *be familiar with the use, storage, hazards and safe disposal of laboratory solvents such as:*

2.5.1 Alcohols - ethanol, methanol and iso-propanol

2.5.2 Acetone

2.5.3 Ether

2.5.4 Hydrocarbons - xylene, toluene, benzene, chloroform and carbon tetrachloride

2.5.5 Xylol substitutes

2.6 *be familiar with the use, storage, hazards and safe disposal of embedding media such as:*

2.6.1 Paraffin waxes

- Paraplast and Paraplast +
- Histosec
- Unilab wax
- Other paraffin wax mixes

2.6.2 Resins

- Methacrylates
- Epoxy resins – aromatic and aliphatic

2.6.3 Celloidin

2.6.4 Gelatin

2.6.5 Water soluble waxes

2.7 *be familiar with the use (and application where appropriate), storage, hazards and safe disposal of laboratory chemicals and reagents such as:*

2.7.1 Liquid and dry chemicals – e.g. peroxide, hydroxide, picric acid

2.7.2 Fixatives – e.g. mercuric chloride, potassium dichromate

2.7.3 Stains and dyes in powder or liquid form – many are carcinogenic (BIOLOGICAL STAINS COMMISION – www.biologicalstainscommission.org)

2.7.4 Organic acids – e.g. acetic acid, formic acid

2.7.5 Inorganic acids – e.g. hydrochloric acid, sulphuric acid

2.7.6 Osmium tetroxide - EM

2.7.7 Uranyl acetate - EM

2.7.8 Lead citrate - EM

2.7.9 DAB – 3, 3'-Diaminobenzidine – IHC / ICC and other carcinogens

2.8 *be aware of:*

2.8.1 The location of all fire extinguishers and firefighting apparatus in the department

2.8.2 The location of all emergency and fire exits in the department

2.8.3 And familiar with fire drill procedures / emergency numbers in the department

2.8.4 and familiar with location and operation of eye wash stations

2.8.5 and familiar with location and operation of emergency showers

2.8.6 and familiar with location and use of First Aid materials

2.8.7 Appropriate actions to be taken when injured on duty (including needle stick injuries)

2.9 *be familiar with the basic rules of laboratory / mortuary safety such as:*

2.9.1 No eating, drinking and smoking in the laboratory

2.9.2 When and how to use the appropriate level of Personal Protective Equipment (PPE)

- laboratory coats
- gloves
- face masks
- hair covering
- rubber boots
- safety goggles
- Mobile telephones

2.9.3 Personal hygiene in the laboratory environment

2.9.4 Spills containment - biohazardous and chemical (large and small)

- Materials (absorbers)
- Equipment
- Neutralisers (specifically for formaldehyde and formalin spills)
- Appropriate disposal techniques of spill material and the materials used to contain spills
- Apply the correct techniques to appropriate spill containment

2.10 *have a thorough knowledge of the regulations relating to the safe packaging and transportation of any chemicals and specimens:*

2.10.1 IATA and road regulations relating to the transportation of the above

2.10.2 Package inserts and SDS

C Evaluation

The intern / student is expected to be competent when he / she is able to:

a Critically evaluate the safety procedures that are in place in the laboratory in which they work. All sections and sub-sections of the facility must be included. 1500-3000 words.

b Critically point out (identify) shortcomings in any of the above and make recommendations for appropriate corrective action.

D Recommended Textbooks and Resources for Module 2

- Theory and Practice of Histological Techniques, John D Bancroft and Marilyn Gamble. 5th or 6th Edition.
- Manual of Histological techniques and their diagnostic applications, John D Bancroft and Harry C Cook, Churchill and Livingstone. 1994
- Laboratory Biosafety Manual, WHO, 1993
- OHASA Regulations
- Government regulations
- IATA regulations
- Road safety regulations –Transportation of goods
- SOPs and SDS

MODULE 3

Light, Fluorescent and Electron Microscopes

A Outcomes

On completion of this module the intern / student must:

a have a working knowledge of the behaviour of light waves, ultra violet light waves and electron waves

b be able to recognise the component parts of a light microscope, polarising, epifluorescent and Transmission electron microscope

c be able to compare / evaluate the similarities and differences between these three types of microscopes

d be able to operate a light microscope, fluorescent and transmission electron microscope

e set up a microscope for Köhler illumination

f have been exposed to a Scanning electron microscope and confocal microscope

B Objectives

3.1 *The intern / student must be able to define and explain the following terms:* (Refer Modules 11, 12 and 13*)*

3.1.1 Amplitude, wavelength and frequency – light; UV light; electrons

3.1.2 Coherent and non-coherent waves

3.1.3 Refraction and refractive index

3.1.4 Chromatic and spherical aberration

3.1.5 Birefringence and dichroism

3.1.6 Primary and secondary fluorescence

3.1.7 Resolving power of microscopes

3.1.8 Kohler illumination

3.1.9 Electron gun

3.1.10 Thermionic emission

3.1.11 Accelerating voltage

3.1.12 Electromagnetic lenses

3.1.13 Vacuum – high and low

3.1.14 Lenses

- In the light microscope
- In the fluorescent microscope – transmission and epifluorescence
- In the electron microscope

3.1.15 calculation of resolving power

3.2 *recognise and compare the essential components of:*

3.2.1 Light microscopes

3.2.2 Fluorescent microscopes

3.2.3 Electron microscopes

3.2.4 Stereo / phase contrast microscopes

3.3 *have a working knowledge of:*

3.3.1 Digital Image capture and archiving

3.3.2 Image analysis and processing

3.3.3 Advantages and disadvantages of whole slide images on computer vs glass slide under a microscope

3.3.4 Legal implications of transmitting images

3.3.5 Legal implications of altering / editing images

3.3.6 Image capture resolutions

3.3.7 Validating digital pathology systems for use in a GLP environment

3.3.8 Cloud technology or Storage as a Service

3.4 *Be able to appropriately store, clean and maintain the basic components of the above microscopes:*

3.4.1 Lenses

3.4.2 Eye pieces

3.4.3 Light bulbs / LEDs

3.4.4 Filters

3.4.5 Fuses

C Evaluation

The intern / student is expected to be competent when he / she is able to:

a Set up and align a light microscope for optimal performance. This may be done with the aid of the microscope manual.

b Set up and align a fluorescent microscope for optimal performance. This may be done with the aid of the microscope manual.

c Set up and align a Transmission electron microscope (TEM) for optimal performance. This may be done with the aid of the microscope manual.

d Draw and comprehensively label the three types of microscopes in order to explain how they work.

e Check the facilities regarding Section 3.3 above and write a short (½ page) report on existing methods of photo archiving for the laboratory.

D Recommended Textbooks for Module 3

- Theory and Practice of Histological Techniques, John D Bancroft and Marilyn Gamble. 5th or 6th Edition.
- Principles and Techniques of electron microscopy, M A Hayat, 2000.

E Other

Websites

- Digitalpathology association.org
- Various vendors
- SOPs
- Instrument instruction manuals

MODULE 4

Fixation and Fixatives

A Outcomes

On completion of this module the intern / student must:

a have a thorough knowledge of fixatives and the components thereof

b have a thorough knowledge of the chemical and physical mechanisms and principles of the fixation process and the impact on staining solutions and staining patterns

c be able to predict the effect of specific fixatives and fixative components on tissues and organs as well as subsequent staining patterns

d be able to recognise poor fixation and fixation related artefacts

e be able to carry out corrective action on artefacts caused by fixation

f be able to carry out appropriate corrective action to restore fixation and fixation related artefacts

B Objectives

4.1 *The intern / student must be able to define and / or explain the following:*

4.1.1 Fixation

4.1.1.1 Describe and evaluate Physical and Chemical methods of fixation

- Heat fixation
- Microwave fixation
- Freeze drying
- Coagulant fixatives
- Dehydrant coagulant fixatives
- Other types of coagulant fixatives
- Non-coagulant cross-linking fixatives
- Cross linking fixatives for Electron microscopy

4.1.2 Outline why tissue needs to be prepared for microscopy

4.1.3 Describe why tissue removed from the body will change

4.1.4 Describe how fixatives stop post-mortem changes

4.1.5 List the functions, properties and actions of a good fixative

4.1.6 List the considerations when choosing a fixative

4.1.7 State the effects of fixation on tissue

4.1.8 List factors that affect quality of fixation

4.1.9 Describe the ways of classifying fixatives

4.1.10 Describe in detail, simple and compound fixatives and give examples of these fixative solutions

4.1.11 Define secondary fixation, its purpose and give examples

4.1.12 Describe how different fixatives have special properties and their uses in histology

4.1.13 Describe how simple fixatives can be used in combination to improve preservation

4.1.14 Describe fixation artefacts and the methods of removal [formalin, mercury, chrome] pigments

4.1.15 Describe the major types of artefacts in sections

4.1.16 Describe how and why several different methods are needed in histology

4.1.17 is able to recognise fixation artefacts and take necessary corrective measures

4.1.18 be able to fix impression smears and squash preparations from fresh tissue

4.1.19 centrifuge, prepare smears and suitably fix body fluids

4.2 *are familiar with and have an understanding of:*

4.2.1 The six main classes of fixatives

4.2.2 The mode of action of aldehyde (formaldehyde and glutaraldehyde) and its effect on cell structures and organelles and subsequent staining patterns

4.2.3 Oxidising agents (osmium tetroxide, potassium dichromate etc) and its effects on cell structures and organelles

4.2.4 The mode of action of alcohol as a fixative and its effect on cell structures and organelles

4.2.5 Special fixatives and fixative mixtures [Dichromates / chromates / mercury (and substitutes) and other metallic ions as fixation supplements]

4.2.6 Factors influencing the quality of fixation (temperature, concentration, buffering, osmolality etc)

4.2.7 Fixatives for nucleic acids (acid alcoholic fixatives etc) the effect of fixatives on cell structures and organelles

4.2.8 Lipid fixation and the effect of fixatives on cell structures and organelles

4.2.9 Fixation for histochemistry and the effect of fixatives on cell structures and organelles

4.2.10 Fixation of muscle and the effect of fixatives on cell structures and organelles

4.2.11 Substitute solutions for mercurial based fixatives using zinc {chloride or sulphate} and barium salts (e.g.Zenker Fixative, Modified, Zinc Chloride)

4.2.12 Special fixation for immunocytochemistry; *in situ* hybridisation, molecular pathology and electron microscopy

- Formaldehyde substitute fixatives
- Advantages and disadvantages of fixatives for the above listed in 4.2 (including substitutes)
- Effects on cell structure and organelles

4.2.13 Preparation of methanol-free formaldehyde

4.2.14 Evaluate the most appropriate fixation reagent(s) and method for frozen sections according to technique

C Evaluation

Evaluation will be in the form of a practical competency test, in which the intern / student must satisfactorily demonstrate fixation of a liver, kidney and skin sample.

The intern / student is expected to be competent when he / she is able to:

a fix a piece of liver, kidney and skin in the following fixatives:

i 10% neutral buffered formal saline

ii 10% phosphate buffered formalin

iii Bouin's fixative

iv 2.5% buffered glutaraldehyde

v 2.5% glutaraldehyde plus 10% formalin (4% formaldehyde) in phosphate buffer pH 7.0; osmolarity 250-400 milliosmoles (recommended TEM fixative).

b Process the tissue, keeping some in reserve, and cut and stain sections with Haematoxylin and Eosin (H & E).

c Process a piece of liver tissue using acetone instead of alcohol. Compare subsequent sections microscopically after staining with H & E, Periodic acid Schiff's reaction (PAS), with and without Diastase digestion (PAS + D).

d Prepare a set of slides of the same tissue mentioned in ''a'' demonstrating the effects of poor / improper fixation with 10% NBFS.

e Critically evaluate all the slides from sections b, c, and d and comment in writing on your findings. State how remedial action should be taken. Include in your evaluation, the effects of these fixatives on histochemical and immunohistochemical techniques – IHC / ICC 2000-3000 words.

D Recommended Textbooks and Resources for Module 4

- Theory and Practice of Histological Techniques, John D Bancroft and Marilyn Gamble. 5th or 6th Edition.
- Fundamentals of Cellular Pathology, E J Truter and W van Wyk. (out of print)
- Wallington, E.A. (1979). Artefacts in tissue sections. *Medical laboratory Sciences* 36, 3-61.
- Kieran, J.A. (1990). Histological and Histochemical Methods. Oxford: Pergamon.
- Hopwood, D. (1996). Fixation and Fixatives.
- Tallitch, R.B. and Guastaferri, R. (2009). Histology and Identification Manual, Mosby and Elsevier.

E Other

Websites

- SOP's from your laboratory
- Product or equipment inserts where applicable.

MODULE 5

Tissue Processing

5.1 Conventional processing

A Outcomes

On completion of this module the intern / student must:

a have a thorough knowledge of tissue processing

b have sound knowledge of the solutions used in tissue processing:

- decalcification agents
- dehydrating agents
- ante-media - ‘clearing’ agents
- embedding media
- appropriate substitutes

c be able to recognise processing artefacts and take appropriate corrective action according to the type of tissue sample

d critically evaluate manual, automated and microwave processing methods

B Objectives

5.1.1 *The intern / student must have a sound knowledge of and be able to:*

5.1.1.1 Define “tissue processing”

5.1.1.2 Describe why tissue needs support during sectioning

5.1.1.3 Describe how tissues are impregnated with wax using intermediate reagents

5.1.1.4 Name several common and useful dehydrating and ante-media – ‘clearing’ agents

5.1.1.5 Describe the preparation of mineralised tissues prior to processing [duplicate under ‘’Bone’’]

5.1.1.6 Critically evaluate different processing schedules [manual and automated] customised for tissue type and size

5.2 *Have a sound knowledge of and be able to:*

5.2.1 Evaluate the four major components of tissue processing

5.2.2 Define and state the purpose of “dehydration”

5.2.3 Evaluate the criteria for choosing a dehydrating agent

5.2.4 Describe and identify several common and useful dehydrating solutions

5.2.5 Describe problems encountered with inadequate dehydration

5.2.6 Evaluate appropriate corrective action

5.3 *Have a sound knowledge of and be able to:*

5.3.1 Define and state the purpose of “clearing”

5.3.2 Clarify the difference between ante-media and ‘clearing’ agents

5.3.3 Evaluate the criteria for choosing a ‘clearing’ agent

5.3.4 Describe and identify several common and useful ‘clearing’ agents

5.3.5 Describe the results of inadequate ‘clearing’

5.2.6 Evaluate appropriate corrective action

5.4 *Have a sound knowledge of and be able to:*

5.4.1 Explain the purpose of infiltration / “impregnation”

5.4.2 Describe the use and effects of vacuum in tissue processing

5.4.3 Describe the advantages and properties of paraffin wax

5.4.4 Evaluate the criteria for choosing a paraffin wax

5.4.5 Explain and motivate why additives such as plastic are used in paraffin wax

5.4.6 Evaluate the effect of wax additives and wax melting point on section cutting

5.4.7 Evaluate the factors influencing the rate of impregnation

5.4.8 Describe how to identify and correct an error in tissue processing

5.4.9 Clarify the disadvantages of tissue processing

5.4.10 Define a universal solvent and cite an example

5.4.11 Evaluate the recycling of processing reagents

5.4.12 Evaluate the recycling of processing reagents on tissue structures and organelles wrt dyes / staining / advanced techniques

5.5 *Have a good understanding and knowledge of:*

5.5.1 Automatic tissue processors

5.5.2 O*pen* and *closed* system tissue processors

5.5.3 The advantages of closed systems

5.5.4 The common difficulties encountered with tissue processors

5.5.5 Appropriate corrective action

5.5.5 The use of a large volume processor

5.5.6 The factors such as heat / agitation / viscosity / vacuum / fixation that may affect the quality and speed of tissue processing

5.5.7 Quality control and instrument maintenance

5.6 *Have a sound knowledge of and be able to:*

5.6.1 Explain the purpose and aims of embedding

5.6.2 Define the characteristics of an ideal embedding medium

5.6.3 Describe the use of paraffin wax versus resins

5.6.4 Describe the use of resins and plastics (Epoxy resins and methacrylates) in Light and Electron microscopy

5.6.5 Correctly embed tissue with special reference to the most appropriate tissue orientation

5.6.6 Describe the process of how to re-embed tissue

5.6.7 Describe alternative embedding media to wax and explain why these may be required

5.6.8 Outline the benefits and problems of water soluble and ester waxes

5.6.9 Outline the use of celloidin as a principle embedding medium as well as its use in double embedding [both used in research applications]

5.6.10 critically consider the requirements of an embedding centre

5.7 *Have a good working knowledge of:*

5.7.1 Dehydrating agents that do not need intermediate 'clearing' agents (i.e. microwave processing, etc) and any precautions in handling these solutions

5.7.2 The toxicity of many reagents used in tissue processing (ethanol, methanol, xylene, toluene, benzene, dioxane, methoxymethanol, acids, decalcification fluids)

5.7.3 The necessity of replacing processing fluids

5.7.4 How to rescue tissue that has dried out in a tissue processor

5.8 *Have an understanding of:*

5.8.1 The Gough and Wentworth technique for whole organ sectioning [Ref: Module 14]

5.8.2 How to restore the colour of previously fixed specimens for photographic and Museum mount purposes

5.8.3 Permanent Museum mounts of gross specimens for teaching purposes

C Evaluation

Evaluation of this section will take place together with the next section of this module.

A Outcomes

On completion of this module the intern / student must:

a have a thorough knowledge of microwave tissue processing

b be familiar with the dehydrating and clearing agents used for this purpose

c be familiar with all aspects of microwave processing

d be able to recognise microwave processing artefacts and take corrective action

B Objectives

5.9.1 *The intern / student must be able to:*

5.9.1.1 Describe and explain microwaves; electromagnetic waves and their properties

5.9.1.2 Cite examples of these electromagnetic waves and where they are used [Module 3: X-Rays, Ultraviolet light; visible light; infrared]

5.9.1.3 Explain the interaction between microwaves and matter

5.9.1.4 Define and explain dipoles

5.9.1.5 Define and explain thermal agitation

5.9.1.6 Define and explain “the microwave effect”

5.9.1.7 Define and explain “the temperature effect”

5.9.1.8 Explain runaway heating and evaluate the effects on tissue and cellular components

5.9.1.9 Define and explain intermedia

5.2.2 *Have a working knowledge of:*

5.2.2.1 Laboratory Microwave ovens and their application in pathology

5.2.2.3 The stabilisation of tissue and cellular components

5.2.2.4 Microwave stimulated fixation of unfixed fresh tissue

5.2.2.5 Microwave decalcification, processing and staining for:

- routine procedures

- histochemical techniques
- immune-techniques
- EM [refer EM section]
- *in-Situ* Hybridisation
- as well as single cells

5.2.2.6 Evaluate the most appropriate choice of an inter-medium for the procedures listed in 5.2.2.1 - 5.2.2.5

5.2.3 *be aware of:*

5.2.3.1 Why conventional microwave ovens are not suitable for tissue processing

5.2.3.2 The dangers of metallic objects in microwave ovens

5.3.3.5 The advantages of microwave processing

C Evaluation

Evaluation will be in the form of a practical competency test, in which the intern / student must satisfactorily demonstrate a thorough knowledge of tissue processing and processors.

The intern / student is deemed competent when he / she is able to:

a Fix and process liver, kidney and skin by conventional and microwave processing. Use microwave assisted fixation for the tissue that will be processed in the microwave.

b Embed, cut and stain the sections with an H & E stain.

c Compare the sections microscopically and report the findings in writing especially noting any differences between conventional and microwave processing.

d Critically evaluate the processing procedures that are in place in the laboratory in which they work. 1500-3000 words.

e Critically point out (identify) shortcomings in any of the above and make recommendations for appropriate corrective action or improvement.

