PROFESSIONAL BOARD FOR
MEDICAL TECHNOLOGY

SYLLABUS

CYTOGENETICS

MEDICAL TECHNOLOGISTS
MEDICAL LABORATORY SCIENTISTS (BHSc)

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

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1. INTRODUCTION

CYTOGENETICS is a term used for a discipline which incorporates the disciplines of standard cytogenetics and molecular cytogenetics which in recent years includes fluorescent-in-situ-hybridization (FISH) and PCR studies. Recently referred to as CYTOGENOMICS. Wherever the term 'laboratory' is used throughout this syllabus it is implicit that this applies to these studies.

The objective of this syllabus is to provide the intern/student technologists or laboratory scientists with a guideline on the essential aspects that must be covered in order to adequately prepare themselves for the HPCSA's Professional Board of Medical Technology examination.

The examination will consist of 2 theory papers written on the same day. Candidates should expect a mixture of essays and short questions. No reference material may be taken into the examination.

Both papers will be broadly based and will cover the entire field of study. Students will be asked to draw on theoretical and practical knowledge acquired during their studies at the University of Technology as well as the 4th year internship. Emphasis will be placed on problem solving and on practical application of theoretical knowledge, as expected from any competent technologist. Candidates will not be expected to memorise specific details and quantities of reagent preparations. They will however be expected to know the principles on which tests are based and how to interpret the test results.

Candidates must achieve a minimum mark of **50% for each paper,** to pass the examination.

NB: Students are reminded that this document is merely a guideline intended to aid the study process. As specialists in a discipline, they are expected to keep their knowledge current and to have an in-depth understanding of their subject.

Each section has an Evaluation section, if no questions are posed, a practical assessment should be done. Also, a portfolio of evidence should be compiled for submission on the completion of the training period for assessment.

Please refer to:

- Nomenclature / Acronyms
- Appendices
    - 11.1 Definitions of acronyms contained in the syllabus
    - 11.2 Recommended text books

HPCSA regulations require that accredited training laboratories perform a minimum of 80% of the tests identified in this syllabus. Laboratories are required to ensure that Interns receive appropriate training in the tests contained within the syllabus but which are not routinely performed on site. (Where practical training at an alternate training facility is not feasible, minimum of theoretical and written assessments are compulsory)

2. STATUTORY REGULATIONS AND ETHICS

Objective

Provide the Intern/ student with information on the regulations and ethical principles which underpin the practice of Medical Laboratory Technology.

Specified outcomes

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

- Demonstrate knowledge of the structure and function of the Health Professions Council of South Africa (HPCSA),
- Demonstrate knowledge of the structure and function of the Professional Board for Medical Technology (PBMT).
- Discuss the regulations relating to the scope of practice for Medical Technologists.
- 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).
- Demonstrate knowledge on the practice/ ethos of how confidentiality in the workplace is achieved and maintained.
- Demonstrate knowledge Acts and Regulations:
  - No. 61 of 2003: National Health Act, 2004
  - Health Professions Act 56 of 1974
  - POPI Act
  - Patients Right Charter
  - Children's Act
- Discuss the ethical rules relating to the practice of Medical Technology with special reference to:

  a) Private practice.

  b) Reporting of results and confidentiality.

  c) Legal and ethical standards of laboratory practice and principles related to the testing of clinical specimens.

- Describe the scope of the profession of medical technology as prescribed by the Professional Board (HPCSA) ●

3. TOTAL QUALITY MANAGEMENT

Objective

A comprehensive study of maintaining a Quality Management System and Laboratory Accreditation in accordance with local (SANAS) and international (e.g. ACC) regulations and guidelines, as well as proficiency testing (e.g. CAP). The study of laboratory safety procedures and regulations. The study of laboratory skills specific to amniotic fluid, chorionic villus sampling, abortus tissue, blood, bone marrow and solid tissue (skin biopsies and products of conception) samples.

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 relating to laboratory safety procedures.

Describe/evaluate/formulate the components involved in a Quality Management System and Laboratory Accreditation (in keeping with the relevant ISO standards) under the following headings:

Specified Outcomes

At the completion of this module, the student will be able to:

