



SYLLABUS

HISTOPATHOLOGICAL TECHNIQUES

MEDICAL TECHNICIANS

Effective from Feb 2019 for exams from Oct 2020

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1. Introduction

Histotechnology is an essential component to the art and science of pathology and plays a crucial role in the diagnosis and treatment of disease. **Histology technicians** and technologists prepare thin slices of tissue for microscopic examination by a pathologist. Histopathology is the study of changes in tissues caused by disease.

The objective of this syllabus is to provide the trainee technician with a guideline on the essential aspects that must be covered in order to adequately prepare themselves for the HPCSA's Professional Board for Medical Technology **Medical Technician's** Board Examination. The examination is in the form of two, two hour, written papers which will be based on the contents of this syllabus and related **theoretical and practical** knowledge gained during training at your laboratory. Candidates are required to attain a minimum of 50% overall and a sub-minimum of 50% for each of the papers that comprise Histology.

Evidence of *"Evaluation"* criteria as detailed within this syllabus must be available prior to the Board Examination and 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 students receive appropriate training in the tests contained within the syllabus but which are not routinely performed on site. In addition, it is expected that the student will have, where applicable, knowledge and understanding of the following:

2. Statutory Regulations and Ethics

Objective:

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

Specified outcomes:

The 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 Technicians.
- > 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 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

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 a laboratory accident or emergency.

Range: Chemical or bio-hazardous spill; fire; flood; bomb threat.

- Describe basic procedures to follow for the prevention, control and management of laboratory acquired infections including the prevention and control of infection by blood borne viruses and tissue types relevant to this syllabus.
- Describe and apply good general housekeeping procedures including the decontamination of equipment.
- Describe the application of laboratory safety procedures for the collection, transport, storage and analysis of biological specimens.
- Describe the purpose and basic content of the safety data 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 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; carcinogens and sharps.

> Define the role of the designated safety personnel.

Range: First aid officer; fire marshal; safety representative.

Recognise and be able to respond to the international 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:

- > 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 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.
- Display knowledge of the optimal storage conditions should testing be delayed and the stability of the specimen for the individual testing process.
- Accurately capture the data and patient demographics that are required for the registration of the specimens at the laboratory.
- > 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; fume cupboards; temperature controlled, ultra, water-baths; stopwatches / timers; thermometers; incubators, knives – disposable and other; knife sharpeners; microtomes – sliding, rotary, sledge, ultratome, cryostat.

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

The 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 basic troubleshooting procedures when optimal operation is not demonstrated by the functional checks.
- Demonstrate a basic understanding of the approach to the validation of new equipment, reagents and testing kits where applicable and within the context of this syllabus.
- Demonstrate knowledge of, and maintain, all equipment records and documentation required for Good Laboratory Practice (GLP).
- Demonstrate a basic knowledge of the working of large apparatus used in histology (Tissue Processors, Microtomes, Coverslipping instrumentation, Embedding machines and automated routine staining machines).

3.4 Laboratory Reagents

Objective:

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

Range: Stock solutions; working solutions; working reagents; controls; reagent kits, where appropriate in this syllabus.

Specified outcomes:

On completion of the syllabus the 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.

3.6 Quality Assurance / Accreditation

Objective:

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

Specified outcomes:

- 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 / contained in this syllabus.
- Demonstrate a *basic* knowledge of all the principles and procedures of all related internal and external, *quantitative* quality control data.
- Apply a *basic* knowledge of all the procedures and principles of internal and external *qualitative* quality control data.
- Demonstrate a *basic* understanding of the potential causes of failed internal and external, quantitative and qualitative quality control.
- > Define basic terminology used in the assessment of quality control results.
 - Range: positive controls for special stains / Immunocytochemistry.

- 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 student to all basic aspects of quality control.

Specified outcomes:

- > Demonstrate an understanding of Quality assurance and Quality control in the correct context.
- Demonstrate 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 basic understanding of the validation process of kits and reagents.

Specific outcomes:

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

3.9 Personnel

Objective:

Demonstrate an understanding of basic requirements for personnel in terms of GLP.