D Recommended Textbooks and Resources for Module 5

- Theory and Practice of Histological Techniques, John D Bancroft and Marilyn Gamble. 5th or 6th Edition.
- Fundamentals of Cellular Pathology, E J Truter and W van Wyk. (out of print)
- Microwave cookbook for microscopists: art and science of visualization, Kok, L.P and Boon, Mathilde E. (1992). 3rd Revised Edition.
- Tissue Processing, microtomy and paraffin sections. Anderson,G and Gordon,K.C. (1996).
- Handbook of Histopathological and Histochemical Techniques. London. Butterworths. Culling, C.F.A. (1975).

MODULE 6

Microtomes and Section cutting

A Outcomes

On completion of this module the intern / student must:

a have a thorough knowledge of microtomes, microtome set up and operation

b have a thorough knowledge of the properties of the various microtome knives and their application for sectioning specific tissue types

c be able to use a microtome safely and ergonomically

d be able to sharpen knives and use microtome knives safely

e be able to assess a microtome knife edge

f be able to recognise cutting artefacts caused by knife edge, wax melting point, wax additives, Freezing compounds, temperature and tissue embedding / orientation and tissue aberrations

B Objectives

6.1 *The intern / student must be able to define and / or explain:*

6.1.1 Wedge and tool edge in regard to microtome knives

6.1.2 Disposable, glass and diamond knives [Refer EM section]

6.1.3 Rake, clearance angle and cutting angle

6.1.4 Effects of slope angle on section cutting

6.1.5 Describe how to evaluate a knife edge and maximum blade life

6.1.6 State how to handle, clean and store knives

6.1.7 Describe a disposable blade and its use, including its advantages and or disadvantages

6.1.8 Differentiate styles of cutting practices

6.1.9 Outline the characteristics of a microtome and microtome knives

6.1.10 Critically evaluate the value and applications of section thickness

6.1.11 Describe the use and purpose of a water / floatation bath

6.2 *have a thorough working knowledge of:*

6.2.1 Glass and metal sharpening plates

6.2.2 Why lubricants are used during sharpening

6.2.3 The use of different abrasives (alumina, carborundum and diamond)

6.2.4 The advantages and disadvantages of disposable knives

6.2.5 Microtome set up and application for sectioning tissue

- sliding
- rotary
- sledge
- freezing (CO_2 and cryostat)
- automated

6.2.6 Evaluate the importance of ergonomic microtome set up in relation to positions associated with injury and repetitive musco-skeletal disorders

6.2.6.1 Disorders associated with:

- Muscles
- Tendons [Ganglion cysts; tendonitis; Tenosynovitis; Shoulder tendonitis; forearm and elbow tendonitis]

6.2.6.2 Disorders associated with nerves:

- Carpal tunnel syndrome
- Ulnar nerve disorders

6.2.6.3 Disorders associated with nerves and blood vessels:

- Thoracic outlet syndrome

6.2.7 The preventative measures / corrective action associated with 6.2.6

6.3 *The intern / student must be able to:*

6.3.1 Evaluate with reasons, the most appropriate methods for cooling blocks for cutting

6.3.2 State the purpose of drying sections

6.3.3 Evaluate methods of section drying on tissue sections and cellular components on frozen and wax sections (Direct heat and dry heat)

6.3.4 Evaluate methods of appropriate section storage [frozen and wax sections; smears and imprints]

6.3.5 Describe the effects of temperature on section drying and cellular compartments

6.3.6 List the commonly used types of adhesives

6.3.7 State the purpose of adhesives (albumen, gelatine poly-l-lysine and 3-amino-propyltriethyloxy silane [APES])

6.3.8 Evaluate the most appropriate adhesive according to techniques performed

6.3.9 Cite examples of situations when adhesives are highly recommended

6.3.10 Evaluate the choice of commercial glass microscope slides

6.4 *The intern / student must be familiar with:*

6.4.1 The different aspects of sectioning (frozen and wax) embedded tissue with special reference to tissue orientation, size and type

6.5 *The intern / student must be able to:*

6.5.1 Recognise faults in wax and frozen sectioning (Macroscopic and microscopic)

6.5.2 Evaluate the cause of the sectioning faults (wax and frozen sections) and recommend appropriate action to remedy these sectioning faults

6.6 *The intern / student must be able to:*

6.6.1 State the purpose / reason for mounting

6.6.2 List the criteria for a good mounting media

6.6.3 Describe resinous and aqueous mounting media and give an example of each. Include temporary mounts.

6.6.4 Describe the procedure of mounting

6.6.5 Describe the automatic coverslipper

6.6.6 Evaluate the use of glass and tape as coverslip mediums

6.6.7 Describe how to properly store stained slides.

6.7 *The intern / student must be familiar with:*

6.7.1 Appropriate Health and Safety measures associated with the use, operation, cleaning / maintenance and / or storage of:

- Blades and knives (including appropriate disposal)
- Sharpening equipment and abrasives
- Microtomes (sliding, rotary, sledge, freezing, automated)
- Waterbaths / ''floatation baths''
- Incubators
- Automated Coverslippers and mountants

C Evaluation

Evaluation will be in the form of a practical competence test, in which the intern / student must satisfactorily demonstrate different cutting artefacts.

a Using the embedded skin from Module 4, cut and stain (H & E) sections to demonstrate the following different cutting artefacts:

- Scores
- Chatters
- Section compression.

b Critically evaluate all the slides from section ‘’a’’ and comment in writing on your findings. State how remedial action should be taken.

c Critically evaluate the microtome set up that you have used in your laboratory to obtain your section in ‘’a’’.

d Critically point out (identify) shortcomings in any of the above and make recommendations for appropriate corrective action or improvement. 1500 – 3000 words.

D Recommended Textbooks and Resources for Module 6

- Theory and Practice of Histological Techniques, John D Bancroft and Marilyn Gamble. 5th or 6th Edition.
- Fundamentals of Cellular Pathology, E J Truter and W van Wyk (out of print)
- Tissue Processing, microtomy and paraffin sections. Anderson, G. and Gordon,K.C.(1996).
- Handbook of Histopathological and Histochemical Techniques. London. Butterworths. Culling, C.F.A. (1975).
- Laboratory SOP’s and Equipment manuals

MODULE 7

Frozen Sections

A Outcomes

On completion of this module the intern / student must have a:

a thorough knowledge of the use and application of frozen section microtomes and cryostats

b thorough knowledge of how to freeze tissues according to tissue type and size, method, temperature and specific special investigations

c thorough knowledge on how to prepare frozen sections for rapid diagnosis including orientation for lesion resection lines of excision verification (inking and Mohs)

d have a working knowledge of freeze-drying and freeze substitution

B Objectives

7.1 *The intern / student must be able to:*

7.1.1 Outline the principles involved in the preparation of frozen sections

7.1.2 Critically compare different methods of freezing fresh unfixed tissues using the following mediums:

- Liquefied nitrogen (-190°C)
- Isopentane (-150°C to -160°C)
- Dry Ice (-70°C)
- Carbon dioxide gas (-70°C)
- aerosol sprays (-50°C)
- OCT compound in the chamber

7.1.3 State the advantages of frozen sections compared to other embedding techniques

7.1.4 Describe the cryostat cutting technique with reference to:

- Freezing mediums (liquefied nitrogen, isopentane, dry Ice, carbon dioxide gas, aerosol sprays, optimal cutting temperature compound {OCT compound})
- Cabinet and temperature
- Microtome
- Blade or knife- angle and temperature
- Anti-roll plate
- Tissue type (Refer 7.1.9)

7.1.5 State the purpose and describe the set-up of the anti-roll plate:

- Height of blade
- Blade angle
- Edge of plate
- Cabinet temperature

7.1.6 Critically evaluate the choice of a cryostat based on the following features:

- Temperature control
- Electronically controlled advance and retraction of the block
- Specimen orientation facility
- Digital visualisation of the chuck and cabinet temperatures
- Mechanical cutting speed control and section thickness
- Automatic defrost features
- Automated decontamination and sterilisation
- Application and portability

7.1.7 Evaluate the uses of a cryostat

7.1.8 Describe the maintenance and disinfection procedures of the freezing microtome and cryostat

7.1.9 Identify cryostat cutting errors and their solutions plus corrective action

7.1.10 Describe ice crystal artefact and describe common methods on how to prevent crystal formation [Cryo-protection]

7.1.11 Know how resection lines of excision of lesions are verified:

7.1.11.1 Inking

7.1.11.2 Mohs technique and complete circumferential peripheral and deep margin assessment (CCPDMA)

7.1.11.3 Tangential sections

7.2 *The intern / student must be able to define and / or explain:*

7.2.1 The principle of the freeze-drying process

7.2.2 Outline the process and stages of freeze drying

- Quenching
- Drying
- Fixation and embedding
- Vapour fixation

7.2.3 Cite the uses of freeze-dried material

7.2.4 Explain the principle / technique of the freeze-substitution process

7.2.5 Clarify the advantages and disadvantages of using freeze-drying and freeze-substitution techniques on cellular components

7.2.6 Evaluate the advantages and disadvantages of the use of cryostat sections

7.2.7 Evaluate when frozen sections are preferable to paraffin sections and why

7.3 The intern / student must be familiar with:

7.3.1 Appropriate Health and Safety measures associated with the use, operation, cleaning / maintenance and / or storage of:

7.3.2 Blades and knives for the freezing microtome and cryostat (including appropriate disposal)

7.3.3 Appropriate decontamination of all equipment used in the preparation procedure of fresh unfixed tissue

7.3.4 Appropriate actions to be taken when injured on duty / cut while performing a frozen section

C Evaluation

Evaluation will be in the form of a practical competency test, in which the intern / student must satisfactorily demonstrate sections cut on a conventional cryostat.

a Cut sections from a piece of fixed liver tissue using a cryostat (use a freezing microtome if a cryostat is unavailable).

b Stain sections and mount in glycerine jelly.

c Stain sections and mount in synthetic resin (DPX™, Entellan™ etc).

d Prepare a rapid section from fresh tissue within a 10 minute time limit.

e Over-freeze your tissue listed in ''a'' and mount and stain the section you obtain by means of H&E.

f View all the sections with a microscope and record your observations. Critically evaluate all the slides and comment in writing on your findings. State how remedial action, if necessary, should be taken. 1500 + words.

g the intern / student must be able to cut a frozen section demonstrating lesion resection lines of excision.

D Recommended Textbooks and Resources for Module 7

- Theory and Practice of Histological Techniques, John D Bancroft and Marilyn Gamble. 5th or 6th Edition.
- Fundamentals of Cellular Pathology, E J Truter and W van Wyk (out of print)
- Peters, Stephen R. Editor: Practical Guide to Frozen Section Technique. Springer.

E Other

- SOP's and Equipment Manuals
- Health and Safety
- First Aid

MODULE 8

Theory of Staining

A Outcomes

On completion of this module the intern / student must:

a Understand why tissues are stained

b have a thorough knowledge of stains and dye structure and associated principles

c have a thorough knowledge of the mechanisms of staining

d be familiar with all staining procedures encountered in a diagnostic histopathology laboratory

e must know the importance of the use of “control tissue” for specific diagnostic staining procedures

f be able to explain the theory of staining in relation to ‘’objective and subjective’’ observation; recognise faults / artefacts / staining aberrations and remedy these faults / artefacts / staining aberrations

B Objectives

8.1 *The intern / student must be able to:*

8.1.1 Describe a dye

8.1.2 Define a chromophore, chromogen, auxochrome

8.1.3 Identify acidic and basic auxochromes

8.1.4 Describe anionic and cationic dyes

8.1.5 Describe mordant dyes, solvent dyes, polychromatic dyes, neutral stains, amphoteric dyes, leuco dyes, and metachromatic dyes

8.1.6 Describe the classification of dyes by chromophores

8.1.7 Define, describe and give examples of the following:

- Physical staining
- Chemical staining

8.1.8 Define, describe and give examples of metallic impregnation

8.2 *The intern / student must be able to:*

8.2.1 State the purpose of the Biological stain commission (BSC)

8.2.2 Explain what a certification number on a stain or dye indicates

8.2.3 Describe WHY tissues need to be coloured with dyes

8.2.4 Explain how and why dyes bind to tissue components to enable staining

8.2.5 Explain why stained components of tissue remain selectively stained

8.2.6 Explain why stains remain in the tissue

8.2.7 Critically evaluate the factors that contribute to dye-tissue affinities

8.2.8 Critically evaluate the effect of decalcification agents, fixatives and fixation on staining outcome [Tissue modification] “Blocking”

8.2.9 Explain the effects of tissue / specimen / section geometry on staining outcomes

8.2.10 Evaluate progressive and regressive staining techniques

8.2.11 Describe the use of mordants [dye lakes], accentuators, accelerators and differentiators

8.2.12 Describe the term ‘’adsorption’’ in staining theory and explain:

- Phenomenon
- Heat exchange
- Temperature
- Rate of reaction
- Concentration

8.2.13 Classify ‘’Adsorption’’

- Physisorption [weak van der Waal’s forces]
- Chemisorption [Covalent and electrostatic bonding]

8.2.14 Explain why / how dyes are coloured

- Dye chemistry
- Dye impurities

8.2.15 Describe the phenomenon of ‘’metachromasia’’ and give examples of its use

8.2.16 Name several common nuclear and cytoplasmic stains

8.2.17 Explain and evaluate the differences between ‘’Direct and Indirect’’ staining

8.2.18 Define the phenomenon of ‘’Orthochromasia’’ and give examples of its use

8.2.19 Define ‘’Trapping agents’’ and its use and application in common staining methods used in the laboratory

8.2.20 critically evaluate the differences between trapping agents and mordants

8.2.21 Select the most appropriate tissue section and preparation for stain control

- Negative controls [various types]
- Positive controls

C Evaluation

Evaluation of this module will take place together with that of Module 9.

D Recommended Textbooks and Resources for Module 8

- Theory and Practice of Histological Techniques, John D Bancroft and Marilyn Gamble. 5th or 6th Edition.
- Histological and Histochemical Methods. Kieran. J.A. (1990). Oxford: Pergamon.

MODULE 9

Staining of Specific Elements

A Outcomes

On completion of this module the intern / student must:

a have a thorough knowledge of the staining and preparative procedures covered in it

b be able to recognise tissues and tissue components

c be able to deduce which stain to use for a specific component / structure

d be familiar with the use of appropriate control tissue specific to tissue element; procedure and technique

e be able to recognise staining artefacts and apply corrective measures

f be able to “troubleshoot” out of the ordinary staining reactions

g Outline the problems associated with identifying and localising chemicals in tissue sections

h know the principles and results of all staining methods in this module

9.1 The Haematoxylins

B Objectives

9.1.1 *The intern / student must be able to define and / or explain:*

9.1.1.1 The origin and extraction process of haematoxylin

9.1.1.2 The oxidation / ripening process of haematoxylin

- Natural
- Chemical

9.1.1.3 Evaluate haematoxylin solutions according to the mordants and oxidants used

- Alum
- Iron
- Tungsten
- lead haematoxylins
- haematoxylin without mordant
- chromium-copper

9.1.1.4 Name the common components of haematoxylin solutions and state their purpose / specific functions. [i.e. Properties of haematoxylin and staining pattern using haematoxylin solutions]

9.1.1.5 Evaluate haematoxylin solutions according to the application / use required and cite an example of the haematoxylin solution

9.1.1.6 Differentiate between iron and alum haematoxylin

9.1.1.7 State the theory of the stain

9.1.1.8 Describe Eosin and its use

- With and without Phloxine
- The differentiation an application for different connective tissue and matrices

9.1.1.9 Describe the regressive method

9.1.1.10 Recognise errors and describe corrective action in the QC process of the routine stain

- On automated staining platforms
- Variations in batch of haematoxylin
- pH of solution
- age of solution
- degree of use of solution
- on acidic and or mercuric fixed tissue sections
- ‘’blueing techniques and choice of blueing solution [Note: incorrect blueing makes archived slides turn brown]

9.1.1.11 Differentiate between a progressive and a regressive stain

9.1.1.12 Name several common cytoplasmic stains

9.1.2 *be conversant with:*

9.1.2.1 The disadvantages of alum haematoxylins

9.1.2.2 Counterstains used after haematoxylin staining (eosin, phloxine, neutral red, kernechtrot, erythrosin etc.)

C Evaluation

Evaluation will be in the form of a practical competence test, in which the intern / student must satisfactorily demonstrate correct fixation, processing, embedding and H & E staining of tissue.

a Collect tissue from the following organs and systems:

- i Circulatory system (aorta, small artery, heart)
- ii Skin
- iii Immune system (lymph node, spleen, thymus, tonsil)
- iv Respiratory system (trachea, bronchus and lung)
- v Digestive system (parotid, sublingual glands, oesophagus, stomach, duodenum, ileum, colon, appendix, liver, gall bladder and pancreas)
- vi Urinary tract (kidney, ureter and bladder)
- vii Endocrine system (pituitary, thyroid, parathyroid and adrenal)
- viii Male reproductive system (testis, vas deferens and prostate)
- ix Female reproductive system (ovary, Fallopian tube, uterus, cervix and breast)
- x Central nervous system (spinal cord, cerebellum and cortex)
- xi Skeletal system (rib, dense bone)
- xii Where possible, source an animal eye [sheep / pig from a butchery]. Ref Module 9.11 and Module 10

b Fix each tissue optimally, process to wax and embed, orientating tissue correctly. *Keep bone specimens in reserve for use at a later date*.

c Cut five sections from each block and stain one section with a standard H & E stain.

d Keep the rest of the sections in reserve for use at a later date.

e Cut four sections from a kidney block. Stain the sections with two iron haematoxylins and two alum haematoxylins.

f Counterstain the four sections with eosin, phloxine, and van Gieson's mixture respectively.

g View the slides microscopically and compare and record the results. Include any corrective / remedial action required to avoid artefacts.

h Critically evaluate all the slides that have been stained. Describe the tissue components and evaluate the quality of the stains. Include in your evaluation, the effects, if any, of these staining solutions on your specific tissues and stain outcome. Comment on any possible remedial action. 2000-3000 words

9.2 Connective Tissue

B Objectives

9.2.1 *The intern / student must be able to describe / explain the structure of:*

9.2.1.1 Connective tissue

- areolar
- adipose
- myxoid
- dense connective tissue
- cartilage
- bone

9.2.1.2 The function of connective tissue listed in 9.2.1.1

9.2.1.3 The three main types of Connective tissue and its function(s)

9.2.1.4 Connective tissue cells and ground substance and function(s)

9.2.1.5 Collagen Types I, II, III, IV, V and VI and cite examples of occurrence and function in the body

9.2.1.6 Describe reticular fibres and its functions

9.2.1.7 Describe elastic fibres and its functions

9.2.1.8 describe amorphous or gel-type intercellular substances (glycosaminoglycans, structural glycoproteins and basement membranes) and their functions

9.2.1.9 Connective tissue cells (fibroblasts, fat cells, etc.) and functions

9.2.1.10 muscular tissue (involuntary smooth muscle, voluntary striated muscle and striated cardiac muscle) and the associated functions of each

9.2.1.11 Fibrin and fibrinoid and its role in the body

9.2.2 *The intern / student must be able to:*

9.2.2.1 Outline the significance of dye size in trichrome staining

9.2.2.2 Discuss and evaluate factors affecting trichrome staining (tissue permeability, dye molecular size, heat and pH)

9.2.2.3 Evaluate the choice of nuclear stains for trichrome methods

9.2.2.4 Discuss the effects of fixation on and the parameters required for optimal trichrome staining

9.2.2.5 Evaluate the effects and of role of phosphotungstic acid [PTA] and phosphomolybdic acid [PMA] on trichrome staining

9.2.3 *The intern / student must be able to:*

For the connective tissue stains listed below:

- Van Gieson
- Masson's trichrome
- Martius scarlet blue (MSB) for fibrin (and muscle)
- Verhoeff V G
- Weigert's resorcin fuchsin method
- Gordon & Sweet's and Gomori's method for reticular fibres
- Southgate's mucicarmine stain
- Sudan III & IV, Oil Red O
- Phosphotungstic Acid Haematoxylin (PTAH)
- Alcian Blue stain

9.2.3.1 State the purpose of the method

9.2.3.2 State the theory of the method

9.2.3.3 List the reagents used and their functions

9.2.3.4 State the recommended fixatives and any effects on staining

9.2.3.5 Describe the staining method

9.2.3.6 Describe the expected results

9.2.3.7 Recognise errors and describe corrective action

9.2.3.8 State any precautions where applicable

9.2.3.9 List appropriate control tissues

C Evaluation

Evaluation will be in the form of a practical competence test, in which the intern / student must satisfactorily demonstrate correct fixation, processing, embedding, post-embedding treatment and staining of connective tissues.