1. Discuss correct procedures to store, handle and dispose of materials/waste and sharp/glass
2. Discuss the procedures required for laboratory safety
3. Follow standard precautions for biological hazards using e.g. PPE and biosafety cabinets
4. Follow Material Safety Data Sheet guidelines
5. Follow emergency procedures (e.g. use of eye wash bottles, spill kit)
6. Practice proper ergonomics (e.g. chair adjustment, posture)
7. Document and investigate all laboratory accidents (e.g. needle sticks, spills, splashes)
8. Document participation in required safety training
9. Distinguish between quality control and quality assurance
10. Describe SANAS accreditation and ISO15189 guidelines. Knowledge of the role it plays in a laboratory's quality.
11. Participate in laboratory proficiency testing and accreditation site inspections.
12. Document training for competency assessment
13. Verify accuracy and reproducibility of results
14. Discuss the basic concepts required for successful tissue culture
15. Demonstrate correct use of laboratory equipment, e.g. centrifuges, pipettes, microscopes etc. – record daily temperatures, % CO_2, humidity)
16. Monitor equipment function and report deviations
17. Perform preventative maintenance of equipment

18. Maintain quality control records of
- Culture failure/contamination
- Growth support for media components
- Reagent performance

19. Record quality indicators as directed (e.g. band level, turnaround time)
20. Demonstrate basic cytogenetic laboratory techniques necessary to prepare tissue samples or cytogenetic diagnosis
21. Perform sterilization/decontamination procedures
22. Follow appropriate cleaning procedures for laboratory instruments, equipment, and work surfaces
23. Monitor adequate stocks and expiry dates of laboratory supplies and chemicals
24. Maintain patient confidentiality and security of patient records
25. Respond to enquiries regarding laboratory tests (e.g. methodology, specimen requirements, reference values, collection procedures)
26. React to requests of on-site laboratory inspectors

Specified outcomes

On completion of this section 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; Hazardous Substances Act; Compensation for Occupational Injuries and Diseases Act*

- 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 the correct procedures for the storage, handling and disposal of laboratory waste.

- Identify and describe the use of all safety equipment:

Fire hose Fire blanket spill kits fire alarms Fire extinguishers Safety shower Eye wash station First aid box

- Describe the application of laboratory safety procedures to the collection, transport, storage and analysis of biological specimens including the International Air Transport Association (IATA) regulations.

- ○ <u>Range</u> - *Biological specimens; Human tissue; Solid and liquid bio-hazardous waste; Radioactive waste; Sharps*
- ○

- Describe the basic principles for the storage, handling and disposal of chemicals; poisons; flammable substances; gases and infectious material.

Biological specimens	Solid and liquid biohazard waste
Human tissue	radioactive waste
sharps	

- Describe procedures to follow for the prevention, control and management of laboratory acquired infections including general housekeeping and decontamination of equipment.
- Describe the purpose and basic content of the material safety data sheets (MSDS).
- Demonstrate knowledge of the protocols to follow in the event of injuries on duty including needle-stick injury.

- Define the role of the designated safety personnel.

Range

Fire marshal
Safety representative
First aid officer

- Recognise the international safety symbols used in the laboratory environment.
- Demonstrate knowledge of all safety and emergency equipment.

Evaluation

1. Show evidence of relevant QC activities performed.

3.2 SPECIMENS / PRE-ANALYTICAL REQUIREMENTS

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 this section the intern/student should be able to :.

- Describe the optimal specimen requirements for the individual tests.
- Describe the conditions under which the specimens must be transported to the laboratory.
- Display knowledge of the optimal storage conditions should testing be delayed and the stability of the specimen for the individual testing process.
- Where applicable, 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 and tracking of the specimen, aliquots and documentation.
- Identify criteria for the rejection of unsuitable specimens.
- Conduct the pre-analytical preparation required for specimen type and test requested.

3.3 LABORATORY EQUIPMENT

Objective

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

Specified outcomes– applicable to all equipment/instruments and analysers
On completion of this section the intern/student should be able to:

- Describe the principle of operation where applicable to discipline specific instrumentation.
- Operate all equipment optimally in accordance with the manufacturers recommended operating 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 within scope, of / for the specific instrumentation.
- Conduct applicable decontamination procedures.
- Apply the appropriate functional checks to ensure optimal operation
- Describe and implement troubleshooting procedures when optimal operation is not demonstrated by the instrument on-board functional checks.
- Demonstrate full knowledge of the maintenance procedures, all equipment records and documentation required for good laboratory practice.

○ Range -

- All glassware – volumetric and graduated
- Pipettes – glass, automated, air displacement and disposable
- Fridges
- Freezers
- Stopwatches/timers
- Thermometers – min/max, electronic and mercury
- Bio-hazardous safety cabinets – Class I and II
- Fume cupboards
- Pipette aids - rubber teats, pro-pipettes and dispensers
- Centrifuges, safety centrifuges

Laboratory instrumentation and automated analysers are included in this range – knowledge of the principals of instruments in use in the current workplace is required.