Specified outcomes:

- > 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 student should be able to:

- Demonstrate a basic understanding of the management of laboratory documentation in terms of Good Laboratory Practice (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 a basic 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 student should be able to:

Demonstrate proficiency in the calculations required for the preparation of solutions for patients' samples. Normal solutions, percentage solutions, molar solutions, titrations.

Laboratory administration

A Outcomes

On completion of this module the student must

- a have a thorough knowledge of the administrative structure of the laboratory that he / she is working in.
- b have a sound knowledge of the collection, logging, distribution, data recording, reporting, accession and retrieval of data.

B Objectives

The student must

- 1.1 have a sound knowledge of:
 - 1.1.1 the hierarchical structure of the laboratory
 - 1.1.2 collection / fixation / receipt / data recording of specimens
 - 1.1.3 data capturing, logging and distribution of specimens to the different laboratories / subsections within histopathology
 - 1.1.4 recording of results into the central computerised programme
 - 1.1.5 communicating the reported result (s) [where permitted]
 - 1.1.6 later accession and retrieval of results
 - 1.1.7 Block and slide filing systems
 - 1.1.8 Gross specimen disposal
- *1.2 be familiar with:*

1.2.1	National Health Act No. 61 of 2003 and subsequent amendments
1.2.2	the Human Tissue Act (65, 1983)
1.2.3	the Human Tissue Amendment Act (51, 1989); and all subsequent updates.
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

- 1.3 Define, describe and understand each of the following:
- *1.3* anatomy, physiology, pathology, histology, histopathology, biopsy, autopsy, neoplasm, benign, malignant
- 1.4 Give a brief description of the technical activities involved with the following specialised branches of histology:
 - 1.4.1 enzyme histochemistry
 - 1.4.2 immunocytochemistry
 - 1.4.3 electron microscopy
 - 1.4.4 neuropathology
 - 1.4.5 renal pathology
 - 1.4.6 cytology
 - 1.4.7 fine needle aspiration cytology

C Evaluation

- a Draw an annotated flow diagram of the hierarchical structure of the department / laboratory.
- b Draw an annotated diagram, following receipt of a specimen in the laboratory until results are reported to the wards / clinicians.

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

Websites

- ✓ <u>http://www.hpcsa.co.za</u>
- ✓ <u>http://www.smltsa.org.za</u>
- ✓ Government Gazettes various
- ✓ Government Acts

Safety in the histopathology laboratory

A Outcomes

On completion of this module the student must

- a be able to recognise the health hazards involved in handling fresh, unfixed tissue.
- b be aware of the storage and safe usage of liquids, chemicals and stains / dyes in the laboratory.
- c be safety competent to work in a laboratory environment.

B Objectives

The student must

- 2.1 *be able to define the following terms:*
 - 2.1.1 Hazard groups 1, 2, 3, and 4 relating to microorganisms
- 2.2 have a basic knowledge of the following groups of microorganisms and / or the diseases they cause, primarily relating to the handling of these specimens
 - 2.2.1 Bacteria and other mycobacteria such as leprosy
 - 2.2.2 HIV and viral hepatitis.
 - 2.2.3 Haemorraghic fevers (Ebola specimens)
 - 2.2.4 Spongiform Encephalopathies (Creuzfeldt-Jacob disease)
- 2.3 be aware of:
 - 2.3.1 the dangers posed by receiving fresh, unfixed tissue and body fluids in the laboratory (universal precautions and specific precautions for those listed above)
 - 2.3.2 how and where to handle the abovementioned tissues and fluids
 - 2.3.3 the danger posed during frozen sectioning of fresh tissue and wearing protective apparatus / clothing
- 2.4 be familiar with the use, hazards and safe disposal of hospital / laboratory antiseptics and disinfectants such as:
 - 2.4.1 methylated spirit and chlorhexidine
 - 2.4.2 phenolic disinfectants and hypochlorites
 - 2.4.3 alcohols and 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) and acetone