Review the slides microscopically and identify / observe the various types of connective tissue. Comment in writing [2000-3000] words on the quality of fixation and staining.

a Use sections cut for the previous assignment or fix, process and embed fresh tissue for this part.

b Stain all the sections with H & E:

Myxoid tissue	Wharton's jelly
Areolar tissue	membranes around brain
Adipose tissue	subcutaneous tissue
Reticular tissue	liver and lymph nodes
Elastic tissue	arteries
Irregular elastic tissue	organ capsules or dermis
Regular elastic tissue	tendons
Hyaline cartilage	trachea
Elastic cartilage	epiglottis
Fibro-cartilage	intervertebral disc
Elastic artery	aorta
Muscular artery	small artery
Fenestrated capillary	kidney
Sinusoids	liver
Large and small muscular veins	kidney and most other tissues
Skeletal muscle	most muscle in arms and legs
Visceral muscle	stomach and uterus
Cardiac muscle	heart tissue

9.3 Proteins and Nucleic acids

B Objectives

9.3.1 *The intern / student must be able to define / explain the following*

9.3.1.1 Nucleic acids

- Phosphate group
- Nitrogenous base of DNA and RNA (purines and pyrimidines)

9.3.1.2 Simple proteins

9.3.1.3 Conjugated proteins and cite examples

9.3.1.4 Prosthetic groups

9.3.1.5 Deoxyribonucleic acid (DNA)

- Double helix DNA
- Active and inert DNA

9.3.1.6 Ribonucleic acid (RNA)

- Ribosomal
- Transfer
- Messenger

9.3.1.7 “pyroninphilia’’

9.3.2 *The intern / student must be familiar with:*

9.3.2.1 The effects of fixation on nucleic acids

- Yield
- Quality

9.3.2.2 Applicability of fixation and the effects thereof in molecular diagnostics, [including reduced material, use of biopsies]

9.3.2.3 The effects of decalcification solutions on DNA and RNA

9.3.2.4 Procedures for tissue preservation for bio-banking

9.3.2.5 Non-formalin based fixatives for DNA and RNA

9.3.2.6 Fixative substitutes

- HOPE fixation (Hepes-glutamic acid buffer mediated organic solvent
  - protection effect
- Acetone-methylebenzoate-xylene (AMeX)
- Universal molecular fixative (UMFIX)
- RCL2 i

9.3.3 *The intern / student must know be able to describe / explain:*

9.3.3.1 The main methods for demonstration of nucleic acids and proteins (histophysical methods, amino acid histochemical methods, enzyme histochemical methods, immuno-fluorescent methods and immuno-histochemical methods) in sections

9.3.3.2 The problems associated with identifying the types of nucleic acids

9.3.3.3 The problems associated with identifying and localising proteins in tissue sections

9.3.3.4 The Feulgen reaction

9.3.3.5 Acid hydrolysis reaction / process

9.3.3.6 “Washed methyl green”

9.3.3.7 The methyl green-pyronin technique

9.3.4 Compulsory stains: Reticulin, Giemsa, Methyl green-pyronin; Feulgen methods and.

Other recommended stains: H & E; Periodic acid Schiff’s and Acridine Orange stains.

For each of the above stains listed in 9.3.4, the intern / student must be able to:

9.3.4.1 State the purpose of the method

9.3.4.2 State the theory / principle of the method

9.3.4.3 List the reagents used and their functions

9.3.4.4 State the recommended fixatives and any effects on staining

9.3.4.5 Describe the staining method

9.3.4.6 Describe the expected results

9.3.4.7 Recognise errors and describe corrective action

9.3.4.8 State any precautions where applicable

9.3.4.9 List appropriate control tissues

C Evaluation

Evaluation will be in the form of a practical competence test, in which the intern / student must satisfactorily demonstrate correct fixation, processing, embedding, post-embedding treatment and staining of lymphoid tissues.

a Use sections cut for the previous assignment or if necessary fix, process and embed fresh tissue for this purpose.

b Use the correct stains to demonstrate general morphology of the following:

- Thymus
- Lymph node
- Palatine tonsil
- Spleen

c View the slides microscopically and write short notes 1500-3000 words to describe each tissue. Identify the different parts correctly and record in writing, the results of each stain, commenting on the quality of the staining.

9.4 Amyloid

B Objectives

9.4.1 *The intern / student must be able to define / explain / describe the following:*

9.4.1.1 Describe the nature of amyloid

9.4.1.2 Discuss / classify the types of amyloid and their underlying pathologies or physiological conditions

- Primary amyloidosis
- Secondary amyloidosis

9.4.1.3 Amyloidosis

9.4.1.4 Appearance of amyloid (light microscopy and ultrastructure)

9.4.1.5 Describe and evaluate the major staining methods used to detect amyloid

- Congo Red
- Alkaline Congo Red
- Standard toluidine blue (STB)
- Crystal or Methyl violet metachromasia
- Thioflavine T
- Sirius Red
- Iodine / Sulphuric acid method for whole organs
- Immuno-techniques

9.4.1.6 State the purpose of the Congo Red method

9.4.1.7 List the reagents and their function

9.4.1.8 State recommended fixatives

9.4.1.9 State the reason for using thicker sections when demonstrating amyloid

9.4.1.10 Describe the staining method

9.4.1.11 Describe the expected results

9.4.1.12 Recognise errors and describe any corrective action

9.4.1.13 State any precautions where applicable

9.4.1.14 List appropriate control tissues

9.4.2 *be familiar with:*

9.4.2.1 The use of the polarising microscope to demonstrate amyloid

9.4.2.2 The advantages and disadvantages of using fluorescent techniques when demonstrating amyloid

9.4.2.3 The ultrastructural configuration of amyloid using electron microscopy

C Evaluation

Evaluation will be in the form of a practical competence test, in which the intern / student must satisfactorily demonstrate amyloid in tissue.

a Obtain a piece of amyloid positive tissue (control tissue used in the laboratory should suffice) for LM.

b Cut sections and stain to demonstrate amyloid.

Note! Compulsory stains: Alkaline Congo Red, Standard toluidine blue (STB), Crystal or Methyl violet.

Other recommended stains: H & E and Thioflavine T (LM and IMF).

c With the aid of a light, polarising and fluorescent microscope, look at the relevant sections (LM, FM {where applicable} and Polarising light microscope).

d. Prepare (fix, dehydrate, embed, cut and stain) amyloid positive tissue for electron microscope studies. Identify the amyloid and describe its structure.

e Critically evaluate the various stains and techniques and record your results. 2000+ words

9.5 Carbohydrates

B Objectives

9.5.1 *The intern / student must be able to:*

9.5.1.1 Evaluate and classify carbohydrates in fixed tissues

- Simple carbohydrates [>mono; >oligosaccharides]
- Glycoconjugates [>proteoglycans,> mucins and >''other glycoproteins'']

9.5.1.2 State the purpose of the carbohydrate methods

9.5.1.3 Discuss the effects of fixation on glycogen relocation within the cell

9.5.1.4 Evaluate the fixation procedure and section preparation to demonstrate carbohydrates

9.5.1.5 Discuss the enzymatic extraction of glycogen

- Diastase
- Sialidase
- Hyaluronidase

9.5.1.6 Describe the Periodic acid-Schiff [PAS] reaction and be able to outline the problems in interpreting the PAS reaction

9.5.1.7 Identify glycogen with the light and electron microscopes (alpha and beta types by ultrastructure)

9.5.2 *The intern / student must be able to define / describe / explain*:

9.5.2.1 A histological classification of mucins

- acid mucins (strongly sulphated, weakly sulphated and sulphated sialomucin)
- neutral mucins

9.5.2.2 How to identify and localise mucins in cells and tissues

9.5.2.3 Why the Alcian dyes and especially Alcian blue is used to stain acid mucopolysaccharides

9.5.2.4 The influence of pH on Alcian blue staining

9.5.2.5 The Alcian blue-PAS method for neutral and acid mucins

9.5.2.6 The digestion techniques

9.5.3 for the following methods: PAS with / and without diastase; Alcian Blue stain pH 1.0 and pH 2.5; Toluidine blue method; *the intern / student must know / be able to explain and critically evaluate:*

9.5.3.1 State the theory / principle of the method

9.5.3.2 List the reagents used and their function

9.5.3.3 State the recommended fixative and any effects on staining

9.5.3.4 Describe the staining method

9.5.3.5 Describe the expected results

9.5.3.6 Recognise errors and describe corrective procedures

9.5.3.7 State the purpose of diastase digestion of glycogen

9.5.3.8 State precautions where applicable

9.5.3.9 List appropriate control tissues

9.5.3.10 Evaluate false positive and false negative PAS reactions

C Evaluation

Evaluation will be in the form of a practical competence test, in which the intern / student must satisfactorily demonstrate glycogen and mucopolysaccharides in tissue.

a Obtain a piece of liver tissue. Autopsy tissue will suffice; however, sections from a liver biopsy would give better results.

b Cut sections and stain to demonstrate glycogen and reticulin.

Note: Compulsory stains-PAS + diastase and any stain for reticulin.

c Cut sections from gastric mucosa, kidney and cartilage and stain to demonstrate glycogen and acid muco-polysaccharides.

Note! Compulsory stains- PAS + diastase / APAS + diastase and Best's carmine stain.

Recommended stains for all the above: H & E.

d Microscopically review the stained sections and evaluate your results commenting on the tissue morphology and results of the staining procedures. 2000+ words

9.6 Fats and Lipids

A. Outcome

On completion of this module the intern / student must:

a be able to critically compare the similarities and differences between lipids, fats and their derivatives

b be able to describe how lipids and fats are demonstrated in tissues

c be able to demonstrate lipids and fats in tissue sections

d be able to critically evaluate how the classification of lipids and fats influences histochemistry

e be able to evaluate how fixation protocols influence lipid histochemistry

f be able to understand the function(s) of lipids and fats in the body

B Objectives

9.6.1 *The intern / student must be able to define / describe / explain the following:*

9.6.1.1 The classification of lipids characterised by their insolubility in water

9.6.1.2 The classification of lipids characterised by their solubility in organic solvents

9.6.1.3 Evaluate the differences between lipids and fats

- Conjugated lipids [neutral fats, waxes, cholesterol esters, phosphoglycerides, sphingomyelins, ceramides, glycolipids]
- Uncongugated lipids [fatty acids and steroids {cholesterol and steroid hormones}]

9.6.1.4 Cite examples of both fats and lipids

9.6.1.5 Free fatty acids

9.6.1.6 Solubility and the physicochemical properties of lipids and fats

- hydrophobic
- hydrophilic
- amphipathic

9.6.1.7 Lipid melting point

9.6.1.8 The biological functions and of lipids and fats

9.6.1.9 The birefringent properties of lipids

9.6.1.10 the metabolism and degradation of lipids and fats

9.6.2 *The intern / student must be able to define / describe / explain and or critically evaluate where necessary, the following:*

9.6.2.1 State the recommended preservation / fixation protocol of lipids and any effects this may have on staining

9.6.2.2 Describe the recommended sections required for the successful demonstration of lipids [type of preparation]

9.6.3 *The intern / student must be able to:*

Describe and evaluate the major histophysical and histochemical staining methods used to detect lipids and fats

- Oil Red O (Fats)
- Sudan dyes:

*Sudan III and IV [lysochrome method]

*Sudan Black B (fats and phospholipids)

*Bromine Sudan Black (lipids)

- Nile Blue sulphate method - neutral and acidic lipids
- Nile blue sulphate method for phospholipids
- Copper rubeanic acid (free fatty acids)
- Perchloric acid-Naphthoquinone (PAN) (cholesterol)
- Marchi technique (myelin)

9.6.4 *For each of the above stains listed in 9.6.3, the intern / student must be able to:*

9.6.4.1 State the purpose of the method

9.6.4.2 State the theory / principle of the method

9.6.4.3 List the reagents used and their functions

9.6.4.4 State the recommended fixatives and any effects on staining.

9.6.4.5 Describe the staining method

9.6.4.6 Describe the expected results

9.6.4.7 Recognise errors and describe corrective action

9.6.4.8 State any precautions where applicable (effects of temperature; fixative)

9.6.4.9 List appropriate control tissues:

- Positive
- Negative
- Delipidised
- Extraction techniques

9.6.5 *The intern / student should be familiar with:*

9.6.5.1 Lipid storage diseases

9.6.5.2 Combination staining techniques used to demonstrate lipids and fats

C Evaluation

Evaluation will be in the form of a practical competence test, in which the intern / student must satisfactorily demonstrate lipids and fats in various tissues.

a Cut frozen or cryostat sections from heart, liver, skin, skeletal muscle and brain.

b Stain sections with the following stains: Oil Red O, Sudan III and IV, Sudan black B, Copper rubeanic acid

Other recommended stains: Bromine Sudan black, Nile Blue sulphate, Perchloric acid-Naphthoquinone (PAN) and Marchi's technique.

c Stain heart and skin sections to demonstrate *free fatty acids and phospholipids.*

d Stain heart sections to demonstrate *cholesterol.*

View the sections under the light microscope, comment and record your results microscopically and record your results.

e Microscopically review the stained sections and evaluate your results commenting on the tissue morphology and results of the staining procedures. 3000+ words.

f Also view the Oil Red O stained section by means of polarised light and compare / evaluate the differences with that seen under conventional light microscope conditions.

9.7 Pigments and Minerals

A. Outcome

On completion of this module the intern / student must:

a Comprehensively describe the classification of pigments

b Describe the formation and production of pigments

c Outline the tests used to identify pigments

d Identify the main minerals found in tissue sections and critically evaluate how they can be identified

B Objectives

9.7.1 *The intern / student must be able to define / describe / explain:*

9.7.1.1 Pigments and their classification

Artefact pigments:

- Formalin (formaldehyde)
- Mercury
- Chrome
- Osmium
- Malarial
- Schistosome
- Starch

Endogenous (heam and non-heamtogenous) pigments and minerals:

- Haemoglobin
- Haemosiderins and ferritin
- Bile pigments
- porphyrins
- Autogenous pigments (Melanin, Lipofuscin, Chromaffin, Argentaffin, Dubin-Johnson, Ceroid-type lipofuscin)

Exogenous pigments and minerals:

- Carbon
- Silica
- Asbestos
- Copper
- Uric acid and urates
- Tatoo
- Amalgam
- Lead
- Aluminum
- Silver

9.7.1.2 How and why the various pigments listed in 9.7.1.1 occur

9.7.1.3 Normal versus pathological classification of pigments

9.7.1.4 Lipofuscin

- production
- classification
- occurrence

9.7.1.5 Haemosiderosis and haemochromatosis

- normal and abnormal iron production and metabolism
- classification
- occurrence

9.7.1.6 The process of calcinosis

9.7.2 Select and critically evaluate the most appropriate method or technique for the demonstration of the pigments listed in 9.7.1.1

9.7.3 Describe the procedure for the identification of an unknown pigment

9.7.4 *The intern / student must be able to:*

For the following methods: Perl's Prussian blue; Masson Fontana and von Kossa; long Ziehl Neelsen (ZN) methods:

9.7.4.1 State the purpose of the method

9.7.4.2 State the theory of the method

9.7.4.3 List the reagents used and their function

9.7.4.4 State the recommended fixative and any effects on the staining

9.7.4.5 Describe the staining method

9.7.4.6 Describe the expected staining results

9.7.4.7 Recognise errors and describe the appropriate corrective action

9.7.4.8 State precautions where applicable

9.7.4.9 List appropriate control tissues

9.7.5 *be familiar with:*

9.7.5.1 Methods and techniques to avoiding artefactual pigment formation in tissues

9.7.5.2 The extraction method for formalin and malarial pigment

9.7.5.3 Staining methods for lipofuscin and associated pathologies

- PAS
- Schmorl's Ferric-Ferricyanide reduction test
- Long ZN
- Sudan Black
- Gomori aldehyde fuchsin
- Masson Fontana silver method
- Basophilia using methyl green
- Churukian's silver method
- Lillie's Nile blue
- Acid phosphatase S
- EM
- Auto fluorescence

9.7.6 *Have a thorough working knowledge of:*

9.7.6.1 Staining methods for calcium (Von Kossa, Alizarin Red S)

9.7.6.2 Staining methods for bile pigments (Fouchet's and Gmelin's method)

9.7.6.3 Staining methods for copper (Rubeanic acid and modified Rhodanine method: Lindquist)

9.7.6.4 Staining methods for asbestos (Perl's method)

9.7.6.5 Staining methods for lead salts (Rhodizonate method)

9.7.6.6 Staining methods for aluminum (Solochrome azurine method)

9.7.6.7 Staining methods for silver (Rhodanine method: Okamota)

C Evaluation

Evaluation will be in the form of a practical competence test, in which the intern / student must satisfactorily demonstrate removal of artefact pigments and staining of other endogenous and exogenous pigments in tissue.

a Fix two pieces of spleen in two different fixatives containing formaldehyde and potassium dichromate. Prepare sections from each block demonstrating artefact pigments produced by each of these fixatives. In parallel, compare the sections after the pigments have been appropriately removed.

b Demonstrate the presence of melanin employing a positive and negative staining method.

c Demonstrate by the appropriate tinctorial method, the presence of haemosiderin in liver tissue.

d Demonstrate the presence of calcium in tissue by staining the sections by means of the van Kossa technique.

e Stain a section of tissue [liver / cardiac muscle] known to have lipofuscin pigment by means of ; PAS and Sudan Black techniques. View by means of light microscopy and under UV light [under any excitation ranging from 360nm to 647 nm]. Record and explain your results.

Note! Compulsory stains: Long Ziehl-Neelsen, Masson Fontana, Perl's Prussian blue method, Von Kossa, Alizarin Red S.