Laboratory equipment:

- Centrifuges
- Biosafety cabinets
- Incubators (aerobic and CO_2)
- Analytical balances
- Automated pipettes
- Water baths
- pH meters
- Microscopes (Fluorescent, inverted, dissecting)
- Adjustable & fixed volume pipettes
- Thermometers
- Thermocyclers
- Gel electrophoresis
- Capillary electrophoresis
- Pipettes (manual and automated)
- Microscopes (automated capture system)
- Spectrophotometer
- DNA extraction instruments

(The systems have been listed generically, to allow for inter-laboratory differences between specific types/makes of instruments used. The student is expected to have a basic understanding **of *one example of each*).**

Microscope and imaging equipment operation and maintenance

Objective

Operate a standard compound microscope, inverted microscope, dissecting microscope, fluorescence microscope and computerised karyotype equipment producing optimal chromosome images.

Specified Outcomes

At the completion of this module, the student will be able to:

1. Describe the parts and functions of the microscope
2. Achieve optimal resolution by
   - establishing Köhler illumination
   - using appropriate immersion oil
   - selecting appropriate magnification, cover glass
   - thickness
3. Troubleshoot microscopy
4. Maintain microscope
5. Describe the parts and functions of computerized imaging equipment
6. Have a working knowledge of the steps in capturing microscope images
7. Capture images of optimal resolution
8. Enhance images
9. Troubleshoot image enhancement
10. Understand Image Archiving

Evaluation

Practical assessment

3.4 LABORATORY REAGENTS

Objective

Provide details of the correct preparation, storage and disposal of laboratory reagents.

Specified outcomes

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

- Differentiate between controls and calibrators.
- Demonstrate knowledge of the objective, use and retention of package inserts/ instructions for use (IFU's).
- Prepare, store, and safely dispose of laboratory reagents including working reagents
- Define terms and solutions used in the laboratory:
  - Range - Physiologically normal saline; Buffer

3.5 STOCK CONTROL

Objective

Outline the processes involved in good materials stock management

Specified outcomes

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

- Demonstrate knowledge of the basic principles to apply when managing merchandise stock.
- 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 for all stock.
- Differentiate between open vial stability and expiry date
- Demonstrate knowledge of workplace 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 control.

Specified outcomes

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

- Discuss 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 of specimen handling.
- Demonstrate general knowledge on the terms accreditation, International Organisation for Standardisation (ISO) and South African National Accreditation System (SANAS).
- Demonstrate general knowledge on the use, performance and evaluation of RISK assessments.
- Define and explain all quality assurance terminology.
  - Range -
    - Non-conformance
    - Corrective action
    - Preventive action
    - Root cause analysis
    - Continual improvement of quality assurance and quality control processes
    - Audits – Internal & External

3.7 QUALITY CONTROL

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

Specified outcomes
On completion of this section the intern/student should be able to:
- Describe and apply the appropriate quality control processes which must be performed and applied to all the analyses as well as equipment and reagents 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 and interpretation of all related internal and external, quantitative quality control data.
- Apply a sound knowledge of all the principles, procedures and interpretation of all related 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 and explain all quality assurance terminology:

Range:

• Non-conformance	Root cause analysis
• Corrective action	Continual Improvement
• Preventative action	Audits – Internal and External

3.8 METHOD VALIDATION

Objective
Expose the Intern/student to all aspects of method validation.

Specified outcomes
On completion of this section the intern/student should be able to:
- Differentiate between validation and verifications in terms of relevant ISO standards.
- Demonstrate an understanding of the approach to the validation and/or verification of new equipment, reagents and testing kits (Qualitative and Quantitative).

  - Validation of new techniques, updating old techniques.
  - Validation of probes, primers, and instruments / equipment

3.9 PERSONNEL

Objective
Provide knowledge of basic requirements for personnel in terms of relevant ISO standards.

Specified outcomes
On completion of this section the intern/student should be able to:
● Describe the personal documents and records which are required for all laboratory personnel which falls within the scope of practice of Medical Technologists.
● Demonstrate an understanding of the terms ‘competency’ and ‘ongoing competency’ in terms of the training of all laboratory personnel which falls within the scope of practice of Medical Technologists. Know when or how competency is measured.

3.10 DOCUMENTATION

Objective

Provide knowledge of basic requirements of documentation in terms of relevant ISO standards.
Specified outcomes
On completion of this section the intern/student should be able to:
● Demonstrate knowledge of document control requirements in terms of relevant ISO standards.
● Demonstrate knowledge of the required content of SOP’s including the minimum content of the cover page.
● Know the process on how to render documents obsolete.
● Demonstrate knowledge on the retention and disposal of this documentation.
● Demonstrate knowledge on document control and regular review of prescribed documentation.
● Differentiate between a record and document.
○ Range - Policies; Procedures (SOPs); Working instructions; Raw data; Equipment records; Quality control records; Personnel records; Package inserts/ IFU’s

4. LABORATORY RELATED MATHEMATICS

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

Specified outcomes
On completion of this section the intern/student should be able to:
● Demonstrate proficiency in the calculations required for the preparation of solutions.
○ Range - Physiological saline; Percentage solutions

5. MOLECULAR BIOLOGY

Objective

Provide intern/student with a foundation knowledge of basic molecular biology as applied to techniques throughout the Medical Laboratory Technology disciplines.