- 2.5.2 Ether
 2.2.3 Hydrocarbons xylene, toluene, benzene, chloroform and carbon tetrachloride
 2.5.4 Xylene substitutes
- 2.6 *be familiar with the use, storage, hazards and safe disposal of embedding media such as:*
 - 2.6.1 paraffin waxes
 - 2.6.2 "Paraplast +"
 - 2.6.3 resins methacrylates; epoxy
- 2.7 be familiar with the use, storage, hazards and safe disposal of laboratory chemicals and reagents such as:

2.7.1	liquid and dry chemicals
2.7.2	picric acid
2.7.3	stains and dyes in powder and liquid form
2.7.4	organic acids (acetic acid, formic acid, etc)
2.7.5	inorganic acids (hydrochloric acid, sulphuric acid, etc.)
2.7.6	Well known carcinogens within the Histology context

2.8 be aware of:

2.8.1	the location of all fire extinguishers and fire fighting 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 safety such as:
 - 2.9.1 no eating, drinking and smoking in the laboratory
 - 2.9.2 when and how to use laboratory coats and other Personal Protective Equipment (PPE)
 - laboratory coats
 - gloves

- face masks
- hair covering
- rubber boots or disposable booties
- safety goggles
- Mobile telephones
- 2.9.3 Personal hygiene in the laboratory environment
- 2.9.4 Spills containment (biohazardous ad 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 correct techniques to the above 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

On completion of this module, the students must

- a critically evaluate safety in the laboratory in which they work.
- b where necessary, point out shortcomings in any of the above and make recommendations for corrective action.

Recommended textbooks 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
- ➢ IATA Regulations
- Road safety regulations Transportation of goods
- SOPs and SDS

Light microscopes

A Outcomes

On completion of this module the student must

- a be able to recognise the component parts of light microscopes.
- b be able to set up and operate a light microscope.
- C set up a microscope for Köhler illumination

B Objectives

The student must be able to:

- 3.1 recognise the essential components of:
 - 3.1.1 light microscopes
- 3.2 appropriately clean the basic components of a light microscope
 - 3.2.1 Lenses
 - 3.2.1 Eye pieces
- 3.3 Know how to store and transport a light microscope

3.4	hav	e a working knowledge of:
	3.4.1	Digital Image capture and archiving
	3.4.2	Legal implications of transmitting images
	3.4.3	Legal implications of altering / editing images
	3.4.4	Image capture resolutions
	3.4.5	Cloud technology or Storage as a Service

C Evaluation

On completion of this module

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

D Recommended textbooks for Module 3

- Theory and Practice of Histological Techniques, John D Bancroft and Marilyn Gamble, 5th or 6th Edition.
- E Other
 - SOPs
 - Instrument instruction manuals

Fixation and Fixatives

A Outcomes

On completion of this module the student must

- a have a thorough knowledge of fixatives, their components and fixation.
- b be able to predict the effect of specific fixatives on tissues and organs.
- c be able to recognise poor fixation and fixation artefacts.
- d be able to carry out appropriate corrective action to restore fixation and fixation related artefacts

B Objectives

The student must

- 4.1 know how to prepare, be familiar with the special purpose, health hazards and associated advantages / disadvantages of the following tissue fixatives:
 - 4.1.1 Formaldehyde / formalin / methanol-free formalin
 - 4.1.2 Glutaraldehyde
 - 4.1.3 Mercuric chloride
 - 4.1.4 Potassium dichromate
 - 4.1.5 Substitute fixatives for mercurials such as zinc sulphate and zinc chloride and barium salts
- 4.2 Be able to define and have a thorough understanding of fixation including the terms:
 - 4.2.1 microanatomical,
 - 4.2.2 cytological and
 - 4.2.3 histochemical fixatives
- 4.3 know why tissues are fixed
- 4.4 be able to recognise macroscopic fixation artefacts and take necessary corrective measures
- 4.5 be able to fix impression smears and squash preparations from fresh tissue
- 4.6 centrifuge, prepare smears and suitably fix body fluids

C Evaluation

Evaluation will be in the form of a theoretical competence test, in which the student must satisfactorily describe aspects of fixation.

Recommended textbooks 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.