Other recommended stains: H & E and PAS, Perl's Turnbull's and Quinke reaction for Total iron.

f Microscopically review the stained sections and evaluate your results commenting on the tissue morphology and results of the staining procedures. 3000+ words

9.8 The Diffuse Neuroendocrine system (DNES) and cytoplasmic granules

A. Outcome

On completion of this module the intern / student must:

a understand the morphology, functionality and biochemical staining characteristics of the dispersed / diffuse neuroendocrine system (DNES) and neuroendocrine amines

b be able to demonstrate neuroendocrine cells (NEC's) containing neurosecretory granules (NSG's) in tissues

c be able to critically evaluate the methods and techniques for the demonstration of NEC's / neurosecretory granules

B Objectives

9.8.1 *The intern / student must be able to define / describe / explain:*

9.8.1.1 The dispersed diffuse / dispersed neuroendocrine system (DNES) and its components

9.8.1.2 Morphology, location and ultrastructure of the DNES

- Features: Argyrophil and Argentaffin reactions
- Bio-marking
- Biochemical characteristics: [Synthesis of regulatory peptides / APUD / Formalin induced amine fluorescence / Cytochrome a561 / Non-specific esterase or cholinesterase]

9.8.1.3 the structure of the endocrine pancreas / ganglia of the autonomic nervous system / adrenal gland / GIT tract / respiratory system / parathyroid / thyroid / pituitary

9.8.1.4 the role of neuroendocrine secretory granules in the endocrine pancreas / ganglia of the autonomic nervous system / adrenal gland / GIT tract / respiratory system / parathyroid / thyroid / pituitary

9.8.1.5 Describe and evaluate the major staining methods used to demonstrate neuro-endocrine tumours

9.8.2 *Have a thorough working knowledge of:*

9.8.2.1 staining methods for neuro-endocrine cells of the organ systems listed in 9.8.1.3 (Giemsa, Grimelius method, Formalin induced fluorescence, Lead haematoxylin method and Masked metachromasia method, Fontana-Masson Argentaffin reaction, Rapid argyrophil procedure for neurosecretory granules)

9.8.2.2 specialised fixation for chromaffin cells

9.8.2.3 Unmasking of metachromasia in chromaffin cells

9.8.2.4 Specialised methods for the demonstration of neurosecretory granules of the DNES; including IHC / ICC / TEM / Molecular (Ref: Modules 11, 12, 13)

9.8.3 *be familiar with:*

9.8.3.1 The type of secretions by chromaffin cells

9.8.3.2 The types of tumours arising from neuro-endocrine cells (phaeochromocytoma, neuroblastoma, carcinoid etc) of the systems listed in 9.8.1.3 and the staining patterns of the predominant cells types

9.8.3.3 What causes formalin-induced fluorescence in chromaffin cells?

9.8.3.4 The choice of appropriate control tissue for argyrophil stains

9.8.4 for each of the stains listed, Giemsa, Grimelius, Lead haematoxylin and masked metachromasia method, Formalin-induced Fluorescence and PAS-Orange G, *the intern / student must be able to:*

9.8.4.1 State the purpose of the method

9.8.4.2 State the theory / principle of the method

9.8.4.3 List the reagents used and their functions

9.8.4.4 State the recommended fixatives and any effects on staining

9.8.4.5 Describe the staining method

9.8.4.6 Describe the expected results

9.8.4.7 Recognise errors and describe corrective action

9.8.4.8 State any precautions where applicable

9.8.5.9 List appropriate control tissues

C Evaluation

Evaluation will be in the form of a practical competence test, in which the intern / student must satisfactorily demonstrate the presence of neuroendocrine cells / neurosecretory granules in tissue.

a Prepare adrenal gland, pyloric antrum and thyroid gland, process and cut sections from the aforementioned tissue.

b Demonstrate chromaffin, argentaffin and argyrophil cells in the above tissues.

Note! Compulsory methods: Giemsa, Grimelius, Lead haematoxylin and masked metachromasia method.

Other recommended methods: H & E, Formalin-induced Fluorescence and PAS-Orange G.

c Microscopically review the stained sections and evaluate your results commenting on the tissue morphology and results of the staining procedures. Include in your comments, the morphology and structure of the two zones of the adrenal gland. 3000+ words.

9.9 Micro-organisms

A. Outcome

On completion of this module the intern / student must:

a be able to identify the types of organisms found in sections and name examples of each type of organism

b be able to describe and critically evaluate the most appropriate methods for detection of micro-organisms in tissue sections

c outline the limitations of histological identification of organisms in tissue sections

d be able to classify organism into hazardous groups

B Objectives

9.9.1 *The intern / student must be able to define / describe / explain (including size, shape and structure, reproduction) of the following groups of microorganisms and / or the diseases they cause:*

9.9.1.1 Bacteria – various gram positive / negative cocci and bacilli; including the cell wall of Gram + and Gram – bacteria

9.9.1.2 Yeasts and fungi

9.9.1.3 Protozoa and other parasites (echinococcus etc)

9.9.1.4 Rikettsia

9.9.1.5 Spirochaetes – leptospira (Weil's disease); *Treponema pallidum* (Syphillis)

9.9.1.6 Mycobacteria - TB; leprosy

9.9.1.7 Bacillus anthracis – anthrax

9.9.1.8 Enterobacteria - Salmonella species

9.9.1.9 Brucella – gram negative coccobacilli

9.9.1.10 Viruses - HIV, viral hepatitis, spongiform encephalopathy (Creuzfeldt-Jacob disease and its variants) and haemorrhagic fevers (Congo-Crimea Fever); Ebola, EBV, CMV, HPV, HSVI and HSVII

9.9.1.11 Fungi – spores and hyphae

9.9.1.12 Protozoa - Amoeba

9.9.1.13 Parasites – intestinal worms; schistosoma ova

The intern / student must:

9.9.2 be able to define and explain the following terms:

9.9.2.1 Hazard groups 1, 2, 3, and 4 relating to classification of micro-organisms and the diseases they cause

9.9.2.2 With reasons / motivate, why histological demonstration of microorganisms may be difficult

9.9.2.3 List precautions used when handling and / or processing infected tissue

9.9.2.4 Outline the method for the detection of micro-organisms in tissue sections with the following staining procedures:

- Ziehl-Neelsen
- Wade-Fite
- Gram's stain
- PAS , Grocott-Gomori and Grimelius' method
- Giemsa
- Phloxine tartrazine stain
- Orcein Shikata
- Gram-Twort
- Kuper & May's method for tubercle bacilli
- Gridley's method
- Macchiavello's / Lendrums / Shikata's method
- Warthin-Starry and Levaditi's method
- H & E

9.9.3 For each of these stains listed in 9.9.2.4, *the intern / student must:*

9.9.3.1 be able to state the purpose of the method and the organism to be demonstrated

9.9.3.2 State the theory / principle of the method

9.9.3.3 List the reagents and their function

9.9.3.4 State recommended fixative and any effects on staining

9.9.3.5 Describe the staining method

9.9.3.6 Describe the expected staining result

9.9.3.7 Recognise errors and describe corrective procedures

9.9.3.8 State any precautions where applicable

9.9.3.9 List appropriate controls tissues for these stains

9.9.4 *be familiar with other methods to demonstrate micro-organisms and viruses such as:*

- HIV, viral hepatitis
- Spongiform encephalopathy (Creuzfeldt-Jacob disease)
- Haemorrhagic fevers (Congo-Crimea Fever); Ebola
- Mycobacteria – TB, HIV, EBV, CMV, HPV, HSVI and HSVII by means of ISH / SISH / IHC / PCR techniques as well as tinctorial methods of demonstration

C Evaluation

Evaluation will be in the form of a practical competence test, in which the intern / student must obtain tissue and satisfactorily demonstrate the presence of microorganisms in tissue sections.

a Obtain tissue infected with the following organisms:

- Bacteria
- Mycobacteria
- Fungi
- Viruses or Rickettsiae (for inclusion bodies)

(Laboratories usually have control blocks available)

b Cut sections from each block and perform the correct stain for each micro-organism.

c Stain one of the fungi infected sections with the aid of a microwave oven in parallel with conventional staining.

Note! Compulsory stains for the different organisms: Ziehl-Neelsen, Wade-Fite, Gram's stain, PAS, Methenamine silver, Giemsa, Phloxine tartrazine stain and Orcein Shikata.

Other recommended stains: H & E, Gram-Twort, Kuper & May's method for tubercle bacilli, Gridley's method and Macchiavello's method.

d View the slides microscopically and record your results. Comment on any differences between the conventionally stained section and that which has been done with the microwave oven.

9.10 Bone and Minerals

A. Outcome

On completion of this module the intern / student must:

a Describe the preparation of mineralised tissues prior to processing

b be able to describe in detail, the total preparation of mineralised tissues for sectioning

c be able to describe and critically evaluate the most appropriate methods for detection of minerals in tissues

d outline the limitations of the above processes and take corrective action

B Objectives

9.10.1 *The intern / student must be able to define / describe / explain:*

- 9.10.1.1 Cortical or compact bone
- 9.10.1.2 Trabecular, cancellous or spongy bone
- 9.10.1.3 The Haversian and non-Haversian system
- 9.10.1.4 Bone collagen
- 9.10.1.5 Bone mineral
- 9.10.1.6 Bone cells: osteoblasts, osteocytes and osteoclasts
- 9.10.1.7 Development of bone (intramembranous ossification and enchondral ossification)

9.10.2 *The intern / student must be able to:*

- 9.10.2.1 State the purpose [the why, and how] of decalcification
- 9.10.2.2 Describe the selection of tissue and fixation requirements for decalcification and / or the demonstration of bone and bone minerals
  - Biopsy specimens
  - Amputation specimens
  - Resection specimens
  - Teeth
- 9.10.2.3 Evaluate what a good decalcifying solution must ensure
- 9.10.2.4 Evaluate criteria for selecting a decalcifying reagent

9.10.2.5 Describe decalcifying agents

- acids : Strong inorganic [e.g. nitric / hydrochloric]; Weak, organic [e.g. Formic, acetic and picric]
- Chelating agents
- Proprietary / commercially available decalcifying fluids
- Combinations of acids in fixative solutions

9.10.2.6 Describe the factors that affect decalcification

- Temperature
- Agitation
- Suspension
- Block size

9.10.2.7 Describe the various methods used to determine the end-point of decalcification

- Physical
- Chemical
- Specimen radiography

9.10.2.8 State the principle of the tests listed in 10.2.7 and evaluate any limitation(s) to these methods

9.10.2.9 Evaluate / describe the treatment of bone specimens following [after] the decalcification process. Motivate the reasons for such treatment(s)

9.10.2.10 State the purpose of surface decalcification and describe how it is done

9.10.2.11 Describe the requirements for the microtomy of bone

- Choice of microtome
- Choice of blade / knife and profile
- Flattening and adhesion of sections [including mounting after staining]

- Celloidin and double embedded / MMA undecalcified specimens
- Frozen sections from trephines and Jamshidi needle biopsies

9.10.2.12 Describe the effect of decalcification on staining solutions

9.10.3 *Have a thorough knowledge of:*

9.10.3.1 Staining methods for decalcified bone sections for the demonstration of specific elements [PAS, a reticulin stain, Von Kossa, Schmorl's Picro-thionin and Goldner's stains]

9.10.4 For each of these stains listed above in 9.10.3.1, *the intern / student must be able to:*

9.10.4.1 State the purpose of the method

9.10.4.2 State the theory of the method

9.10.4.3 Evaluate the reagents and their function

9.10.4.4 State recommended fixative and any effects on staining

9.10.4.5 Describe the staining method

9.10.4.6 Describe the expected staining result

9.10.4.7 Recognise errors and describe corrective procedures

9.10.4.8 State any precautions where applicable

9.10.4.9 List appropriate controls tissues for these stains

9.10.4 *be familiar with:*

9.10.4.1 The use of different haematoxylins in staining of bone (Ehrlich's haematoxylin)

9.10.4.2 Alternative methods for embedding and sectioning of bone (undecalcified, grinding and resin embedding)

9.10.4.3 The preparation and demonstration of mineralised bone components

9.10.4.4 IHC techniques on methyl methacrylate sections

9.10.4.5 Techniques for the preparation of specimens requiring demonstration of elements such as Aluminium

9.10.4.6 The technical specifications / requirements, use, and appropriate handling of

- Bone saws
- grinding / polishing equipment

C Evaluation

Evaluation will be in the form of a practical competence test, in which the intern / student must obtain an unfixed bone specimen and satisfactorily decalcify and stain the aforementioned specimen.

a Obtain a bone specimen and compare the effects of four different compound decalcifying fluids on similar sized pieces (rib is recommended).

b Using one of the decalcifying fluids, perform microwave assisted decalcification on one of the pieces of bone.

c Check for completion of decalcification by:

i mechanical means

ii chemical means and compare the advantages and disadvantages.

d Process, embed and cut four sections from the each piece of bone and stain with an H & E stain to compare the quality of the sections obtained after the four different decalcifying fluids.

e Compare the quality of decalcification of the microwave assistance to that of conventional decalcification.

f Stain sections from the best quality block to demonstrate the morphology of the bone to its best advantage.

Note! Compulsory stains: PAS, a reticulin stain, Von Kossa.

Other Recommended stains: Schmorl's Picro-thionin and Goldner's stains.

g Microscopically review the stained sections and evaluate your results commenting on the tissue morphology and results of the staining procedures - 3000+ words**.**

h Critically evaluate the challenges of receiving a bone marrow trephine biopsy in Helly's Fluid on a Tuesday afternoon at 14h30. The biopsy has been in the fixative for one (1) hour. Explain how you will prepare this specimen for Histopathological diagnosis (include all procedures from receipt up to embedding of the tissue only).

9.11 Neuropathology

A. Outcome

On completion of this module the intern must:

a be able to describe the geographic divisions of the central and peripheral nervous systems [CNS and PNS] and the major cellular structures of each

b be able to critically evaluate the most appropriate methods for receipt, handling and preparation of specimen from the CNS and PNS

c be able to describe and critically evaluate the most appropriate methods for demonstration of structures and cellular components within the CNS and PNS

d outline the limitations of the above processes and take corrective action

B Objectives

9.11.1 *The intern / student must be able to define / describe / explain:*

9.11.1.1 The main geographic divisions of the central and peripheral nervous systems

CNS: Brain [including cerebellum]
Brain stem
Spinal cord
PNS: Nerves

9.11.1.2 The major cellular structures found in the nervous system

- Neurons [nerve cells]: cell body, axon, dendrites

  Motor
  Sensory
  Interneurons

- Oligodendrocytes (CNS) or Schwann cells (PNS)(myelin production)
- Astrocytes [supporting cells]
- Ependymal cells [cells lining ventricles and spinal canal]
- Microglia [monocyte-macrophage-type cells]

9.11.1.3 Nissl substance / granules

9.11.1.4 chromatolysis

9.11.1.5 neurofibril complexes [including cytoskeletal components]

9.11.1.6 neurofribillary tangles

9.11.1.7 Gliosis

9.11.1.8 Classify dementias [including motor neuron disease]

- Alzheimers disease
- Vascular dementia
- Dementia with Lewy bodies
- Frontotemporal dementias

9.11.1.9 Classify neurodegenerative disease

- Inclusion bodies
- transmissible
- Prion disease detection and its variants (Ref: Module 2, 11, 12,13, 14)

9.11.2 *Have a thorough knowledge of:*

9.11.2.1 The morphology of the structures listed in 9.11.1.2 when demonstrated by means of H&E as well as appropriate special stains and other investigations

9.11.2.2 Staining techniques for Nissl substance (Cresyl fast violet)

9.11.2.3 Staining techniques for axons and neuronal processes (Bielschowsky's silver stain and Glees & Marsland's stain)

9.11.2.4 Techniques for staining axons in peripheral nervous system (Palmgren's method)

9.11.2.5 Staining techniques for myelin (Kluver & Barrera and Swank-Davenport methods)

9.11.2.6 Stains for neuroglia [support cells]

- atrocytes (PTAH, Cajal's and Hortega's methods; IHC: GFAP and markers)
- ependymal cells [H&E, PTAH and IHC: vimentin / GFAP / S100protein and ISH for polysomy of Chromosome 7 {ependymal tumour variants}]
- oligodendrocytes [IHC; FISH for 1p/19q deletion; Penfield's combined method: 1928; Weil and Davenport

- microglia [Weil and Davenport; Penfield's combined method: 1928]

9.11.2.7 Staining techniques for *degenerate* nerve fibres and *degenerate* myelin [Eager's method for nerve fibres and Marchi's for degenerate myelin]

9.11.2.8 Identify and demonstrate pigments within neurons by the most appropriate method [lipofuscin, neuromelanin]

9.11.2.9 Techniques for the vital staining of nerve fibres and endings [methylene blue]

9.1.2.10 the appropriate applicable techniques for the histological investigation of dementias as listed in 9.11.1.8 [Thioflavine S; Gallay's; Modified Bielschowsky]; appropriate IHC antibodies

9.1.2.11 the appropriate applicable techniques for the histological investigation of neurodegenerative diseases as classified in 9.11.1.9 [primarily with IHC markers and methods to detect protein prions]

9.11.3 *The intern / student must*; for each of these stains [Kluver & Barrera, Swank-Davenport, Palmgren's, PTAH, Cajal's and Bielschowsky's methods, Eager's , Marchi's method and Methylene blue] ; *be able to:*

9.11.3.1 State the purpose of the method

9.11.3.2 State the theory of the method

9.11.3.3 Evaluate the reagents and their function

9.11.3.4 State recommended fixative and any effects on staining.

9.11.3.5 Describe the staining method

9.11.3.6 Describe the expected staining result

9.11.3.7 Recognise errors and describe corrective procedures

9.11.3.8 State any precautions where applicable

9.11.3.9 List appropriate controls tissues for these stains

9.11.4 *Have a thorough knowledge of:*

9.11.4.1 The handling, preparation, orientation and fixation of neurological specimens

- Tumour samples from neurosurgery

- Brain biopsies from neurosurgery
- Whole brain from autopsy
- Spinal cord from autopsy
- Peripheral nerve biopsy
- Pituitary gland from neurosurgery
- Skeletal muscle biopsies
- Smear preparations for rapid diagnosis

9.11.4.2 Prevention of artefacts

9.11.5 *The intern / student must be able to critically evaluate the applications for and limitations of the procedures for the successful enucleation, grossing of ocular specimens; fixation, processing, impregnation, embedding and sectioning of these specimens as well as troubleshoot problems and causes of poor results with resultant corrective action:* [Ref 9.1 *''Evaluation'': a xii*]

9.11.5.1 Whole eyes [anterior and posterior]

9.11.5.2 Biopsies

9.11.5.3 Retinal prostheses

9.11.6 The intern / student must be conversant with:

9.11.6.1 Fixation

- Submersion technique [Davidson's solution, Bouins solution and Zenkers solution]
- Injection or window technique [2-6% gluteraldehyde / post fixation in 10% NBF; paraformaldehyde for TEM and 10% NBF]

9.11.6.2 Inking of ocular specimens

9.11.6.3 Appropriate and suitable processing schedules and reagents [automated and manual methods]

9.11.6.4 Selection of appropriate clearing / ante-media

9.11.6.5 Embedding techniques and correct orientation of specimens

9.11.7 *The intern / student must be able to identify:*

9.11.7.1 Cornea

- Stromal clefts
- Vacuolation of endothelial cells

9.11.7.2 Lens

- Shattering
- Artefactual vacuoles

9.11.7.3 Retina

- Artefactual detachment
- Minimal artefactual spaces
- Correct orientation, clearly defined rods and cones

9.11.8 *The intern / student must* for each of these tinctorial, histochemical and immunochemistry antibody stains listed below, *be able to:*

- Cresyl Violet
- Biebrich Scarlet- Acid Fuchsin
- Aniline Blue
- Fast Blue
- Periodic Acid Schiffs
- Glial Fibrillary Acid Protein (GFAP)
- Neuro- filament
- Glutamine Synthetase

9.11.8.1 State the theory / principle of the method

9.11.8.2 List the reagents used and their function

9.11.8.3 State the recommended fixative and any effects on staining

9.11.8.4 Describe the staining method

9.11.8.5 Describe the expected results and the structures demonstrated

9.11.8.6 Recognise errors and describe corrective procedures

9.11.8.7 State precautions where applicable

9.11.8.8 List appropriate control tissues

C Evaluation

Evaluation will be in the form of a practical competence test, in which the intern must satisfactorily demonstrate neurological elements.

a Fix, process and embed a specimen from the central nervous system.

b Cut sections and stain to demonstrate myelin, neurones and neuroglia.

Recommended stains:

- Kluver & Barrera
- Swank-Davenport
- Palmgren's
- PTAH
- Cajal's
- Bielschowsky's methods

c Microscopically review the stained sections and evaluate your results commenting on the specific cells structures, morphology and results of the staining procedures - 3000+ words.

9.12 Enzyme Histochemistry

A. Outcome

On completion of this module the intern / student must:

a Understand the function and role of enzymes

b Discuss the difficulties of retaining enzyme activity in sections

c Describe examples of methods for demonstrating hydrolase, dehydrogenase and oxidase enzymes

d Outline the problems involved in quantifying enzyme activity in sections and take corrective action

B Objectives

Discuss the difficulties of retaining enzyme activity in sections

9.12.1 *The intern / student must be able to define / describe / explain:*

9.12.1.1 The classification of enzymes and their role / function in the body

- Hydrolytic enzymes [Alkaline-, acid- and specific phosphatase]
- Oxidative enzymes [Oxidases, Peroxidases, Dehydrogenases, Diaphorases]
- Transferases
- Hydrolases [esterases / lipases / phosphatises / glycosidases / peptidases / pyrophosphatases]
- Lyases

9.12.1.2 The process of hydrolysis and cite examples of this reaction

9.12.1.3 The role of co-enzymes and cite examples

9.12.1.4 The effect of and / or limitations of fixation of tissue / smears and imprints on organelles prior to enzyme detection.