Specified outcomes

At the end of this training the intern/student should be able to:

- Describe workflow dynamics in a molecular biology laboratory.
- Demonstrate and apply knowledge of the methods used for the prevention of contamination in a molecular laboratory.
- Demonstrate a fundamental knowledge of the function of DNA in terms of structure, replication, transcription and translation.
- Discuss the principle of the polymerase chain reaction (PCR) and the steps involved.
  - Range - Denaturation; Annealing; Extension
- List the components of a PCR master mix and explain the purpose and action of each component.
- Discuss the role of primers used within a PCR laboratory.
- Demonstrate knowledge of the quality controls used in the assay procedure.
- Identify the potential causes of false positive and negative results.
- Identify potential causes of interference in the PCR process.
- A basic understanding of what probes are and how they are used in real-time PCR.
- A basic understanding of the PCR graph and Ct values (how the Ct values are used in quantitative and semi-quantitative PCR's).
- Understand the difference between conventional PCR and real-time PCR.
- Understand the principle and purpose of reverse transcription PCR (cDNA synthesis).
- Understand the difference between multiplex and single-plex PCR's.
- Demonstrate basic practical knowledge of the techniques utilised for the automated extraction, amplification and detection.
- Explain the principle and basic introductory level information of agarose gel electrophoresis.
- Demonstrate an understanding of medical disorders that are tested for by using Molecular techniques.

6. SPECMEN RECEPTION

Objective

Identify appropriate specimens for study and methods of collection, preservation and transport, assess acceptability of specimens – type and quality, verification and recording of patient data, tracking of specimens, documentation.

Specified Outcomes

At the completion of this module, the student will be able to:

1. Identify appropriate samples: Amniotic fluid, Chorionic villi samples, blood, Bone marrow aspirate / blood smears, trephine imprints (slides),POC,skin.
2. Identify factors important for the transport of specimens, e.g. delivery time, transport media and containers, condition (e.g. temperature, time, sterility)

- Assess and handle specimens by **type**:
- peripheral blood, cord blood via periumbilical blood sampling (PUBS) / cordocentesis
- bone marrow and core biopsy
- amniotic fluid
- solid tissues (e.g., skin biopsies, products of conception)
- chorionic villi
- lymph nodes, tumors, malignant effusions

3. Assess and handle specimens by **quality**:

- volume
- viability
- bacterial or fungal contamination
- presence of clots
- colour

4. Completion of relevant documentation, laboratory information system and traceability of samples
5. Troubleshoot compromised / unacceptable specimens
6. Notify appropriate authorities (e.g supervisor) of compromised/unacceptable specimens,
7. Split samples appropriately for multiple tests
8. Enter or verify appropriate data:

- Patient information (e.g., name, identification number, date of birth, sex, clinical

  history, indication for study, referring physician, billing information)

- Specimen information (eg., type of specimen, quality, date/time of collection)
- Confirm appropriate test, priority status, and patient data based on reason for referral

Evaluation

1. What is the appropriate specimen for constitutional Cytogenetic blood analysis? Discuss appropriate storage and transport of the specimen.
2. Mention effects of quality of the specimen on culture, e.g. haemolysis.
3. Illustrate a completed referral form containing all the appropriate data required.
4. Describe the laboratory information system and how samples can be traced.
5. When is a sample unacceptable? Explain troubleshooting in the case of receiving such a sample.
6. Discuss when, how and why you would split an amniotic fluid where chromosome culturing and FISH or PCR were requested.

7. SPECIMEN SET UP AND CULTURE

Objective

The use of aseptic techniques, preparation of appropriate media for specimens, apply appropriate culture techniques for specimens, monitor cell growth and trouble-shooting.