Tissue processing

5.1 Conventional processing

A Outcomes

On completion of this module the student must:

- a have a thorough knowledge of tissue processing and the solutions used within the scope of this syllabus
- b be familiar with decalcification agents
- c be familiar with dehydrating and ante-media / clearing agents
- d be able to recognise macroscopic processing artefacts and take corrective action

B Objectives

The student must

5.1.1 be able to define and / or explain:

5.1.1.1	"tissue processing"
5.1.1.2	the four major components of tissue processing
5.1.1.3	the purpose of "dehydration"
5.1.1.4	the purpose of "clearing"
5.1.1.5	the purpose of "impregnation"
5.1.1.6	factors influencing the rate of impregnation
5.1.1.7	the aims and purpose of embedding and embedding media (waxes & resins)
5.1.1.8	why alternative embedding media may be required
5.1.1.9	automated tissue processors
5.1.1.10	the necessity of replacing processing fluids

5.1.2 be familiar with:

5.1.2.1	the commonly used dehydrating solutions
5.1.2.2	selection of suitable clearing agents
5.1.2.3	why additives are included in waxes for processing
5.1.2.4	the use and effects of vacuum impregnation in tissue processing.
5.1.2.5	ways of increasing the speed of conventional processing
5.1.2.6	automated processing schedules.
5.1.2.7	manual processing schedules
5.1.2.8	the recycling of processing reagents (how, why, advantages and disadvantages)

5.1.3 be aware of:

- 5.1.3.1 dehydrating agents that do not need intermediate clearing agents (i.e. microwave processing, etc)
- 5.1.3.2 the toxicity of many reagents used in tissue processing (ethanol, methanol, xylene, toluene, benzene, dioxane, methoxymethanol, acids and commercial decalcification fluids etc)
- 5.1.3.3 why tissue must be oriented for embedding
- 5.1.3.4 the necessity of replacing processing fluids
- 5.1.3.5 the use of alternative embedding media
- 5.1.3.6 how to rescue tissue that has dried out in a tissue processor

5.1.4 have knowledge of:

5.1.4.1 How to restore the colour of previously fixed specimens for photographic and Museum mount purposes (Kaiserling solutions and procedure)

C Evaluation

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

5.2 Microwave processing

A Outcomes

On completion of this module the student must

- a have a thorough knowledge of microwave tissue processing
- b be familiar with microwave dehydrating and clearing agents
- c be familiar with all aspects of microwave processing
- d be able to macroscopically recognise microwave processing artefacts and take corrective action

B Objectives

The student must

- 5.2.1 be able to define and / or explain:
 - 5.2.1.1 microwaves
 - 5.2.1.2 microwave ovens
 - 5.2.1.3 safety aspects of microwaving

5.2.2 be familiar with:

5.2.2.1	microwave fixation of unfixed fresh tissue
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- 5.2.2.2 microwave stimulated fixation and microwave-assisted fixation
- 5.2.2.3 microwave processing
- 5.2.2.4 choice of an inter-medium
- 5.2.3 be aware:

5.2.3.1	of why conventional microwave ovens are not suitable for tissue processing
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- 5.2.3.2 of the dangers of metallic objects in microwave ovens
- 5.3.3.3 that microwaves can be used to aid staining
- 5.3.3.4 of the advantages of microwave processing
- 5.3.3.5 of the need for melted wax in the microwave processor

5.2.4 Know how to:

- 5.2.4.1 Perform routine cleaning and maintenance on:
 - Routine tissue processors and related components
 - Microwave tissue processors and related components
 - Embedding machines and related components and equipment

5.2.4.2 Safely discard reagents and chemicals from the equipment listed in 5.2.4 as well as apply appropriate Health and Safety measures associated with the above tasks

C Evaluation

Evaluation will be in the form of a theoretical competence test, in which the student must satisfactorily demonstrate a thorough knowledge of tissue processing and processors.

Recommended textbooks 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.
- Microwave cookbook for microscopists: art and science of visualization, L P Kok and Mathilde E Boon.