9.12.1.5 The appropriate methods of storing fresh unfixed and fresh fixed tissue / smears and imprints on organelles prior to enzyme detection

9.12.1.6 The appropriate choice of fixative solution for the specimens mentioned in 9.12.1.4 and 9.12.1.5

9.12.1.7 The types of histochemical reactions with examples of applications

- simultaneous capture [coupling, conversion and chelation]
- post-incubation coupling [conversion and chelation]
- self-coloured substrate [solubility change]
- intra-molecular rearrangement
- diazonium salts

9.12.2 *Have a thorough knowledge of:*

9.12.2.1 The techniques for the demonstration of Phosphatases:

- Alkaline phosphatase: metal precipitation; Azo dye, Naphthols, (Gomori calcium, azo dye coupling and naphthol AS-B1 methods)
- Acid phosphatase: (Gomori lead, azo dye coupling and naphthol AS-B1 methods)
- Adenosine tri-phosphatase (ATP-ase method)

9.12.3 *be familiar with:*

9.12.3.1 The techniques for the demonstration of Oxidative Enzymes

- Tyrosinase-DOPA reaction
- Monamine oxidase: tetrazolium method
- Cytochrome oxidase
- Tetrazoliun salt methods [e.g. NBT]

9.12.3.2 The diagnostic applications of histochemical techniques for:

- Skeletal muscle biopsies
- Ganglia and nerves in suspected cases of Hirschsprung's disease
- Lactase / sucrose deficiencies in jejunal biopsies
- Demonstration of various white blood cells
- Demonstration of mast cells [alternative methods by IHC]
- miscellaneous

9.12.4 *For each of these stains listed below the intern / student must be able to:*

- Gomori calcium; azo dye coupling and naphthol AS-B1 methods
- Gomori lead; ATP-ase method
- Cytochrome oxidase method
- Tetrazoliun salt method
- Chloroacetate esterase (pararosanilin method)
- Cholinesterase (Karnovsky and Roots)

9.12.4.1 State the purpose of the method

9.12.4.2 State the theory of the method

9.12.4.3 Evaluate the reagents and their function

9.12.4.4 State recommended fixative and any effects on staining

9.12.4.5 Describe the staining method

9.12.4.6 Describe the expected staining result

9.12.4.7 Recognise errors and describe corrective procedures

9.12.4.8 State any precautions where applicable

9.12.4.9 List appropriate control tissue and conditions for these techniques

- Positive controls
- Negative controls
- Inhibitors [heat, specific- and competitive - inhibitors]

C Evaluation

Evaluation will be in the form of a practical competence test, in which the intern / student must satisfactorily demonstrate enzyme activity in tissue.

a Obtain unfixed tissue specimens (kidney is recommended) and cut cryostat sections.

b Demonstrate alkaline and acid phosphatase in these sections.

c Microscopically review the stained sections and evaluate your results commenting on the staining pattern and localisation of positivity.
Record your results. 2000+ words

9.13 Muscle and Rectal biopsies

B Objectives

9.13.1 *The intern / student must be able to define / describe / explain:*

- 9.13.1.1 Open muscle biopsies
- 9.13.1.2 Needle muscle biopsies
- 9.13.1.3 Fibre type grouping
- 9.13.1.4 Hirschsprung's disease
  - Aetiology / cause / origin / symptoms
  - Importance of full thickness bowel biopsies

9.13.2 *be familiar with* H + E for morphology; Myosin ATPase; SDH; αGPD for all below **

- 9.13.2.1 Handling and preparation of muscle biopsies. NB here on snap freezing with isopentane or Tween 22 for muscle histochemistry
- 9.13.2.2 Detection of ATP-ase in muscle biopsies (metal precipitation method at high and low pH) **
- 9.13.2.3 Detection of Cytochrome oxidase in muscle biopsies (Seligman's method)
- 9.13.2.4 Detection of NADH in muscle biopsies**
- 9.13.2.5 Detection of cholinesterase in rectal biopsies (Karnovsky & Root's method)
- 9.13.2.6 All histochemistry methods to detect and identify muscle fibre types for nerve atrophy **
  - 9.13.2.6.1 Fast and slow twitch
  - 9.13.2.6.2 Oxidative and non-oxidative
  - 9.13.2.6.3 Glycolytic and non-glycolytic

*NOTE: Often ganglia and nerves are detected by serials sections stained by IHC when Hirschprung's disease is suspected. See D below.

C Evaluation

Evaluation will be in the form of a practical competence test, in which the intern / student must satisfactorily demonstrate enzyme activity in muscle and rectal biopsies.

a Obtain a fresh skeletal muscle specimen and cut cryostat sections.

b Demonstrate fibre type grouping with two different methods.
Recommended enzyme methods: Metal precipitation at high and low pH and Seligman's method.

c Obtain colon or rectal tissue and cut cryostat sections.

d Demonstrate ganglion cells in the specimen. *See *Note* above
Recommended method: Karnovsky & Root's method or if available, appropriate immunochemistry methods such as S100 protein.

e Microscopically review the stained sections and evaluate your results commenting on the tissue morphology, artefacts, staining patterns and results of the staining procedures.

D Recommended Textbooks and Resources for Module 9

- Theory and Practice of Histological Techniques, John D Bancroft and Marilyn Gamble. 5th or 6th Edition.
- Histological and Histochemical Methods. Kieran. J.A. (1990). Oxford: Pergamon.
- Lipids. Bayliss-High, O.B and Lake, B. (1996).
- Manual of Histological Demonstration Techniques. Cook, H.C. (1974). London: Butterworths.

MODULE 10

Histology of Tissues

A Outcomes

On completion of this module the intern / student must be able to:

a identify the four basic tissue types

b identify the basic structure of each organ system

c identify the structures specific to each organ or system

d Microscopically identify all normal cell and tissue structures by the most appropriate tinctorial stain, including immunochemistry and or in-situ hybridisation as well as structures necessary for diagnosis. E.g micro-organisms immune complexes in renal disease, amyloid, muscle abnormalities and neuronal disorders / inclusions [Ref Modules 9, 11 and 12]

e identify all normal ultrastructural (TEM) cell and tissue structures as well as ultrastructural entities necessary for diagnoses. E.g. immune complexes in renal disease; amyloid; glycogen; demyelination of nerves; muscle abnormalities; abnormal mitochondria; filaments; micro-organisms; etc. [Ref Module 13]

B Objectives

10.1 *The intern / student must be able to identify and describe the following structures within the circulatory system:*

- heart ventricle (epicardium, myocardium, endocardium, cardiac muscle cells, capillaries and Purkinje fibres)
- aorta (elastic lamina, tunica media and tunica adventitia)
- large muscular arteries (tunica intima, tunica media, tunica adventitia and elastic lamina)

10.2 *The intern / student must be able to identify and describe the following structures within the integument.*

10.2.1 Skin:

- thin skin (epidermis, dermis and hypodermis)
- the layers of the epidermis and the cells found in each layer

- dermal structures (hair follicle, sebaceous gland, merocrine sweat glands, small artery and veins)
- thick skin

10.2.2 Structure of muscle:

- smooth muscle
- striated muscle
- cardiac muscle
- muscle cells / fibres / sarcomere / banding
- muscle filaments – thick and thin
- muscle proteins

10.2.3 Bone

- compact cortical bone
- trabecular, spongy, cancellous bone
- osteoblasts, osteoclasts and osteocytes
- Organic matrix of bone – collagen type 1; glycosaminoglycans; osteocalcin; osteonectin; bone sialo protein; osteopontin
- Woven bone – haphazard collagen and lamellar bone consisting of collagenous support tissue embedded in glycosaminoglycans
- Inorganic matrix of bone – bone minerals

10.3 *The intern / student must be able to identify and describe the following structures within the immune system:*

- thymus (capsule, cortex, medulla and Hassall's corpuscle)
- lymph node (trabeculae, cortex, paracortex, medulla and sinuses)
- lymphoid follicle with germinal centre (predominant cells). Primary and secondary follicles
- high endothelial venule in the paracortex (medullary cords and sinuses, predominant cells)
- palatine tonsil (luminal surface)
- the capsule of the tonsil (type of tissue)
- lymphoid follicle in the tonsil (differentiate between these cells and cells in a lymph node)
- spleen (capsule, red and white pulp)
- T cell and B cell areas in both white pulp
- venous sinuses and parenchyma

10.4 *The intern / student must be able to identify and describe the following structures within the respiratory system:*

- trachea and primary bronchus (mucosa, submucosa, cartilage rings and adventitia)
- respiratory epithelium
- goblet cells
- glands in the submucosa
- lung and visceral pleura
- a bronchiole (differentiate from bronchus)
- alveoli and alveolar cells
- alveolar wall
- pleura (mesothelial cells lining pleura)

10.5 *The intern / student must be able to identify and describe the following structures within the digestive system:*

- parotid gland (secretory acini and excretory ducts)
- sublingual gland
- oesophagus (epithelium, lamina propria, muscularis mucosae, submucosa, muscularis propria, and serosa)
- stomach (epithelium, lamina propria, muscularis mucosae, submucosa, muscularis propria, and serosa as well as gastric pits and underlying glands)
- duodenum (epithelium, lamina propria, muscularis mucosae, submucosa, muscularis propria, and serosa plus crypts of Lieberkuhn and Brunner's glands)
- ileum (epithelium, lamina propria, muscularis mucosae, submucosa, muscularis propria, and serosa plus mucosal lymphoid tissue [MALT] and Peyer's patches)
- colon (epithelium, lamina propria, muscularis mucosae, submucosa, muscularis propria, and serosa)
- appendix (epithelium, lamina propria, muscularis mucosae, submucosa, muscularis propria, and serosa
- liver (portal tracts, central vein, hepatocytes and sinusoids, space of Disse and Kupffer cells)
- gall bladder (epithelium, lamina propria, muscularis mucosae, submucosa, muscularis propria, and serosa)
- pancreas (secretory acini, excretory ducts, centroacinar cells and Islets of Langerhans)

10.6 *The intern / student must be able to identify and describe the following structures within the urinary system:*

- kidney (capsule, cortex, medulla, glomeruli, proximal tubules, distal convoluted tubules, collecting ducts, thick and thin loops of Henle and the juxtaglomerular apparatus)
- ureter (epithelium, lamina propria, muscularis mucosae, submucosa, muscularis propria, and serosa)
- bladder (epithelium, lamina propria, muscular layers, and advetitia)

10.7 *The intern / student must be able to identify and describe the following structures within the endocrine system:*

- pituitary (anterior, intermediate and posterior)
- thyroid (thyroid follicles and parafollicular cells)
- parathyroid (chief and oxyphil cells)
- adrenal (capsule, cortex, medulla)

10.8 *The intern / student must be able to identify and describe the following structures within the male reproductive system:*

- testis (epididymus, tunica albuginea, seminiferous tubules and Leydig cells)
- ductus deferens (epithelium, lamina propria, muscular layers, and adventitia)
- prostate (capsule, prostatic glands and periurethral glands)

10.9 *The intern / student must be able to identify and describe the following structures within the female reproductive system:*

- ovary (ovarian cortex, covering epithelium, primordial follicles, primary follicles, secondary follicles, Graafian follicles, corpus luteum and corpus albicans)
- oviduct / Fallopian tube (epithelium, lamina propria, muscular layers, and adventitia)
- uterus in proliferative phase (surface epithelium, basal layer, stratum spongiosum, stratum compactum and glands of the endometrium and the myometrium)
- uterus in secretory phase (surface epithelium, basal layer, stratum spongiosum, stratum compactum and glands of the endometrium and the myometrium)
- menopausal uterus (surface epithelium, basal layer, stratum spongiosum, stratum compactum and glands of the endometrium and the myometrium)

- uterine cervix (epithelial lining, endocervical canal, ectocervix, ecto-endocervical junction, endocervical glands and cervical stroma)
- breast (breast lobule, acini, myoepithelial cells, terminal ducts, lactiferous ducts and interlobular breast stroma)

10.10 *The intern / student must be able to identify and describe the following structures within the central nervous system:*

- spinal cord (white matter, grey matter, ventral horns, large motor neurons, central canal and ependymal cells)
- cerebellum (grey matter, white matter and meninges)
- cerebral cortex (pyramidal cells)

10.11 *The intern / student must be able to identify and describe the following structures within the peripheral nervous system:*

- Somatic nerves – sensory; motor; cranial; spinal – nerve trunk structure
- Autonomic – sympathetic; parasympathetic; enteric
- Connective tissue/myelin (Schwann cells/axons)
- PNS – CNS transitional zone with astrocyte involvement

10.12 *The intern / student must be able to identify and describe the following structures within the eye (ocular):*

- Cornea; conjunctiva; sclera; vitreous; lens; iris; ciliary muscle / epithelium; pupil; retina; optic nerve

C Evaluation

Evaluation will be in the form of a practical competency test, in which the intern / student must satisfactorily demonstrate a thorough knowledge of the appearance and function of specific tissue elements.

- Tissue for this module must be obtained from suitable sources. To assemble a complete set, tissue collected for Module 9 may be used for this purpose.
- Process, cut and stain H & E sections of each type of tissue.
- Other special stains or appropriate and relevant techniques contained in this syllabus, together with those from the previous module may also be used to aid recognition of tissues and organ systems.

a. Circulatory system

i Identify epicardium, myocardium, endocardium, cardiac muscle cells, capilliaries and Purkinje cells.

ii Identify elastic lamina, tunica media and tunica adventitia.

iii Identify large muscular arteries and corresponding veins, the tunica intima, tunica adventitia and elastic lamina.

b. Skin

i Thin skin: Identify the epidermis, dermis, hypodermis, the layers of the epidermis and the cells that are found in each layer. Identify the following dermal structures: hair follicle, sebaceous gland, merocrine sweat gland and a small artery and vein in the dermis and compare them to the large artery and vein in a.iii

ii Thick skin: Compare the epidermis and dermis with that of the thin skin.

c. Immune system

i Thymus: Identify the capsule, cortex and medulla. Which cells dominate each zone? Identify a Hassall's corpuscle.

ii Lymph node: Identify the capsule and trabeculae, cortex, paracortex, medulla and sinuses (subcapsular, trabecular/cortical and medullary). In the cortex identify a lymphoid follicle with a germinal centre. Which cells are dominant in the cortex? Identify a high endothelial venule in the paracortex. Which cells dominate the paracortex? Identify the medullary cords and sinuses. Which cells are found in the medulla?

iii Palatine tonsil: Identify the covering epithelium (luminal surface). How is this epithelium different to that seen in thin skin

i.e.? Identify the capsule of the tonsil. What type of tissue does the capsule consist of? Identify a lymphoid follicle with a germinal centre. Do these follicles differ from those seen in the cortex of the lymph node?

iv Spleen: Identify the capsule, red and white pulp. In the white pulp identify the T cell and the B cell areas. In the red pulp identify the venous sinuses and the parenchyma.

d. Respiratory system

i Trachea or primary bronchus: Identify the mucosa, submucosa, cartilage rings and adventitia. Classify the luminal epithelium called respiratory epithelium. Give the function of goblet cells. Identify and classify the glands in the submucosa.

ii Lung and visceral pleura: Identify a bronchiole. How does a bronchiole differ from a larger bronchus? Identify the alveoli. Which cells line the alveoli? What are dust cells? What does the alveolar wall consist of? Identify the pleura. What does the pleura consist of? Which cells line the surface of the pleura?

e. Digestive system

i Parotid gland: Identify the secretory acini and excretory ducts. Distinguish between the secretory acini and ducts.

ii Sublingual gland: Identify the secretory acini and excretory ducts. Compare the secretory acini in the sublingual gland to those of the parotid gland in ‘*’e.i”*

iii Oesophagus (lower 1/3): Identify and classify / describe the epithelium, the lamina propria, muscularis mucosae, submucosa, muscularis propria and adventitia (serosa).

iv Stomach (body): Identify and classify / describe the epithelium, the lamina propria, muscularis mucosae, submucosa, muscularis propria and adventitia (serosa). Identify the gastric pits and underlying glands. Identify the cells found in the gastric glands.

v Duodenum: Identify and classify / describe the epithelium, the lamina propria, muscularis mucosae, submucosa, muscularis propria and adventitia (serosa). Identify the villi and crypts of Lieberkuhn, Brunner’s glands. Compare the covering epithelium to that of the stomach. Are goblet cells present in the stomach?

vi Ileum: and classify / describe the epithelium, the lamina propria, muscularis mucosae, submucosa, muscularis propria and adventitia (serosa). Identify the villi and crypts of Lieberkuhn and mucosal lymphoid aggregations (MALT). What are Peyer’s patches?

vii Colon: Identify and classify/describe the epithelium, the lamina propria, muscularis mucosae, submucosa, muscularis propria

and adventitia (serosa). Are there villi and crypts of Lieberkuhn in the colon? What are the taeniae coli?

viii Appendix (cross section): Identify and classify / describe the epithelium, the lamina propria, muscularis mucosae, submucosa, muscularis propria and adventitia (serosa).

ix Liver: Identify a liver lobule, portal tracts, central vein (terminal hepatic venule), plates of hepatocytes and sinuses. Where is the space of Disse found? What are Kuppfer cells? Identify an artery, vein and bile ductule in the portal tract.

x Gall bladder: Identify and classify / describe the epithelium, the lamina propria, muscular layer and adventitia (serosa).

xi Pancreas: Identify the secretory acini and excretory ducts. Compare the secretory acini and ducts to those seen in the parotid gland. What is a centroacinar cell? Identify the Islets of Langerhans. Which cells are found in the islet? What is the function of these cells?

f. Urinary system

i Kidney: Identify the capsule, cortex and medulla. Identify the glomeruli, proximal and distal convoluted tubules, collecting ducts, thick and thin loops of Henle and the juxtaglomerular apparatus.

ii Ureter (cross section): Identify the epithelium, lamina propria, muscle layers and adventitia.

iii Bladder: Identify the epithelium, lamina propria, muscle layers and adventitia. Compare the epithelium with that seen in the ureter.

g. Endocrine system

i Hypothalamus: anterior, median and posterior nuclei

ii Pituitary: Identify the anterior, intermediate and posterior pituitary. Name the cells found in each part of the pituitary.

ii Thyroid: Identify a thyroid follicle. What cell type lines the follicle? What are parafollicular cells?

iii Parathyroid gland: Identify the chief and oxyphil cells.

iv Adrenal: Identify the capsule, cortex and medulla. Identify the three layers of the cortex. Which hormones are secreted in each layer? Identify the medulla. Which important hormones are secreted in the medulla?

v Pancreas: see GIT

vi Testes and ovaries: See male and female reproductive systems.

h. Male reproductive system

i Testis: Identify the epididymus, tunica albuginea, and seminiferous tubules. In the seminiferous tubules identify the sertoli cells, spermatogonia, spermatocytes and spermatids. Identify Leydig cells.

ii Ductus deferens: Identify the epithelium, lamina propria, muscular layers and adventitia.

iii Prostate: Identify the capsule, the main prostatic glands and the periurethral glands. What are corpora amylacea?

i. Female reproductive system

i Ovary: Identify and describe the ovarian cortex, covering epithelium, primordial follicles, primary, secondary and Graafian follicles, a corpus luteum and a corpus albicans.

ii Oviduct (Fallopian tube): Identify and describe the epithelium, lamina propria, muscular layer and serosa.

iii Uterus (body) in proliferative phase: Identify and describe the surface epithelium, basal layer (stratum basalis), functional layer (stratum spongiosum and compactum), endometrial glands of the endometrium, and the myometrium.

iv Uterus (body) in secretory phase: Identify and describe the surface epithelium, basal layer (stratum basalis), functional layer (stratum spongiosum and compactum), endometrial glands, spiral arteries of the endometrium, and the myometrium.

v Menopausal uterus (body): Identify and describe the surface epithelium, basal layer (stratum basalis), functional layer (stratum spongiosum and compactum), endometrial glands of the endometrium, and the myometrium. Compare the endometrial glands in the proliferative phase, and the secretory phase to those seen in this section.

vi Uterine cervix: Identify and describe the epithelium lining the endocervical canal, the ectocervix, ecto-endocervical junction, endocervical glands and cervical stroma.

vii Breast: Identify and describe a breast lobule, acini, myoepithelial cells, terminal ducts, lactiferous ducts and interlobular breast stroma.

j. Central nervous system

i Spinal cord (transverse section): Identify and describe the white matter and grey matter. In the ventral horns identify the large motor neurons. Identify the central canal and the ependymal cells.

ii Cerebellum: Identify the grey matter, the white matter and the meninges. Identify the 3 layers of the grey matter and describe the cells that are present in each layer.

iii Cerebral cortex: Identify a pyramidal cell (layers 3 or 5). Name the other cell types found in the cerebral cortex.