Specified Outcomes

At the completion of this module, the student will be able to:

1. Outline the criteria for acceptable specimens – at end of this module, student will be able to:
    a. Outline the criteria for acceptable specimens and rejection criteria,
    b. Evaluate cultures for appropriateness of request,

2. Select an appropriate culture system:

- Method – suspension, *in situ*, or monolayer.
- Appropriate Tissue – products of conception, maternal vs decidua.
- Specimen Preparation – mince, enzymatic

3. Prepare media and solutions for culture:

- prepare media and supplements (e.g., buffers, growth factors, fetal bovine serum, mitogens, essential amino acids, and antibiotics)
- perform tests to assess sterility and ability to support growth prior to use
- acquire a thorough working knowledge of the purpose and function of media and solutions

4. Monitor environmental and culture variables and their effect on cell growth:

- Maintain cultures
- Detect, identify, culture contamination
- Evaluate and subculture monolayer cells
- Select for harvest
- Recognize and troubleshoot culture failures

5. Select culture conditions / additives for

- routine cytogenetics; molecular / biochemical testing
- special tests (e.g., breakage syndromes, B cell mitogens, growth factors)

6. Select/determine appropriate number of cultures and inoculation based on cell count and cellularity eg.leukeamic specimens.

7. Select incubation period
8. Perform aseptic culture techniques:

- Employ measures that protect samples from microbial contamination e.g., bacterial, fungal, or mycoplasma).
  - Prevent cross-contamination between cultures by using appropriate labelling techniques and physical isolation techniques (e.g. handling single specimens)

Evaluation

1. Explain aseptic technique.
2. Discuss buffer systems used in cell culture.
3. Discuss the appropriate conditions required for amniotic fluid culture.
4. What is the function of the following reagents during culture: PHA and ethidium bromide?
5. Describe the conditions that would alert a technologist to the presence of an infected culture. Explain the steps that should be taken to treat the infection and prevent the spread within the culture system.

8. Principles and techniques for harvesting specimens of cell cultures

Objective

Determine optimal timing of harvest, use appropriate harvest procedures for specimen or culture, and prepare slides with analysable metaphases

Specified Outcomes
At the completion of this module, the student will be able to:

1. Employ knowledge of cell cycle and synchronizing agents during the harvest of cell cultures
2. Select an appropriate harvest type:

- suspension (e.g., direct, 24-hour, 72-hour)
- *in situ* (colonies)
- culture flask of an adherent cell population (monolayer)

3. Understand the different steps in the set up and harvest of different kinds of samples

4. Understand and use chromosome elongation techniques:

- synchronization and release agents (e.g., thymidine, MTX)
- intercalation agents (e.g., ethidium bromide, actinomycin D)

5. Understand and use mitotic inhibitors, hypotonic solutions and fixation process
6. Prepare media and solutions for harvesting
7. Store cell pellets
8. Adjust for the effects of humidity, slide drying time, and temperature with respect to chromosome spreading

9. Assess slide quality by phase microscopy on mitotic index, chromosome morphology and appropriate spreading (e.g., intact cells free of cytoplasm)
10. Age slides appropriately
11. Assess harvest quality

12. Troubleshoot harvest errors associated with

- outdated or improperly prepared reagents
- missing or improper reagent steps
- mixed/ contaminated specimens

Evaluation

1. What is the function of the following reagents during harvest: colcemid, potassium hydroxide and fixative?

2. Summarise the cell cycle, explaining mitosis and meiosis, and how these stages produce trisomies, mosiacism, monosomies, balanced and unbalanced translocations.

9. Chromosome banding and staining techniques

Objective
Understanding of banding and staining methods which permits identification of each chromosome pair, at an appropriate level, slide storage, trouble-shooting of unacceptable or unanalysable results for all banding/staining procedures.

Specified Outcomes
At the completion of this module, the student will be able to:

1. Prepare solutions for staining
2. Demonstrate knowledge and ability to perform chromosome banding techniques
3. Perform G-banding using an enzyme (e.g. trypsin)
4. Store slides as required by regulation
5. Assess and troubleshoot banding or staining quality:
6. Slide aging
7. Reagent exposure time (enzyme and stain)
8. Stain intensity
9. Environmental factors (temperature, humidity, pH)
10. Perform Q-banding
11. Perform C-banding
12. Perform NOR banding
13. Understand Reverse Banding and why it may be used in the laboratory

Evaluation

1. What is the function of trypsin during staining?
2. Discuss the extra staining techniques which could be used to establish whether extra material on an acrocentric chromosome is satellite material or not. Explain the principles of the methods.

10. Chromosome analysis

Objective
Select suitable metaphases cells for analysis, performing accurate microscopic counts and analyses of banded and non-banded chromosomes, recording microscope identification, verniers, and cell analysis data on the selected cells, preparation of accurate karyotypes from computer images, identification of numerical and structural chromosome abnormalities, reporting according to relevant guidelines.

Specified Outcomes

At the completion of this module, the student will be able to:
1. Select and analyze suitable metaphases in a systematic manner including:

- chromosome morphology (e.g. stain intensity (band quality), background staining, number of chromosome overlaps)
- suitability: banding level for referral diagnosis and number of metaphases to analyse
- Identification of heterochromatic regions.
- Identify chromosomes and classify them into groups by sizes and centromere location.