Microtomes and section cutting

A Outcomes

On completion of this module the student must

- a have a thorough knowledge of the use of microtomes and microtome knives
- B be able to set up a microtome work area ergonomically
- c be able to use a microtome; and sharpen and use microtome knives safely
- d be able to recognise cutting artefacts and employ corrective measures

B Objectives

The student must

- 6.1 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
 - 6.1.3 rake, clearance angle and cutting angle
 - 6.1.4 effects of slope angle

6.2 have a 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	microtomes (sliding, rotary, sledge, cryostat)
6.2.5	advantages of disposable knives

6.3 have a good understanding of:

- 6.3.1 the importance of ergonomic microtome set up in relation to positions associated with injury and repetitive motion disorders
- 6.3.2 The preventative measures / corrective action associated with 6.3.1

6.4 *be familiar with:*

- 6.4.1 the different aspects of sectioning embedded tissue
- 6.4.2 adhesives (albumen, gelatine poly-l-lysine and 3-amino-propyltriethyloxy silane [APES])

6.4.3 the use of charged glass slides – and where appropriate to use depending on the application

6.5 be able to:

6.5.1	Recognise faults in wax sectioning
6.5.2	Remedy faults in wax sectioning
6.5.3	Understand and explain the most appropriate methods for cooling blocks for cutting
6.5.4	State the purpose of and describe the procedure for drying sections
6.5.5	Select the most appropriate adhesive according to the technique performed
6.5.6	Cite examples of situations when adhesives are recommended
6.5.7	Explain the effects of drying sections on the quality of tissue staining result

- 6.6 *The student must be familiar with:*
 - 6.6.1 The criteria for orientating tissue correctly for embedding and subsequent sectioning
 - 6.6.2 The different aspects of sectioning (frozen and wax) embedded tissue with special reference to tissue orientation, size and type (lumens, skins, curetting's, prostatic chips etc)
- 6.7 The student must be able to:
 - 6.7.1 State the purpose / reason for mounting sections
 - 6.7.2 List the criteria for a good mounting media
 - 6.7.3 Describe resinous and aqueous mounting media and give an example of each
 - 6.7.4 Describe the procedure of mounting
 - 6.7.5 Describe the automatic coverslipper (tape and glass coverslips)
 - 6.7.6 Describe the advantages and disadvantages of 6.7.5 in relation to:
 - The tape / glass (use and removal)
 - Cleaning and maintenance
 - Long-term storage of archived slides
 - Impact on optical resolution
 - Specimen type (cytology etc)

6.8 *The student must be familiar with:*

- 6.8.1 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, cryostat, automated)
- Waterbaths / "floatation baths"
- Incubators
- Automated Coverslippers and mountants

C Evaluation

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

Recommended textbooks 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.
- Laboratory SOPs and Equipment manuals

Frozen sections

A Outcomes

On completion of this module the student must

- a have a thorough knowledge of frozen section microtomes and cryostats
- b have a working knowledge of freeze-drying and freeze substitution

B Objectives

The student must

7.1 be able to define and / or explain:

7.1.1	the advantages and disadvantages of cryostat sections
7.1.2	when frozen sections are preferable to paraffin sections and why
7.1.3	staining of frozen sections for urgent diagnosis
7.1.4	Recognise faults in frozen sectioning
7.1.5	Remedy faults in frozen sectioning
7.1.6	Understand and explain the most appropriate methods for cooling blocks for cutting
7.1.7	the advantages of frozen sections compared with other embedding techniques
7.1.8	the cabinet and temperature
7.1.9	the blade / knife temperature
7.1.10	the set-up and use of the anti-roll plate

- 7.2 be able to:
 - 7.2.1 Describe the basic daily maintenance procedures of the cryostat
 - 7.2.2 Describe disinfection procedures of the cryostat
 - UV light
 - Solutions and vapourisation

7.3 *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 cryostat (including appropriate disposal)
- 7.3.3 Appropriate decontamination of all equipment used in the preparation procedure of fresh unfixed tissue

7.3.4 Procedures to follow in the event of an injury on duty involving sharp instruments

C Evaluation

Evaluation will be in the form of a theoretical competence test, in which the student must satisfactorily demonstrate the procedure for producing sections cut with cryostat.

Recommended textbooks 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.