D Recommended Textbooks and Resources for Module 10

- Functional Histology: A Text and Colour Atlas. Wheater, PR. (2000).
- An Atlas of Human Histology. Di Fiore, MS. (1967).
- Histology – An Identification Manual. Tallitsch, R and Guastaferri, R. (2009).

All Histology atlases will be useful for this section, including the adjuvant use of EM / ICC / ISH techniques and atlases.

E Other

Websites

There are numerous websites which may be accessed. Google is an excellent search engine for all the microscopical structures required for this Module.

MODULE 11

Advanced Staining: Immunocytochemistry (ICC) / Immunohistochemistry (IHC) / Microarray (TMA)

A Outcomes

On completion of this module the intern / student must be able to:

a describe how antibodies can be used as histological reagents

b outline the principle of the direct and indirect immunofluorescent techniques

c explain how optimal antibody dilutions are established with reference to checkerboard matrix / signal to background noise issues

d describe effects of fixation and processing on immunofluorescent and immunoperoxidase methods.

e describe the principles and applications of ICC / IHC and the advantages of these techniques

f critically evaluate the most appropriate methods for receipt, handling and preparation of specimens for these techniques

g describe and critically evaluate the most appropriate methods for demonstration of structures and cellular components

h Microscopically identify all normal cell and tissue structures by the most appropriate tinctorial stain, including immunochemistry and / or in-situ hybridisation as well as structures necessary for diagnosis. E.g. micro-organisms' immune complexes in renal disease, amyloid, muscle abnormalities and neuronal disorders / inclusions [Ref Modules 9, 10]

i Microscopically recognise / identify technically induced aberrant staining / artefact(s)

i outline the limitations of the above processes and take corrective action

B Objectives

11.1 *The intern / student must be able to define / describe / explain, and where applicable, outline the uses / benefits and / or limitations of:*

11.1.1 Immunocytochemistry / immunohistochemistry

11.1.2 Immunoglobulins

11.1.3 Antigens

11.1.4 Antibodies

- Polyclonal antibodies
- Monoclonal antibodies

11.1.5 antigen-antibody binding

- Avidity
- Affinity
- Specificity
- Sensitivity

11.1.6 The production of primary reagents

- Polyclonal antibodies
- Monoclonal antibodies
- Lectins
- Secondary antibodies [link antibodies]
- Polymers

11.1.7 Labels

- Enzyme labels
  - Horseradish peroxidise [HRP]
  - Alkaline Phosphatase [AP]
  - {Miscellaneous others such as Glucose oxidase and Bacterial-derived β-D- galactosidase}
- Colloidal metal labels
  - Gold
  - Silver
- Fluorescent labels
  - Fluorescein (FITC)
  - Rhodamine
  - Phycoerythrin
  - AMCA
- *Other* labels
  - Radioisotopes
  - Hapten sandwich
  - Biotin

11.1.8 The term ‘’*conjugation*’’

11.1.9 Optimisation Vs validation

11.1.10 Avidin

11.1.11 Biotin

11.1.12 Dextran polymers

11.1.13 Chromogen substrates [choice / application / preparation / visible reaction product / limitations]

- 3,3'-Diaminobenzidine tetrahydrochloride (DAB)
- 3-amino-9-ethylcarbozole (AEC)
- 4-chloro-1-napthol (CN)
- Hanker-Yates reagent
- Napthol AS-MX Phosphate
- New Fuchsin
- Fast red
- BCIP
- NBT

11.2 *The intern / student must have a thorough knowledge of antigen retrieval techniques, the impact of and artefacts caused to tissue structure(s) and antigens, the reagents used and explain why antigen retrieval may be require**:***

11.2.1 Enzyme retrieval

- Pepsin
- Trypsin
- Protease
- Pronase
- Proteinase K

11.2.2 Heat retrieval (with solutions pH 6, 8 and 9)

- Microwave
- Pressure cookers [manual and electronic]
- Steamers / water-baths / autoclaves etc
- Semi-automated platforms and automated platforms performing this procedure

11.3 *The intern / student must have a thorough knowledge of the principles of the following techniques and evaluate when each may be used, including causes and corrections of problems in the techniques:*

11.3.1 Direct techniques

- Traditional direct
- New direct techniques

11.3.2 Indirect techniques

- Dextran labelled polymers
- Unlabelled antibody enzyme-complex technique [PAP and APAAP]
- Avidin-biotin
- Immunogold silver staining technique

11.3.3 Double staining techniques

- Sequential double staining
- Parallel double staining
- Antibody cocktails
- Pitfalls in designing protocols for multiple stains on a single slide (advantages and disadvantages)

11.4 *The intern / student must have a thorough knowledge of the collection of; principles applied, reagents used and effect of fixation for immunochemistry techniques:*

11.4.1 Wax sections

11.4.2 Frozen sections

11.4.3 Smears / imprints [blood smears, centrifuged cells, buffy coats, etc.]

11.4.4 Cell - blocks prepared from cytology specimens

11.4.5 Previously stained; decolourised sections, smears etc

11.5 *The intern / student must be able to describe causes of undesirable positive and / or spurious background staining and the techniques and reagents required to limit these:*

11.5.1 Endogenous enzyme activity

11.5.2 Highly charged connective tissue

11.5.3 Naturally occurring IgG

11.5.4 Charged avidin

11.5.5 Endogenous biotin

11.5.6 Antigen diffusion

11.5.7 Pigments

11.5.8 Auto-fluorescence

11.6 *The intern / student must be able to describe and where appropriate, microscopically recognise procedural errors encountered with:*

11.6.1 Deparaffinisation

11.6.2 Tissue drying (wetting agents)

11.6.3 Retrievals methods

11.6.4 Temperature

11.6.5 Dilutions

11.6.6 Washing steps and buffers

11.6.7 Incubations (Slide drying, reagent incubations)

11.6.8 Antibody concentrations

11.6.9 Reagent contamination

11.6.10 Chromogen incompatibility

11.7 *The intern / student must be able to describe / explain and critically evaluate the importance of antibody quality:*

11.7.1 Concentration of antibody

11.7.2 Type of immunoglobulin (Ig) and / or subclass used [structure]

11.7.3 Stability of structure and chemical complexity

11.7.4 Preparation: hybridoma supernatant / ascites / serum / culture supernatant

11.7.8 Molecular weight

11.7.9 Immunogen used

11.8 *The intern / student must be able to describe and explain the importance of control mechanisms and procedures in ICC / IHC:*

11.8.1 Positive controls

11.8.2 Negative controls

11.8.3 Absorption controls

11.8.4 Iso-typic controls

11.8.6 Multi-tissue control blocks

11.8.6.1 Sausage blocks

11.8.6.2 Tissue-micro array (TMA): construction techniques, equipment used {manual /semi-automated and fully automated arrayers}; advantages and disadvantages of the technique(s) and appropriate application of the type of TMA used

- Prevalence TMA
- Progression TMA
- Prognostic TMA
- Experimental TMA

11.8.7 Antibody batches / Lot numbers

11.8.8 Intra- and inter- laboratory control

11.8.9 External Quality Assurance programmes

11.9 *The intern / student must be conversant with the following:*

11.9.1 The appropriate manner in which to store antibodies

- Liquid format
- Lyophilised format
- Reconstituted aliquots

11.9.2 the most appropriate methods and techniques for drying and storing blocks and slides to preserve antigenicity and localisation of antigens

- Wax
- Frozen
- Smears
- Decolourising previously stained sections, smears etc

11.10 *The intern / student must be able to recognise antibody staining patterns / localisation according to the specific antibody / cell structure / tumour type / infectious agent:*

11.10.1 Cytoplasmic

11.10.2 Membrane

11.10.3 Nuclear

11.11 *The intern / student must have thorough knowledge of the following:*

11.11.1 Statutory markings on reagents: IVD, CE, FDA markings, etc.

11.11.2 Companion diagnostics / Theranostics / Targeted therapy and associated testing algorithms as per International guidelines [Breast / GIT / Myelomas etc.]

11.11.3 Class I-IV of the different types of IHC antibodies / reagents

11.11.4 Antibody panels / testing algorithms used for diagnosis

11.11.5 Cluster of Differentiation nomenclature

11.11.6 Sample exclusion criteria [Theranostics / Targeted therapy]

11.12 *The intern / student must be aware of:*

11.12.1 Enhancement of standard methods

- Build–up methods

11.12.2 Intensification of the DAB / H_2O_2 product

11.12.3 Tyramide signal amplification (TSA)

11.12.4 Signal amplification (CSA)

11.13 *The intern / student must be familiar with automated advanced staining platforms in the ICC / IHC laboratory:*

11.13.1 Output [slide capacity / protocol flexibility]

11.13.2 Reproducibility and quality / impact of lean histology

11.13.3 Labour costs and material

11.13.4 Standardisation

11.13.5 Open and closed systems

11.14. *The intern / student must have a thorough knowledge of:*

11.14.1 Validation vs Optimisation

11.14.2 American Society of Clinical Oncology / College of American Pathologists [ASCO/CAP] [Ref 11.11]

C Evaluations

Evaluation will be in the form of a practical competency test, in which the intern / student must satisfactorily demonstrate cytoplasmic filaments in tissue specimens.

a Obtain suitable tissue [formalin fixed or frozen sections] and using *any manual* immunocytochemical method available to you, stain for any two types of cytoplasmic filaments. Include the appropriate control sections.

b Microscopically review the stained sections. Record in writing (1500+ words), commenting on your results by critically evaluating the specific method you have chosen.

c Comment on the tissue morphology, any artefacts in; and staining patterns of your tissue as well as the control sections.

D Recommended Textbooks and Resources for Module 11

- Theory and Practice of Histological Techniques, John D Bancroft and Marilyn Gamble. 5th or 6th Edition.
- Fundamentals of Cellular Pathology. Truter, EJ and van Wyk, W. (out of print)
- Wheater's Functional Histology: A Text and Colour Atlas. Wheater, PR. (2000).
- Introduction to Immunochemistry. Polak, JM and van Noorden.
- Manual of Diagnostic Antibodies for Immunohistology. Leong, A. *et al*. 2nd Edition.
- Immunohistochemistry – Methods Express. Renshaw, S. (Ed). Abcam Ltd, Cambridge, UK.
- Advanced Techniques in Diagnostic Cellular Pathology. Hannon-Fletcher, M. and Maxwell, P. (Ed). Wiley-Blackwell.

E Other

Websites

- http://www.Leica-biosystems-biosystems.com
- http:// www.dako.com
- http://ebiz.thermofisher.com/flash_movies/fse_p_basic/index.php
- http://ebiz.thermofisher.com/flash_movies/fse_s_buffer_prep/index.php
- http://www.vectorlabs.com/tutorials.aspx

MODULE 12

Molecular Pathology – *in Situ* Hybridisation

A Outcomes

On completion of this module the intern / student must have:

a thorough applicable knowledge of *in Situ* hybridisation (ISH), variations to the procedure, applications, limitations and know how to take corrective action for failure of the technique

b thorough knowledge of DNA, RNA, nucleotides, etc.

c an understanding of molecular cell biology techniques as applied to Histopathology

B Objectives

12.1 *The intern / student must be able to define / describe / explain:*

12.1.1 The principle of *in Situ* hybridisation

12.1.2 Amino acids, polypeptides, nucleotides

12.1.3 Nucleic acid structure

12.1.4 Replication

12.1.5 Transcription

12.1.6 Translation

12.1.7 DNA and RNA

12.1.8 Denaturation of DNA

12.1.9 Melting temperature

12.1.10 annealing

12.1.11 prokaryotes and eukaryotes and cite examples

12.1.12 Cytogenetics

12.1.13 intrinsic fluorescence

12.1.14 Probes and their choice for a specific application

- Probe type and means of synthesis
- Oligonucleotide probes
- Single stranded DNA probes
- Double stranded DNA probes
- RNA (cRNA probes or riboprobes)

- Commercially available probes as Analyte-specifc reagents (ASRs)

12.1.15 Probe preparation and labelling

- Direct labelling: enzyme, radio-isotope or fluorescent marker [SpectrumGreen™, SpectrumOrange™, Texas Red, SpectrumAqua™ SpectrumGold™]
- Indirect labelling: hapten binding {biotin, digoxygenin or Fluorescein}

12.1.16 the effect of probe length on ISH

- Nick translated probes
- Random priming
- Probe size

12.2 *The intern / student must have a thorough working knowledge of all the procedural steps of ISH including; specimen type and preparation, equipment, reagents and reagent preparation (including probes, fixatives, buffers, detection kits and ancillary reagents for visualisation, counter-stains and appropriate mounting media); as well as the reasons for use and how manipulation of these factors may influence the final reaction product and result*

12.2.1 Specimen type:

- Cultured cells such as amniocytes; chorionic villi cells, lymphocytes from BM aspirates, cells from solid tumours (metaphase spreads)
- Disaggregated cells from FFPE sections, touch preps (interphase cells)
- Tissue sections

12.2.2 Specimen fixation: [specimen / section type: frozen and / or fixed]

- Type of fixative
- Duration of fixation
- Temperature of fixative
- Maintenance of morphological detail
- Specimen storage

12.2.3 Slide / section preparation:

- Section thickness / drying procedures
- Microtomy procedure to limit contamination
- Slide adhesion / coating selection
- Deparaffinisation
- Slide storage pre- and post ISH procedure
- Use and interpretation of control material / slides / tissue

12.2.4 Pre-treatment: Proteolytic digestion and denaturation

- Selection of enzyme [type and grade]
- Concentration of enzyme

12.2.5 Hybridisation: [rate of, stability and stringency]

- Length, composition, quality and sequence of the probe / DNA
- Hybridisation buffer composition (ionic strength)
- Hybridisation temperature
- Agitation rate
- Hybridisation time
- Concentration of DNA [target strand and probe]
- Surface charge
- Linker length and flexibility
- Solid phase or solution based assay

12.2.6 Visualisation / detection systems - Link a “visual” molecule to the probe {See IHC section}

- Direct visualisation: e.g. Probe conjugated with Fluorescein (FISH)
- Indirect (colourimetric i.e. bright-field microscopy)
- CISH (chromogenic) based on enzyme-conjugated antibodies that recognise the target of interest
- e.g. biotin-free (multimer-based) system for detecting hapten labeled probes via an anti-hapten rabbit primary antibody
- Reaction of substrates with enzymes such as horseradish peroxidase (HRP) and / or alkaline phosphatase (AP) Appropriate endogenous blocking required

- Metallographic *in Situ* hybridisation SISH (silver-enhanced *in Situ* hybridisation) [also bright-field microscopy]
- Gold for EM
- BDISH (bright field double in *Situ* hydridisation): combinations of the above to visualise reference gene and target on the same slide

12.2.7 Counterstain and coverslipping:

- Propidium iodide
- DAPI (4', 6-diamidino-2-phenylindole) etc

12.2.8 Enumeration and nomenclature of signals: (Ref: Module 3, 10)

- FISH: metaphase scoring / interphase scoring [Single and dual probes]
- Microscopes and filters required (excitation ranges of probes and counterstains)
- Scoring / reading target area criteria: signal / ratio / control / morphology, etc
- SISH enumeration
- Sample exclusion / rejection criteria for ISH testing

12.3 *The intern / student must be able to critically evaluate the applications for and limitations of the procedures listed above as well as troubleshoot problems and causes of poor results with resultant corrective action:*

- Slide background
- Weak or no signal
- Distorted chromosome morphology
- Contaminants / debris

12.4. *The intern / student must be familiar with:*

12.4.1 The Polymerase Chain Reaction (PCR) technique

12.4.2 Principles and basic procedure of the technique

- Initialisation step
- Denaturation step
- Annealing step
- Extension (elongation step)
- Final elongation
- Final hold

- Reasons for failure of the PCR technique

12.4.3 Application of PCR technique in diagnosis of diseases

12.4.4 The precautions that should be taken when working with any of the chemicals involved in the above reaction(s) / techniques (i.e. formamide, DAB, etc)

12.5. *The intern / student must have a thorough knowledge of:*

12.5.1 Quality control procedures for ISH techniques

12.5.2 Quality Control programmes for ISH [intra and inter-laboratory]

12.5.3 Validation vs Optimisation

12.5.4 American Society of Clinical Oncology / College of American Pathologists [ASCO/CAP] as per 11.11[Ref Module 10 and 11]

C Evaluations

Draw a comprehensively annotated flow diagram to trace the path of a FFPE cervical tissue biopsy with suspected Human Papillomavirus (HPV) infection through the different processes of CISH. Select and include the appropriate controls for the reaction. Describe all the chemicals / components used in the various steps of the process and record any special precautions with regard to safely handling the reagents as well as limiting any contamination of the procedure. This can be done with the aid of a textbook.

Should you have been able to perform the procedure, microscopically review the stained sections. Record in writing (2000+ words), commenting on your results by evaluating the specific method you have chosen. Comment on the tissue morphology, any artefacts in and staining patterns of your tissue as well as the control sections.

D Recommended Textbooks and Resources for *in-situ* Hybridisation Module 12

- Theory and Practice of Histological Techniques, John D Bancroft and Marilyn Gamble. 5th or 6th Edition.
- Molecular Biology of the Cell. Alberts, B. *et.al.* 2nd Edition or later.
- *In Situ* Hybridization. Leitch, AR. *et.al.* (1994). 1st Edition.
- Gene Cloning. Brown, TA. (1986).

E Other

Websites

- http://www.Leica-biosystems.com
- http://www.dako.com
- http://roche.com

MODULE 13

Electron Microscopy

A Outcomes

On completion of this module the intern / student must:

Scanning Electron Microscopy (SEM)

a know the principle of the scanning electron microscope (SEM)

b to know the methods of preparing specimens for the SEM

c to know the difference between SEM and Transmission electron Microscope (TEM) and their use

Transmission Electron Microscopy (TEM)

a know the specific cut up, fixation, processing and microtomy requirements for samples requiring TEM.

b know the various components of an TEM and describe their function

c have good knowledge of the operation of transmission electron microscope

d be able to evaluate the quality of stain, artefacts and suitability of the section for diagnostic evaluation

e be able to recognise the normal morphology and pathological alterations in the cell fine structure

f be able to identify the abnormal ultrastructure in human tumours and various non-neoplastic human diseases specified in this syllabus

g be able to decide the right magnification for specific structures for a correct micrograph

h know how images can be recorded

i to be able to correlate the light microscopy, immunohistochemistry and electron microscopy for a reliable diagnosis

j immune-electron microscopy principle and applications

k nanotechnology and electron microscopy applications and use

B Objectives

13. *After studying this module the intern / student must be able to:*
Describe the operation of the various types of microscopes used in Histology and its specialised branches plus in Cytology and Cytogenetics.