2. Review previous results when available
3. Analyze cells from the appropriate number of cultures
4. Document analysis including
- Chromosome count
- Chromosome analysis
- Vernier coordinates

5. Troubleshoot difficulties in analysis
6. Repeat culture after evaluating the need for additional studies
7. Arrange G-banded chromosomes in a systematic manner, ie. Karyotyping.
8. Perform karyotyping using an automated karyotyping system:
- Select good quality images
- Arrange chromosomes using approved format – Classify the major chromosome groups by size and centromere location.
- Prepare an appropriate number of karyotypes
- Provide permanent copy of final karyotype

9. Describe metacentric, submetacentric and acrocentric chromosomes
10. Identify abnormal chromosomes and classify the abnormality ie. Duplication, deletion.
11. Identify numerical and structural abnormalities of autosomal and sex chromosomes from metaphase spreads
12. Classify the major chromosome groups by size and centromere location
13. Describe the major landmark bands for each chromosome
14. Classify metaphase chromosomes according to band level e.g. <400, 400, 500, 550, 650, >800
15. Understand and use various techniques to identify and evaluate clinical implications:
- Techniques: G-, Q-, C- and NOR banding
- Abnormalities: numerical, structural, mosiacism-vs-chimerism, cultural artefacts (e.g. pseudomosaicism), marker chromosomes and normal variants
16. Understand the rules of ISCN nomenclature used to describe both normal and abnormal chromosome constitution
17. Use the ISCN nomenclature to record results
18. Report and document preliminary results
19. Refer to previous studies and/or literature searches

Evaluation

1. Give the qualities of a well-stained metaphase that would be suitable for karyotyping.
2. Is high resolution banding more important in samples with possible trisomy, or in samples with a possible translocation, and why?
3. What could be the problem in the following circumstances: no cell growth, no metaphases,

low metaphase yield, poor spreading of metaphases, fuzzy or bloated chromosomes, infected cultures?

11. Prenatal Cytogenetics

Objective

A study of indications for prenatal diagnosis and the procedures used to obtain specimens for such diagnoses: ultrasonography, cordocentesis, amniocentesis and CVS. Problems encountered in prenatal chromosome analysis and the demonstration of prenatal cytogenetic techniques and procedures.

Specified Outcomes

At the completion of this module the student will be able to:

1. Identify the indications for prenatal diagnosis
2. Identify important quality issues in prenatal sample culture
3. Define different levels of chromosome mosaicism
4. Define maternal cell contamination and evaluate techniques employed in prevention and detection
5. Know the principles and use of relevant antenatal biochemical tests e.g. AFP, First trimester test, “triple test”, etc.
6. Have a basic knowledge of *in vitro* fertilization and pre-implantation genetics
7. Have a knowledge of specimen preparation and culture specific methods:

    - Macroscopic evaluation of specimen eg.size, turbidity, amount,
    - Appropriate culture media and incubation conditions,
    - Knowledge of specimen preparation and culture specific to prenatal specimen.
    - Macroscopic evaluation of specimen. Eg.size, turbidity, amount.
    - Assessment of cultures eg. Subculturing,
    - Evaluate cultures for appropriateness of harvesting.
    - Explain the role of CO_2 in the incubation of prenatal cultures.

8 Have an understanding and knowledge of principles of non-invasive prenatal testing (NIPT). Its limitations and advantages.

Evaluation

1. List the antenatal test which can be used to assess risk of foetal abnormality. Explain the theory and how it may be used as an indicator for amniocentesis.
2. Explain the use of a contrast-inverted microscope.
3. What are the pitfalls associated with CVS results?
4. Name limitations an advantages of NIPT

12. Syndromes and other clinical indications

Objective

A comprehensive study of disease aetiology, terminology and general principles of clinical genetics.

Specified Outcomes
At the completion of this module, the student will be able to:

1. Differentiate the abnormalities of chromosome number/structure and/or molecular abnormality with regard to terminology, aetiology and mechanisms in various syndromes including, but not limited to:

- Down syndrome
- Edward syndrome
- Patau syndrome
- Turner syndrome
- Klinefelter syndrome
- Prader Willi syndrome
- Angelman syndrome
- Di George syndrome
- Smith Magenis syndrome
- Miller Dieker syndrome
- Williams Beuren syndrome
- Wolf Hirschhorn syndrome
- Cri du Chat syndrome
- Fragile X syndrome
- Duchenne Muscular Dystrophy
- Cystic fibrosis

2. Triplet-repeat disorders – Huntington's disease, Muscular dystrophy, Spinocerrebellar ataxia,

3. Discuss the chromosomal abnormalities with regard to terminology, aetiology and mechanisms associated with, but not limited to:

- Recurrent miscarriages
- Infertility
- Azoospermia, Oligospermia
- Ambiquous genitalia
- Maternal age
- Familial reciprocal translocation
- Mosaicism

Evaluation

1. Discuss all the above with regards to terminology, aetiology and mechanisms.

13. Molecular cytogenetic techniques

Objective

A comprehensive understanding of molecular cytogenetic techniques: FISH, and QF-PCR, with basic understanding of CGH and MLPA.