E Other

- SOP's and Equipment Manuals
- OHASA
- Laboratory Health and Safety
- > First Aid

Theory of staining

A Outcomes

On completion of this module the student must

- a have a basic knowledge of the theory of staining
- b understand why tissue are stained
- c be familiar with all staining procedures encountered in a diagnostic histopathology laboratory (as contained in this syllabus)
- d be aware of the importance of the use of "control tissue" for specific diagnostic staining procedures
- e be able to make up most staining solutions and associated buffers

B Objectives

The student must

- 8.1. be able to define and / or explain:
 - 8.1.1 effect of fixation on staining
 - 8.1.2 progressive and regressive staining
 - 8.1.3 mordants and differentiators
- 8.2. be able to:

8.2.1	Operate automated <i>routine</i> staining instruments (e.g. H+E linear strainers)
8.2.2	Load reagents appropriately
8.2.3	Perform routine cleaning and maintenance on these instruments
8.2.4	Apply appropriate Health and Safety measures associated with the above tasks

C Evaluation

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

Recommended textbooks for Module 8

Theory and Practice of Histological Techniques, John D Bancroft and Marilyn Gamble, 5th or 6th Edition.

Staining of specific tissue elements

A Outcomes

On completion of this module the student must

- a have a thorough knowledge of the staining and preparative procedures covered in this syllabus
- b be able to logically reason / decide / deduce which stain to use for a specific component / structure
- c be familiar with the use of "control" tissue

9.1 The haematoxylins

B Objectives

The student must

- 9.1.1 be able to define and / or explain:
 - 9.1.1.1 The origin of haematoxylin
 - 9.1.1.2 The oxidation / ripening process of haematoxylin
 - Natural
 - Chemical
 - 9.1.1.3 Alum haematoxylins
 - 9.1.1.4 Iron haematoxylins
 - 9.1.1.5 Tungsten haematoxylin
 - 9.1.1.6 State the principle of the Haematoxylin + Eosin [H+E] stain
 - 9.1.1.7 Describe the H+E regressive and progressive staining method
 - 9.1.1.8 Describe Eosin and its use with and without Phloxine
 - 9.1.1.9 'blueing techniques and choice of blueing solution
 - 9.1.1.10 Describe the use of mordants [dye lakes], accentuators, accelerators and differentiators
 - 9.1.1.11 Describe the phenomenon of "metachromasia" and give examples of its use
 - 9.1.1.12 Negative and positive tissue controls

9.1.2 *be familiar with:*

9.1.2.1 The disadvantages of alum haematoxylins
9.1.2.2 Counterstains used after haematoxylin staining (neutral red etc.)
9.1.2.3 The Biological stain commission (BSC)
9.1.2.4 The purpose of a certification number on a stain or dye and what this indicates

9.1.2.5 Common nuclear and cytoplasmic stains

9.2 Special staining techniques

B Objectives

The student must

- 9.2.1 Describe each of the following staining techniques giving i.e. the name of the stain, elements stained, principle of the stain, major reagents, method outline and expected results as well as any hazards and special precautions with handling of stain / solution components
 - 9.2.1.1 Gram stain
 - 9.2.1.2 Long Ziehl Neelsen
 - 9.2.1.3 Perl's Prussian blue
 - 9.2.1.4 Verhőeff's method
 - 9.2.1.5 Van Gieson
 - 9.2.1.6 Masson's trichrome (three solution and single solution Trichrome stains)
 - 9.2.1.7 Mallory's Phosphotungstic acid haematoxylin [PTAH]
 - 9.2.1.8 Silver reticulin stain (Gordon and Sweet)
 - 9.2.1.9 Oil red O
 - 9.2.1.10 Von Kossa
 - 9.2.1.11 Masson-Fontana
 - 9.2.1.12 Congo red
 - 9.2.1.13 Periodic acid-Schiff (PAS)
 - 9.2.1.14 Alcian blue/PAS and PAS/Alcian Blue
 - 9.2.1.15 PAS with diastase digestion
 - 9.2.1.16 Giemsa
 - 9.2.1.17 Rhodanine for copper
 - 9.2.1.18 Southgates' Mucicarmine
- 9.2.2 be able to explain / describe / define:

9.2.2.1	The classification of pigments
9.2.2.2	The causes of pigment formation
9.2.2.3	Extraction methods for the following pigments:
	Formalin
	Mercury

Mercury Melanin Dichromates

9.2.3 be familiar with

- 9.2.3.1 factors affecting special staining (tissue permeability, mordants, precipitation, dye molecular size, heat and pH)
- 9.2.3.2 nuclear stains for trichrome methods
- 9.2.3.3 fixation factors for optimal staining

C Evaluation

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

Recommended textbooks for Module 9

Theory and Practice of Histological Techniques, John D Bancroft and Marilyn Gamble, 5th or 6th Edition.

Histology of tissues

A Outcomes

On completion of this and the previous module the student will be able to

a identify the four basic tissue types

B Objectives

The student must

- 10.1 be able to identify, describe and classify:
 - 10.1.1 Epithelial tissue
 - 10.1.2 Connective tissue
 - 10.1.3 Muscular tissue
 - 10.1.4 Nervous tissue

C Evaluation

Evaluation will be in the form of a theoretical competence test, in which the student must satisfactorily demonstrate a thorough knowledge of the basic tissue types.

Recommended textbooks for Module 10

- > Wheater's Functional Histology: A text and colour atlas, Paul R Wheater, 2000.
- > An Atlas of Human Histology, Mariano S. di Fiore, 1967.

IMPORTANT NOTICE

THE STUDENT TECHNICIAN MUST BE DEEMED COMPLETELY COMPETENT IN ALL PRACTICAL AND THEORETICAL ASPECTS BEFORE THE TRAINING SUPERVISOR SIGNS THE TRAINEE OFF AS READY FOR EXAMINATION. THE HPCSA, AS THE REGULATORY BODY, HAS THE RIGHT TO CALL FOR THE STUDENT'S WRITTEN EVALUATIONS REQUIRED AFTER EACH MODULE OF THE SYLLABUS AS WELL AS EVIDENCE OF COMPETENCY (COMPETENCY RECORDS), BOTH PRACTICAL AND THEORETICAL. A LABORATORY TRAINING PROGRAMME FOR EACH STUDENT TECHNICIAN MAY ALSO BE REQUESTED.

APPENDIX 1 - RESOURCES

Useful textbooks / References / literature for students and Trainers

<u>Useful Links</u>

<u>Leica</u>

http://www.leicabiosystems.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/

Histotechnology

stainsfile.info/StainsFile/jindex.html Useful information about dyes, stains and staining

<u>www.ihcworld.com/index.htm</u> 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): Junqueira'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). Wheatear'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 Tchniques. London. Butterworths.
- Bancroft, John D and Cook, Harry C. (1994). Manual of histological techniques and their diagnostic applications. Churchill and Livingstone.
- > Bancroft, John D and Gamble. Theory and practice of histological techniques. 5th or 6th Edition.
- Bayliss High, O.B and Lake, B. (1996). Lipids.
- Glauert, Audrey M and Lewis, Peter. (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.
- > Truter, E J and van Wyk, W. Fundamentals of Cellular Pathology.
- > Wallington, E.A. (1979). Artefacts in tissue sections. *Medical laboratory Sciences* 36, 3-61.

Websites:

- ✓ <u>http://www.Leica-biosystems.com</u>
- ✓ <u>http://www.dako.com</u>
- ✓ <u>http://roche.com</u>
- http://ebiz.thermofisher.com/flash_movies/fse_p_basic/index.php
- <u>http://ebiz.thermofisher.com/flash_movies/fse_s_buffer_prep/index.php</u>
- <u>http://www.vectorlabs.com/tutorials.aspx</u>
- ✓ <u>http://www.hpcsa.co.za</u>
- ✓ http://www.smltsa.org.za
- ✓ <u>http://www.cdc.gov</u>
- ✓ http://eppendorfna.com

Other:

- Government Gazettes.
- OHASA legislation / Acts and ISO / SANAS.
- All laboratory SOPs.
- All instrument manuals.
- ✤ All product inserts and Safety Data sheets.