KEY TERMS AND CONCEPTS

➢ Chromatic aberration	➢ Filament (tungsten)
➢ Spherical aberration	➢ Thermionic emission
➢ Resolution	➢ Backscattered electrons
➢ Electromagnetic lenses	➢ Nicol prism (polariser)
➢ Cathode shield	➢ T E M / S E M
➢ Fluorescent screen	➢ Bias resistor
➢ Accelerating voltage	➢ Anode plate
➢ Birefringence	➢ 2^{o} electrons
➢ Transmitted light fluorescence	➢ Detector
➢ Incidence light fluorescence	➢ Amplitude
➢ Electrons	

13.1 *The intern / student must be able to define / describe / explain:*

13.1.1	The principle of the transmission electron microscope (TEM)
13.1.2	How specimens can be prepared and stained for the TEM
13.1.3	The principle of the scanning electron microscope
13.1.4	Outline the methods of preparing specimens for the SEM
13.1.5	The principles of X-ray analysis of EM
13.1.6	Critically evaluate differences between the electron and light microscopes (Refer Module 3)
13.1.7	Evaluate the differences of plastics and resins in EM and their uses
13.1.8	Different action of wax [solid-liquid-solid] Vs plastics [converting liquid monomer- solid polymer]
13.1.9	Polymerisation [speed of polymerisation]
13.1.10	why clearing is not always needed when plastic embedding?
13.1.11	Epoxy resin

13.1.12 why acrylic resins for light microscopy / epoxy resins are preferred for EM

13.1.13 Diamond and glass knives

13.1.14 Electrons, accelerating voltage, waves

13.1.15 Electromagnetic lenses

13.1.16 Elastic and inelastic scatter

13.2 *The intern / student must have a thorough knowledge of:*

13.2.1 Specimens submitted for TEM evaluation: solid tumours, Percutaneous fine needle aspirations, effusions, peripheral blood, bone marrow, cultured cells, wax-removed tissue, nasal scrapings, tissue biopsies (e.g. muscle, renal biopises)

13.2.2 Fixation and post-fixation for transmission electron microscopy

13.2.3 Processing and embedding of TEM specimens

13.2.4 Cutting and staining TEM specimens

13.2.5 The advantages and disadvantages of using paraffin embedded material for TEM

13.2.6 Theoretical applications of IHC techniques on TEM specimens [including technical challenges, advantages and disadvantages]

13.2.7 How the image is formed, produced, recorded and stored in an electron microscope (Refer Module 3)

13.2.8 How information is recorded in the electron microscope

13.3 *The intern / student must be aware of:*

13.3.1 The dangers posed by osmium tetroxide

13.3.2 The danger of epoxy resins

13.3.3 The dangers posed by uranium and lead salts

13.4 *The intern / student must have a thorough knowledge of the stages of ultrastructural evaluation of TEM specimens and normal ultrastructure of the cells:*

13.4.1 Glycogen, lipids, fibrin, crystalline and viral inclusion

13.4.2 *Cell Membrane and Junctions*

13.4.2.1 Cell membrane and coat

13.4.2.2 Cell junctions

13.4.2.3 Alteration of cell junctions in neoplasia

13.4.2.4 Cell junction in connective tissue

13.4.2.5 Intracytoplasmic desmosomes

13.4.3 *Endocytotic Structures and Cell Processes (* be aware of)*

13.4.3.1 Endocytotic vesicules and vacuoles

13.4.3.2 Micropinocytotic vermiformis

Stages of ultrastructural evaluation

	FEATURE
1	Topographic cellular relationships
2	Basal lamina
3	Cell contour (membrane)
4	Intercellular junction
5	Cytoplasmic organelle patterns
6	Cytoplasmic granules
7	Cytoplasmic filaments
8	Cytoplasmic vacuoles and vesicles
9	Nuclear and nucleolar morphology
10	Stroma

13.5 *Nucleus*

13.5.1 Nuclear shape

13.5.2 Chromatin in normal neoplastic and necrotic cells

13.5.3 Inter-chromatin and peri-chromatin granules

13.5.4 Nuclear envelope and pores, thickening, proliferation and Reduplication

13.5.4 Evaginations or blebs of nuclear envelope

13.5.6 Intra-nuclear and intra-nucleolar lamellae, tubules and vesicles

13.5.7 Fibrous lamina

13.5.8 Nucleolus, structure and variation in size, shape and Numbers

13.5.9 Nucleolar margination

13.5.10 Segregation of nucleolar components

13.5.11 Pseudo-inclusion and true inclusion

13.5.12 Intra-nuclear glycogen, lipid, crystalline, haemoglobin, concentric laminated, viral inclusions

13.5.13 nuclear projections and pockets

13.5.14 nuclear bodies

13.6 *Centrioles*

13.6.1 Structure and function

13.7 *Mitochondria*

13.7.1 Mitochondrial morphology and enzyme content

13.7.2 Concentration of cristae

13.7.3 Lamellar, tubular, concentric, zig-zag, fenestrated cristae

13.7.4 Longitudinally orientated cristae

13.7.5 Mitochondrogensis

13.7.6 Mitochondrial involution, elimination and associations

13.7.7 Intermitochondrial herniation, whorls and bridges

13.7.8 Variations in dense granules and calcification

13.7.9 Swollen or hydropic mitochondria

13.7.10 mitochondrial hypertrophy and hyperplasia

13.7.11 Giant mitochondria

13.7.12 Ring-shaped and cup-shaped mitochondria

13.7.13 Intra-mitochondrial glycogen, lipidic, crystalline and iron inclusion

13.8 *Golgi Apparatus and Secretory Granules*

13.8.1 Structure and function

13.8.2 Golgi apparatus in cell differentiation and neoplasia

13.8.3 Hypertrophy and Atrophy of Golgi apparatus

13.8.4 Lipid and lipoprotein in Golgi apparatus

13.9 *Endoplasmic Reticulum*

13.9.1 Rough endoplasmic reticulum (RER)

13.9.2 Smooth endoplasmic reticulum (SER)

13.9.3 Dilatation and vesiculation of endoplasmic reticulum

13.9.4 Degranualtion of RER

13.9.5 Disaggregation of polyribosomes

13.9.6 Helical polyribosomes and ribosomes crystals

13.9.7 Endoplasmic reticulum and ribosomes in cell differentiation and neoplasia

13.9.8 Protein granules and crystalline inclusion in RER

13.9.9 Lipid in endoplasmic reticulum (liposomes)

13.9.10 Viruses in endoplasmic reticulum

13.9.11 Undulating tubules

13.9.12 Concentric lamellar bodies

13.9.13 Hypertrophy of SER in hepatocytes

13.10 *Annulate Lamellae*

13.10.1 Structure and function

13.11 *Lysosomes*

13.11.1 Classification and nomenclature

13.11.2 Multivesicular bodies

13.11.3 Myelin figures and myelinoid bodies

13.11.4 Erythrophagosomes

13.11.5 Siderosomes

13.11.6 Lysosomes in erythrocyte, neutrophils, eosinophils

13.11.7 Lysosomes in melanosis coli

13.11.8 Lysosomes in rheumatoid arthritis

13.11.9 Lysosomes in the liver

13.11.10 Metals, collagen and glycogen in lysosomes

13.12 *Microbodies (Peroxisomes)*

13.12.1 Structure, function and variation

13.13 *Melanosomes*

13.13.1 Morphology and normal variations

13.13.2 Pathological alterations

13.14 *Rod-Shaped Tubulated Bodies*

13.14.1 Structure, distribution and variations

13.15 *Myofilaments and Other Intracytoplasmic Filaments* (Ref: Module 9.13)

13.15.1 Myofilaments in striated muscle

13.15.2 Ring fibres

13.15.3 Myofibrillary Degeneration

13.15.4 Morphological alteration of the Z-line

13.15.5 Myofilaments in smooth muscle

13.15.6 Myofilaments in other cells

13.15.7 Other intracytoplasmic filaments

13.16 *Microtubules*

13.16.1 Structure, function and variations

13.17 *Cytoplasmic Inclusions*

13.17.1 Glycogen, lipids, fibrin, crystalline and viral inclusion

13.18 *Microvilli* (* be aware of)

13.18.1 Morphological alterations in microvilli

13.18.2 Cilia, flagella and sperm tails

13.18.3 Single, primary or oligocilia

13.18.4 Atypical cilia

13.18.5 Langerhans cell granules*

13.18.6 Cell processes in hairy cell leukaemia*

13.18.7 Cell processes in Erythrocytic cells*

13.18.8 Psammoma bodies*

13.19 *Extracellular Constituents* (Refer Module 9)

13.19.1 Collagen

13.19.2 Elastic fibres

13.19.3 Amyloid and amyloidoma

13.19.4 Basement membranes

13.20 *Non-neoplastic human diseases*

13.20.1 *Evaluation of renal biopsies*

- tubular structures such the organelles
- peri-tubular capilliaries
- glomeruli structures such as mesangial cells and matrix
- endothelial and epithelial cells
- capilliary loops and their arrangements
- glomerular basement membrane (GBM) aspect-Alport's syndrome (irregular and lamellar aspect), Amyloid-non-branching), extracellular, fibrils of 8-10nm
- inter-positioning of loops
- immune complex deposits in the GBM:

A. Immune complex deposits position in the basement membrane in:

- Membrano-proliferative glomerulonephritis – Sub-endothelial inner medullary collecting duct (IMCD) and reduplication of the BM
- Membranous glomerulonephritis – Sub-epithelial immune complex deposits
- Post-infectious glomerulonephritis- sub-epithelial immune complexes-humps
- Lupus nephritis (LN) and immune-complex pattern that influence the classification of LN: mesangial, sub-endothelial, intramembranous, sub-epithelial

B. Dense deposit disease (ribbon disease) - do not contain immunoglobulin, occurs in children and young adults and progress to renal failure

13.20.2 *Skeletal muscle biopsies – Myopathies* (Ref: Module 9.13)

- The bands
- sub-sarcolemmal and intermyofibrilar mitochondria
- nuclear displacement
- cores
- inclusions
- membrane atrophy characteristics
- glycogen accumulations
- Endomyocardial biopsies (structural differences from skeletal muscle)

13.20.3 *Peripheral nerves biopsies*

- Myelin sheath modification
- Bands of Büngner (collapsed Schwann cells)
- Clusters

13.20.4 *Epidermolysis Bullosa types*

- genetic - blister formation occurs within basal layer of epidermis
- dystrophic (dermolytic) - and acquired separation occurs above lamina densa

13.20.5 *Ciliary dysmorphology*

- KARTAGINER'S SYNDROME (triad-sinusitis, bronchiectasis and situs inversus)
- compound cilia
- 9+2 microtubuli pattern disturbed
- dynein arms missing

13.21. The student / intern must be familiar with and have a theoretical understanding of:

13.21.1 *Storage Diseases*

- lysosomal disease – Tay-Sachs disease
- CADASIL (cerebral autosomal dominant arteriopathy with subcortical infarcts and leucoencephalopathy), with extracellular electron-dense granular material that is in contact with the vascular smooth muscle cells sited in a small indentation
- Pompei's disease

13.21.2 *Rapid diagnosis of viral diseases*

- rapid diagnosis on negative stain with 2%phosphotungstic acid-polyoma virus, herpes virus, etc

13.21.3 *Alveolar cell carcinoma*

- Classification and description
- Incidence
- Characteristic ultrastructural differentiation
- Lamellar inclusion bodies-myelinosomes
- Light microscopy and Immunohistochemistry findings correlation
- Differential diagnosis

13.21.4 *Mesothelioma* (Ref: Module 9.7)

- Classification and description
- Incidence
- Characteristic ultrastructural differentiation
    - Lumen
    - Long slender microvilli
    - Tonofilaments and tonofibrils
- Light microscopy and Immunohistochemistry findings correlation
- Differential diagnosis

13.21.5 *Alveolar soft part sarcoma*

- Classification and description

- Incidence
- Characteristic ultrastructural differentiation
- Membrane-bound rhomboid crystals
- Light microscopy and Immunohistochemistry findings correlation
- Differential diagnosis

13.21.6 *Carcinoid tumours* (Refer Module 9.8)

The intern / student must be able to define / describe / explain:

- Incidence
- Characteristic ultrastructural differentiation (Neurosecretory granules – size and shape)
- Light microscopy and Immunohistochemistry findings and correlation
- Differential diagnosis

C Evaluation

1. Draw an annotated flow diagram to trace the path of a renal biopsy from the time it is taken through the preparatory steps for electron microscopy to the final product ready for diagnosis. Describe all the chemicals / components used in the various steps of the process and record any special precautions with regard to safely handling the reagents as well as limiting any contamination of the procedure. This can be done with the aid of a textbook.

   Should you have been able to perform the procedure, microscopically review the stained sections. Record in writing (+/-5000 words), commenting on your results by evaluating the methods you have used. Comment on the tissue morphology and ultrastructure, any artefacts and staining patterns of your tissue. If you have not been able to perform the procedure, please review an anonymised archived case for your review. Record that you have used an archived case for future audit purposes.

2. Describe in detail, the aspect and positions of immune-complex deposits in a nephropathy according to the disease classification - supply examples of micrographs.

D Recommended Textbooks and Resources for Module 13

- Theory and Practice of Histological Techniques, John D Bancroft and Marilyn Gamble. 5th or 6th Edition.
- Biological specimen preparation for transmission Electron Microscopy, Audrey M Glauert and Peter Lewis, 1998.
- Principles and techniques of Electron microscopy, M A Hayat, 2000
- Using the Transmission Electron Microscope in the Biological Sciences. Griffin, R.L. (1990). London: Ellis Horwood.
- Light and Electron Microscopy. Slayter. E.M. and Slayter, H.S (1992). Cambridge: Cambridge University Press.

- Ultrastructural Pathology of the Cell and Matrix, Feroze N. Ghadially, 1988 Third Edition, Butterworths
- Diagnostic Transmission Electron Microscopy of Tumours, 1994, Robert A. Erlandson, Raven Press
- Textbook of Histology, 2017, Leslie P. Gartner. Fourth Edition, Elsevier
- Heath JR, Davis ME. *Nanotechnology and cancer*. Annu Rev Med 2008; 59: 251-65.
- Sanvicens N, Marco MP. *Multifunctional nanoparticles - Properties and prospects for their use in human medicine*. Trends Biotechnol 2008; 26:42
- Ferrari M. *Cancer nanotechnology: Opportunities and challenges*. Nat Rev Cancer 2005;5:161-71. Back to cited text no. 7 [PUBMED]
- Mansoori GA, Mohazzabi P, McCormack P, Jabbari S. *Nanotechnology in cancer prevention, detection and treatment: Bright future lies ahead*. World \Rev Sci Technol Sustain Dev 2007;4:226-57.
- Yezhelyev MV, Gao X, Xing Y, Al-Hajj A, Nie S, O′Regan RM. *Emerging use of nanoparticles in diagnosis and treatment of breast cancer.* Lancet Oncol 2006; 7: 657-67.

MODULE 14

Mortuary, museum and other specialised techniques

Mortuary Practice

A Outcomes

On completion of this module the intern / student must be able to:

a describe the five functions which may be fulfilled by a mortuary and post-mortem facility

b list the naturally occurring processes that occur after death

c understand the work of the Human Tissue Authority (HTA) and how it impacts on the mortuary

d understand which Acts and Regulations affect the work and what can be done in the mortuary

e understand the difference between consented, or authorised, post-mortem examinations and those instructed by legal process

B Objectives

14. *The intern / student must be able to define / describe / explain:*

14.1 Mortuary

14.1.1 Mortuary Function

14.1.2 Mortuary Staff and their specific roles

14.2 Death and decomposition

14.3 Certification of death as required by South Africa law

14.3.1 Cremation legislation and regulations

14.4 Documentation related to:

14.4.1 Identification

14.4.2 Infection status [Advisory Committee on Dangerous Pathogens]

14.4.3 Confidentiality wrt immuno-compromised persons

14.5 Post-mortem Examinations

14.5.1 Licence holders

14.5.2 Human Tissue Act (HTA)

14.5.3 Organ Transplant Act and time delays affecting transplants

14.5.4 Post-mortem examinations carried out under instruction from legal authorities

14.5.5 Post-mortem examinations with consent or authorisation [medical interest post-mortems examinations]

14.6 *The intern / student must have a thorough knowledge of:*

14.6.1 Post-mortem Preparation

14.6.1.1 Evisceration methods:

- Letulle or Rokitanski method
- Gon method [en bloc method]

14.6.1.2 Health and Safety

- SOPs
- PPE [appropriate and relevant levels of PPE]
- Occupational health
- Sealing of puncture wounds
- Stout plastic shrouds and body bags
- Importance and relevance of the National institute for Communicable disease [NICD]

14.7 *The intern / student must have a thorough knowledge of:*

14.7.1 The Post-mortem procedure

14.7.1.1 Documentation procedures checks

14.7.1.2 Description procedures: external

14.7.1.3 Body charts

14.7.1.4 Evisceration methods:

- Letulle or Rokitanski method
- Gon method [en bloc method]

14.7.1.5 How organ weights are taken and documented

14.7.1.6 Harvesting / preparing / transporting and preserving common samples taken at post-mortem for appropriate investigative procedures
- Urine
- Blood
- Cerebrospinal fluid
- Vitreous humour
- Stomach contents

14.7.1.7 Harvesting / preparing / transporting and preserving ''other samples'' taken at post-mortem for appropriate investigative procedures
- Bile
- Brain
- Liver: poisons
- Lung
- Hair
- Nail and Bone

14.8 *The intern / student must be familiar with:*

14.8 1 Reconstruction procedures of the deceased

14.9 *The intern / student must have a thorough knowledge of:*

14.9.1 Disinfection

14.9.1.1 Techniques / protocols: Post-mortem room and instruments

14.9.1.2 Disinfection Solutions: applications / use / Health and Safety of the following:
- Phenolics and aldehydes
- Alcohol
- Chlorine–releasing agents
- Quaternary ammonium compounds and
- halogenated tertiary amines
- UV

Museum techniques

Introduction

All teaching / academic hospitals and schools of Pathology have Museums which serve many functions: permanent exhibition of common specimen for undergraduate and postgraduate teaching purposes, illustrating specimens of rarity, permanent source of histologic material and for gross and microscopic photography.