Specified Outcomes

At the completion of this module, the student will be able to:

1. Describe how molecular cytogenetics complements conventional cytogenetics,
2. Perform, analyse, trouble-shoot and compile diagnostic reports for each assay.

A: Fluorescence *in situ* hybridization (FISH)

Objective

A study of the use of fluorescence *in situ* hybridization for antenatal and postnatal diagnosis, the procedures and techniques involved, and the recognition of relevant clinical indications related to specific syndromes and available probes.

Specified Outcomes

At the completion of this module the student will be able to:

1. Evaluate the analysis type (e.g. interphase or metaphase)
    - Sample preparation of different sample types.
2. Knowledge of sample preparation of different sample types.
3. Understand and perform denaturation
4. Understand and perform hybridize to target
5. Understand and perform post-hybridization
6. Use an appropriate counterstain
7. Troubleshoot FISH failures
8. Select the correct filter(s) for fluorescence microscopy
9. Score the appropriate type and number of cells including:
    - Interphase (e.g. prenatal, haematologic, tumour)
    - Metaphase (e.g. microdeletion, markers)
10. Capture appropriate FISH images
11. Recognise clinical indications
    - Appropriate reasons for performing antenatal FISH
    - Clinical features associated with microdeletion syndromes
    - Understand the choice of probe according to indications
    - Knowledge of types of probe: centromeric, locus specific, telomeric, whole chromosome paints,
12. Understand and be able to use short-term ISCN nomenclature.

Evaluation

1. Briefly summarise the steps used in the FISH protocol, explaining the theory of each step.
2. What influence does formamide have in the denaturation buffer?
3. Why is the sample washed in a stringency buffer after hybridisation?

B: QF-PCR

Objective

A study of the use of Quantitative fluorescent PCR for antenatal and post-natal diagnosis, the procedures and techniques involved, and the recognition of relevant clinical indications related to specific syndromes and available probes.

Specified Outcomes

At the completion of this module the student will be able to:

1. Understand and perform PCR
    - Analyse and compile diagnostic reports for each assay
    - Understand and be able to use the short form of ISCN reporting accurately.
    - Understand the advantages and limitations of the technique
    - Understand and perform DNA extraction from different sample types (manual and automation)
    - Understand the clinical indications of QF-PCR
    - Understand the advantages and limitations of the technique,
    - Undertand and perform DNA extraction from different sample types (manual and automated)
2. Understand and perform capillary electrophoresis using the relevant instrumentation
3. Troubleshoot failures
4. Recognise clinical indications

Evaluation

1. Give examples of the clinical use of QF-PCR.
2. Discuss the possible reasons why there is no PCR product detected on the electropherogram.
3. Discuss the principle of QF-PCR
4. Explain the concept of capillary electrophoresis,
5. Explain the function of HIDI in capillary electrophoresis

C: Cytogenomic Microarray(CMA)- array CGH (aCGH)

Objective

A study of the diagnostic use of CMA, the procedures and techniques involved, and the recognition of relevant clinical indications related to specific syndromes.

Specified Outcomes

At the completion of this module the student will be able to:

1. Understand the principles and the use of the relevant instrumentation of CMA.
2. Recognise clinical indications and limitations of CMA versus conventional cytogenetics – advantages and disadvantages.
3. Knowledge of DNA extraction and quality checks. Ie. Gel electrophoresis,
4. Perform, analyse, troubleshoot and compile diagnostic reports.
5. Understand and be able to use short form ISCN accurately.

Evaluation

1. Give examples of the clinical use of CMA,
2. Implications of inadequate washing conditions,
3. The role of electrophoresis step in this technique

D: MLPA

Objective

A study of the diagnostic use of MLPA and the procedures and techniques involved, and the recognition of relevant clinical indications related to specific syndromes.

Specified Outcomes

At the completion of this module the student will be able to:

At the completion of this module the student will be able to:

1. Understand the principles and the use of the relevant instrumentation of MLPA
2. Recognise clinical indications
3. Perform, analyse and trouble-shoot and compile diagnostic reports for the use of each indication.
4. Recognize clinical indications.