A Outcomes

Upon completion of this part of the Module, the intern / student must be able to:

a explain the methods used in handling museum specimens

b describe the techniques of specimen preservation

B Objectives

14.10 *The intern / student must be able to define / explain / evaluate the following steps for any specimens received for museum techniques*

14.10.1 Museum Techniques

- 14.10.1.1 Reception and recording of specimens for receipt
- 14.10.1.2 Appropriate preparation of the specimen for museum purposes
- 14.10.1.3 Wet specimens / fresh unfixed / fixed specimens / delicate structures / friable tissue / special precautions

14.10.2 Fixation

- Kaiserling I Solution

14.10.3 Restoration

- Kaiserling II Solution
- Stabilising / Rejuvenator Solution solutions

14.10.4 Preservation

- Kaiserling III solution

14.10.5 Presentation

14.10.5.1 Mounting: orientation / plastic sheets / threads / wax

14.10.5.2 Jars [making of Perspex / glass and other containers for display]

14.10.5.3 Labeling of Specimen Jars

14.10.5.4 Cataloguing of Specimens

14.10.6 *The intern / student must be able to explain / prepare the most appropriate museum exhibit areas / sections as follows:*

14.10.6. 1 Exhibiting of Specimens:

- History of Medicine Section
- Normal Anatomy Section
- Morbid (pathological) Anatomy Section
- Histology Section
- Haematopathology Section
- Radiology and Osteology Section
- Microbiology and Parasitology Section
- Interactive Corners (Interactive Corner for Human Diseases)
- Interactive Corner for Educational Visits Interactive
- Corner for Virtual Anatomy

14.11 *The intern / student must be able to define / explain the purpose of:*

14.11.1 Medical Graphics

14.11.2 Paper and art works

14.11.3 Chart layouts

14.11.4 Captions

14.11.5 Drawings and laminating

14.11.6 Drawings and laminating [digital camera to take pictures of pictures of specimens or to convert analog images to digital as in slides]

14.11.7 Scanning of images from photographs or books

14.12 *The intern / student must be able to define / explain the purpose of:*

14.12.1 Dry techniques – bone where the tissue is boiled off the bone or maggots are allowed to eat the flesh off the bone

14.12.2 Gross staining of specimen – amyloid, calcium, lipid and iron

14.12.3 Alizarin Red staining for foetal skeleton after clearing of the soft tissue with an ante-medium / clearing agent

14.12.4 Injection techniques using latex or resin of different colours particularly useful for vasculature

14.12.5 Plastinisation

14.13 *The intern / student must be able to define / explain the purpose of:*

14.13.1 Gough and Wentworth Whole Organ Sections

14.13.1.1 Fixation and preparation of the tissue

14.13.1.2 Inflation of whole lungs

14.13.1.3 Impregnation

- Gelatin solutions
- Incubator / equipment required

14.13.1.4 Embedding

14.13.1.5 Section cutting

14.13.1.6 Mounting of sections

14.13.1.7 Preservation of the mounted sections

14.14 *The intern / student must have a thorough knowledge of:*

14.14.1 Health and Safety

14.14.1.1 Where, when and how to wear appropriate levels of Protective clothing

14.14.1.2 Decontamination

14.14.1.3 Safe use of equipment such as saws etc

14.14.1.4 Maintenance and cleaning of equipment used for all the above techniques

14.14.2 The chemical hazards and related precautions as detailed in the techniques above

14.15 *The intern / student must be familiar with:* (Ref Modules 1 and 2)

14.15.1 National Health Act No. 61 of 2003 and subsequent amendments

14.15.2 Human Tissue Act (65, 1983)

14.15.3 Human Tissue Amendment Act (51, 1989)

14.15.4 Patient Rights Charter (108, 1996); and all subsequent updates

14.15.5 Births and Deaths Registration Amendment Act 67, of 1997

14.15.6 HPSCA regulations relevant to the Profession; including the Health Professions Act 56 of 1974 and subsequent updates

14.15.7 Data Protection within the framework of the South African Constitution

14.15.8 Protection of Personal Information Act, No 4 of 2013 (POPI); Government Gazette Notice 37067

C Evaluation

a Draw an annotated flow diagram to trace the path of a fresh whole lung from the time it is taken from the body, through the preparatory steps for whole section mounting by the Gough and Wentworth technique.

Write short notes detailing any limitations of the procedure and include any precautions that may be required.

b Describe in detail, the legislation, required procedure(s) and documentation for registering a death and obtaining a death certificate in South Africa. 1000 + words.

D Recommended Textbooks and Resources for Module 14

- Handbook of Histopathological Techniques (including museum technique) 3rd Edition. CFA Culling. 1963. Butterworths London [ISBN: 978-0-407-72901-8]
- Histopathology. 2012 Ed Orchard and Nation. Oxford University Press. [ISBN: 978-0-19-957434-6]

E Other

Websites

- http://www.ptrust.co.za/documents/Brochures/Bereavement
- http://www.cmej.org.za/
- http://www.westerncape.gov.za/documents/#policies
- http://www.westerncape.gov.za/general-publication/your-questions

7. Clinical Applications

Medical technologists and BHSc:MLS are not clinicians, and clinical symptoms and presentation will not be specifically asked in the examination, unless relevant to the tests detailed within this syllabus. It is however, important to have a sound foundation knowledge of the clinical presentation of diseases related to the special investigations [Special stains / Advanced Techniques] as detailed in this syllabus. These clinical symptoms are given as a guide to interpretation.

It is recommended that case studies within the Clinical Laboratory Training are used as a teaching tool.

8. Reference Material - APPENDIX 1

Useful textbooks / References / literature for intern / students and Trainers

Useful Links

http://www.leica-biosystemsbiosystems.com/pathologyleaders/

http://ebiz.thermofisher.com/flash_movies/fse_p_basic/index.php

http://ebiz.thermofisher.com/flash_movies/fse_s_buffer_prep/index.php

http://www.vectorlabs.com/tutorials.aspx

histology.leeds.ac.uk/what-is-histology/

www.pathologyoutlines.com/

www.path.uiowa.edu/virtualslidebox/

library.med.utah.edu/

ventana-slidebox.pathxchange.org/

Histotechnology

stainsfile.info/StainsFile/jindex.html
Useful information about dyes, stains and staining

www.ihcworld.com/index.htm
Information Source for immunohistochemistry

www.bristol.ac.uk/vetpath/cpl/histmeth.htm
Useful information on staining techniques

Useful Text Books

- Young B, O'Dowd G, Stewart W, (2010): Wheater's Basic Pathology: A Text, Atlas and Review of Histopathology (5th Ed).
- Kerr JB. (2010): Functional Histology (2nd Ed).
- Mescher AL. (2009): Jun queira's Basic Histology (12th Ed).
- Eroschenko VP. (2007): diFiore's Atlas of Histology with Functional Correlations (11th Ed).
- Young B, Lowe JS, Stevens A, Heath JW (2006). Wheater's Functional Histology (5th Ed).
- Wheater, PR. (2000). Wheater's Functional Histology: A Text and Colour Atlas
- Mariano S. di Fiore (1967). An Atlas of Human Histology

Histotechnology

- Anderson,G and Gordon,K.C. (1996). Tissue Processing, microtomy and paraffin sections
- Carson F, Hladik C. (2009): Histotechnology: A Self-Instructional Text. (3rd Ed).
- Cook, H.C. (1974). Manual of Histological Demonstration Techniques. London: Butterworths
- Culling, C.F.A. (1975). Handbook of Histopathological and Histochemical Techniques. London. Butterworths
- John D Bancroft and Harry C Cook, Churchill and Livingstone. (1994). Manual of histological techniques and their diagnostic applications
- Bancroft, John D and Gamble. Theory and practice of histological techniques. 5th or 6th Ed.
- BaylissHigh, O.B and Lake,B. (1996). Lipids
- Audrey M Glauert and Peter Lewis. (1998). Biological specimen preparation for transmission Electron Microscopy
- Griffin, R.L. (1990). Using the Transmission Electron Microscope in the Biological Sciences. London: Ellis Horwood
- M A Hayat. (2000). Principles and techniques of electron microscopy
- Hopwood,D. (1996). Fixation and Fixatives
- Kieran. J.A. (1990). *Histological and Histochemical Methods*. Oxford: Pergamon
- Kiernan J. (2008): Histological and Histochemical Methods: Theory and Practice. (4th Ed).
- Kok, L.P and Boon, Mathilde E. (1992). Microwave cookbook for microscopists: art and science of visualization, 3rd Revised Edition
- Laboratory Biosafety Manual, WHO, 1993
- Peters, Stephen R. Editor: Practical Guide to Frozen Section Technique. Springer
- Slayter. E.M. and Slayter, H.S (1992). Light and Electron Microscopy. Cambridge: Cambridge University Press
- E J Truter and W van Wyk. Fundamentals of Cellular Pathology
- Wallington, E.A. (1979). Artefacts in tissue sections. *Medical laboratory Sciences* 36, 3-61

Websites:

- http://www.Leica-biosystems-biosystems.com
- http://www.dako.com
- http://roche.com
- http://ebiz.thermofisher.com/flash_movies/fse_p_basic/index.php
- http://ebiz.thermofisher.com/flash_movies/fse_s_buffer_prep/index.php
- http://www.vectorlabs.com/tutorials.aspx
- http://www.hpcsa.co.za
- http://www.smltsa.org.za
- http://www.cdc.gov
- http://eppendorfna.com
- www.pathguy.com/histo
- http://ventana-slidebox.pathxchange.org/

Other

- Government Gazettes
- OHASA legislation / Acts and ISO / SANAS
- All laboratory SOPs
- All instrument manuals

****NOTE: The Professional Board for Medical Technology and the SMLTSA require that intern / students use British (UK) English and not USA English**

9. Nomenclature - APPENDIX 2

a-, *a-,* without: avascular
ab-, *ab,* from: abduct
-ac, *-akos,* pertaining to: cardiac
acr-, *akron,* extremity: acromegaly
ad-, *ad,* to, toward: adduct
aden-, adeno-, *adenos,* gland: adenoid
adip-, *adipos,* fat: adipocytes
aer-, *aeros,* air: aerobic respiration
af-, *ad,* toward: afferent
-al, *-alis,* pertaining to: brachial
alb-, *albicans,* white: albino
-algia, *algos,* pain: neuralgia
allo-, *allos,* other: allograft
ana-, *ana,* up, back: anaphase
andro-, *andros,* male: androgen
angio-, *angeion,* vessel: angiogram
ante-, *ante,* before: antebrachial
anti-, ant-, *anti,* against: antibiotic
apo-, *apo,* from: apocrine
arachn-, *arachne,* spider: arachnoid
arter-, *arteria,* artery: arterial
arthro-, *arthros,* joint: arthroscopy
-asis, -asia, *asis,* state, condition: homeostasis
astro-, *aster,* star: astrocyte
atel-, *ateles,* imperfect: atelectasis
aur-, *auris,* car: auricle
auto-, *auto,* self: autonomic
baro-, *baros,* pressure: baroreceptor
bi-, *bi-,* two: bifurcate
bili-, *bilis,* bile: bilirubin[
bio-, *bios,* life: biology
blast-, -blast, *blastos,* precursor: blastocyst
brachi-, *brachium,* arm: brachiocephalic
brachy-, *brachys,* short: brachydactyly
brady-, *bradys,* slow: bradycardia
bronch-, *bronchus,* windpipe, airway: bronchial
carcin-, *karkinos,* cancer: carcinoma
cardi-, cardio-, -cardia, *kardia,* heart: cardiac
-cele, *kele,* tumor, hernia, or swelling: blastocoele
-centesis, *kentesis,* puncture: thoracocentesis
cephal-, *cephalos,* head: brachiocephalic
cerebr-, *cerebros,* brain: cerebral hemispheres
cerebro-, *cerebrum,* brain: cerebrospinal fluid
cervic-, *cervicis,* neck: cervical vertebrae
chole-, *chole,* bile: cholecystitis
chondro-, *chondros,* cartilage: chondrocyte
chrom-, chromo-, *chroma,* color: chromatin
circum-, *circum,* around: circumduction
-clast, *klastos,* broken: osteoclast
colo-, *kolon,* colon: colonoscopy
contra-, *contra,* against: contralateral
corp-, *corpus,* body: corpuscle
cortic-, *cortex,* rind or bark: corticospinal
cost-, *costa,* rib: costal
cranio-, *cranium,* skull: craniosacral
cribr-, *cribrum,* sieve: cribriform
-crine, *krinein,* to secrete: endocrine
cut-, *cutis,* skin: cutaneous
cyan-, *kyanos,* blue: cyanosis
cyst-, -cyst, *kystis,* sac: blastocyst
cyt-, cyto-, *kyton,* a hollow cell: cytology
de-, *de,* from, away: deactivation
dendr-, *dendron,* tree: dendrite
dent-, *dentes,* teeth: dentition
derm-, *derma,* skin: dermis
desmo-, *desmos,* band: desmosome
di-, *dis,* twice: disaccharide
dia-, *dia,* through: diameter
digit-, *digit,* a finger or toe: digital
dipl-, *diploos,* double: diploid
dis-, *des,* apart, away from: disability
diure-, *diourein,* to urinate: diuresis
dys-, *dys-,* painful: dysmenorrhea
-ectasis, *ektasis,* expansion: atelectasis
ecto-, *ektos,* outside: ectoderm
-ectomy, *ektome,* excision: appendectomy
ef-, *ex,* away from: efferent
emmetro-, *emmetros,* in proper measure: emmetropia
encephalo-, *enkephalos,* brain: encephalitis
end-, endo-, *endos,* inside: endometrium
entero-, *enteron,* intestine: enteric
epi-, *epi,* on: epimysium
erythema-, *erythema,* flushed (skin): erythematosis
erythro-, *erythros,* red: erythrocyte
ex-, *ex,* out, away from: exocytosis
extra-, outside, beyond, in addition: extracellular
ferr-, *ferrum,* iron: transferrin
fil-, *filum,* thread: filament
-form, *-formis,* shape: fusiform
gastr-, *gaster,* stomach: gastrointestinal
-gen, -genic, *gennan,* to produce: mutagen
geniculа-, *geniculum,* kneelike structure: geniculate nuclei
gest-, *gesto,* to bear: gestation
glosso-, -glossus, *glossus,* tongue: hypoglossal
glyco-, *glykys,* sugar: glycogen
-gram, *gramma,* record: myogram
gran-, *granulum,* grain: granulocyte
-graph, -graphia, graphein, to write, record: electroencephalograph
gyne-, gyno-, *gynaikos,* woman: gynecologist
hem-, hemato-, *haima,* blood: hemopoiesis
hemi-, *hemi-,* half: hemisphere
hepato-, *hepaticus,* liver: hepatocyte
hetero-, *heteros,* other: heterozygous
histo-, *histos,* tissue: histology
holo-, *holos,* entire: holocrine
homeo-, homo-, *homos,* same: homeostasis
hyal-, hyalo-, *hyalos,* glass: hyaline
hydro-, *hydros,* water: hydrolysis
hyo-, *hyoeides,* U-shaped: hyoid
hyper-, *hyper,* above: hyperpolarization
hypo-, *hypo,* under: hypothyroid
hyster-, *hystera,* uterus: hysterectomy
-ia, state or condition: insomnia
idi-, *idios,* one's own: idiopathic

ile-, *ileum:* ileocecal valve
ili-, ilio-, *ilium,* flank, groin: iliac
in-, *in,* within, or denoting negative effect: inactivate
infra-, *infra,* beneath: infraorbital
inter-, *inter,* between: interventricular
intra-, *intra,* within: intracellular
ipsi-, *ipse,* itself: ipsilateral
iso-, *isos,* equal: isotonic
-itis, *-itis,* inflammation: dermatitis
karyo-, *karyon,* body: megakaryocyte
kerato-, *keros,* horn: keratin
kino-, -kinin, *kinein,* to move: bradykinin
lact-, lacto-, *-lactin,* lac, milk: prolactin
lapar-, *lapara,* flank or loins: laparoscopy
-lemma, *lemma,* husk: sarcolemma
leuko-, *leukos,* white: leukocyte
liga-, *ligare,* to bind together: ligase
lip-, lipo-, *lipos,* fat: lipase
lith-, *lithos,* stone: otolith
lyso-, -lysis, -lyze, *lysis,* dissolution: hydrolysis
macr-, *makros,* large: macrophage
mal-, *mal,* abnormal: malabsorption
mamilla-, *mamilla,* little breast: mamillary
mast-, masto-, *mustos,* breast: mastoid
mega-, *megas,* big: megakaryocyte
melan-, *melas,* black: melanocyte
men-, *men,* month: menstrual
mero-, *meros,* part: merocrine
meso-, *mesos,* middle: mesoderm
meta-, *meta,* after, beyond: metaphase
micr-, *mikros,* small: microscope
mito-, *mitos,* thread: mitosis
mono-, *monos,* single: monocyte
morpho-, *morphe,* form: morphology
multi-, *multus,* many: multicellular
-mural, *murus,* wall: intramural
myelo-, *myelos,* marrow: myeloblast
myo-, *mys,* muscle: myofilament
narc-, *narkoun,* to numb or deaden: narcotics
nas-, *nasus,* nose: nasolacrimal duct
natri-, *natrium,* sodium: natriuretic
necr-, *nekros,* corpse: necrosis
nephr-, *nephros,* kidney: nephron
neur-, neuro-, *neuron,* nerve: neuromuscular
oculo-, oculus, eye: oculomotor
odont-, *odontos,* tooth: odontoid process
-oid, *eidos,* form: odontoid process
oligo-, *oligos,* little, few: oligodendrocyte
-ology, *logos,* the study of: physiology
-oma, *-oma,* swelling: carcinoma
onco-, *onkos,* mass, tumor: oncology
oo-, *oon,* egg: oocyte
ophthalm-, *ophthalmos,* eye: ophthalmic nerve
-opia, *ops,* eye: optic
orb-, *orbita,* a circle: orbicularis oris
orchi-, *orchis,* testis: orchiectomy
orth-, *orthos,* correct, straight: orthopedist
-osis, *-osis,* state, condition: neurosis
osteon, osteo-, *os,* bone: osteocyte
oto-, *otikos,* ear: otolith
para-, *para,* beyond: paraplegia
patho-, -path, -pathy, *pathos,* disease: pathology
pedia-, *paidos,* child: pediatrician
per-, *per,* through, throughout: percutaneous
peri-, *peri,* around: perineum
phag-, *phagein,* to eat: phagocyte
-phasia, *phasis,* speech: aphasia
-phil, -philia, *philus,* love: hydrophilic
phleb-, *phleps,* a vein: phlebitis
-phobe, -phobia, *phobos,* fear: hydrophobic
phot-, *phos,* light: photoreceptor
-phylaxis, *phylax,* a guard: prophylaxis
physio-, *physis,* nature: physiology
-plasia, *plasis,* formation: dysplasia
platy-, *platys,* flat: platysma
-plegia, *plege,* a blow, paralysis: paraplegia
-plexy, *plessein,* to strike: apoplexy
pneum-, *pneuma,* air: pneumonia
podo, *podon,* foot: podocyte
-poiesis, *poiesis,* making: hemopoiesis
poly-, *polys,* many: polysaccharide
post-, *post,* after: postganglionic
pre-, *prae,* before: precapillary sphincter
presby-, *presbys,* old: presbyopia
pro-, *pro,* before: prophase
proct-, *proktos,* anus: proctology
pterygo-, *pteryx,* wing: pterygoid
pulmo-, *pulmo,* lung: pulmonary
pulp-, *pulpa,* flesh: pulpitis
pyel-, *pyelos,* trough or pelvis: pyelitis
quadr-, *quadrans,* one quarter: quadriplegia
re-, *re-,* back, again: reinfection
retro-, *retro,* backward: retroperitoneal
rhin-, *rhis,* nose: rhinitis
-rrhage, *rhegnymi,* to burst forth: hemorrhage
-rrhea, *rhein,* flow, discharge: amenorrhea
sarco-, *sarkos,* flesh: sarcomere
scler-, *sclero-,* skleros, hard: sclera
-scope, *skopeo,* to view: microscope
-sect, *sectio,* to cut: transect
semi-, *semis,* half: semilunar valve
-septic, *septikos,* putrid: antiseptic
-sis, state or condition: metastasis
som-, -some, *soma,* body: somatic
spino-, *spina,* spine, vertebral column: spinodeltoid
-stalsis, *staltikos,* contractile: peristalsis
sten-, *stenos,* a narrowing: stenosis
-stomy, *stoma,* mouth, opening: colostomy
stylo-, *stylus,* stake, pole: styloid
sub-, *sub,* below; subcutaneous
super-, *super,* above or beyond: superficial
supra-, *supra,* on the upper side: supraspinous fossa
syn-, *syn,* together: synthesis
tachy-, *tachys,* swift: tachycardia
telo-, *telos,* end: telophase
tetra-, *tettares,* four: tetrad
therm-, thermo-, *therme,* heat: thermoregulation
thorac-, *thorax,* chest: thoracentesis
thromb-, *thrombos,* clot: thrombocyte
-tomy, *temnein,* to cut: appendectomy
tox-, *toxikon,* poison: toxin
trans-, *trans,* through: transport
tri-, *tres,* three: trimester
tropho-, *trophe,* nutrition: trophoblast
-trophy, *trophikos,* nourishing: atrophy
-tropic, *trope,* turning: adrenocorticotropic
tropo-, *tropikos,* turning: troponin
uni-, *unus,* one: unicellular
uro-, -uria, *ouron,* urine: glycosuria
vas-, *vas,* vessel: vascular
zyg-, *zygotos,* yoked: zygote