Evaluation

1. Give examples of the clinical use of MLPA.

E: Next-Generation Seqencing (NGS)

Objective

A study of the diagnostic use of NGS, the procedures and techniques involved, and the recognition of relevant clinical indications related to use.

Specified Outcomes

At the completion of this module the student will be able to:

1 Understand the principles and the use of the relevant instrumentation of NGS.

2 Recognize clinical indications

Evaluation

1 Give examples of the clinical use of NGS,

3 Briefly explain the principle of NGS and clinical indications.

13. Molecular genetics

Objective

The student will achieve upon completion of this course the basic ability to:

1. Explain specimen processing, collection
2. Explain steps to DNA extraction and purification
3. Explain the steps to PCR reaction
4. Identify steps to Real Time PCR
5. Have an understanding of Southern Blotting
6. Explain triple repeat tests e.g. SCA, etc.
7. Explain different resolution platforms e.g. agarose electrophoresis, capillary electrophoresis, etc.

Specified Outcomes

On the completion of this module, the student will be able to:
1. Explain DNA replication, transcription and translation\
2. Describe the process of PCR.
3. Describe the process of Real Time PCR
4. Describe the process of Southern Blotting
5. Describe the role of the promoter, transcription factors, and chromatin structure in determining gene expression
6. Demonstrate proper pipetting techniques
7. Demonstrate an understanding of the interpretation of results

Evaluation

1. Explain the steps in DNA replication, transcription, and translation.
2. Explain the steps involved in Southern Blotting. Discuss the reason for each.

14. Haematology chromosome analysis

Objective

A comprehensive study of the principles and procedures used in the genetic analysis of peripheral blood and bone marrow in the study of malignant processes, especially haematological. Emphasis will be made on the molecular and chromosome abnormalities occurring in leukaemia and lymphomas and their clinical significance.

Specified Outcomes

Upon completion of this module, the student will be able to:
1. Provide advice on appropriate specimen for study, its collection, preservation, and transport
2. Select and prepare appropriate media for specific specimens

3. Judge quality of bone marrow specimens through the use of visual inspection, haematology reports and cell counts
4. Use appropriate culture techniques for blood and bone marrow
5. Use appropriate harvest procedures for specimen type, and obtain analyzable chromosome preparations
6. Understand and perform G-banding by trypsin
7. Produce computer images displaying optimal specimen definition by mastering capture, enhancement and karyotyping software
8. Perform accurate counts and analyses of banded chromosomes under the microscope
9. Prepare accurate karyotypes
10. Recognize abnormalities of chromosome number and structure and their implications for hematopoietic studies.
11. Understand and be able to use accurately the short form nomenclature for human chromosomes
12. Determine what constitutes a complete cytogenetic analysis of haematopoietic tissues
13. Understand the reporting procedures for cytogenetic analyses of haematopoietic tissues
14. Understand the Quality Control and Quality Assurance procedures required in a Cytogenetics laboratory

Evaluation

1. Explain the principle of blocking the cell cycle with methotrexate and the benefits of this culture technique.
2. How would cell count affect culture success, and why would this vary in a bone marrow sample?
3. Why might there be 2 cell lines, 46,XX /46,XY, post-transplant? What would be the significance of the ratio?

15. Haematology FISH

Objective

A comprehensive study of principles and procedures used in the genetic analysis of peripheral blood and bone marrow in the study of malignant processes, especially haematological ones using fluorescence in situ hybridization techniques. Emphasis will be made on the molecular and chromosome abnormalities occurring in leukaemias and lymphomas and their clinical significance.

Specified Outcomes

Upon completion of this module, the student will be able to:

1. Provide advice on appropriate specimen for study, collection, preservation and transport.
   1.1 Prepare different sample types,

2. Prepare appropriate reagents and know their functions.
3. Differentiate by chromosome abnormality, probe type and basic disease description of various haematological diseases including but not limited to:
    a. Myeloid neoplasia – CML, AML, MDS
    b. Lymphoid neoplasia – ALL, CLL, Myeloma, Burkitt's, Diffuse large B-cell, Mantle cell and Follicular lymphoma.
4. Have a knowledge of which disorder/s the probes are associated with.
5. Prioritise specimens according to urgency e.g. APL, new leukaemia.
6. Judge the quality of samples during pre-treatment
7. Be able to make acceptable smears from blood and bone marrow aspirates,
8. Understand the different types of FISH probes available e.g.Break-apart, Dual-colour, dual-fusion and deletion probes
9. To recognize colocalization and split signals,
10. Be able to identify the signal patterns in both normal and abnormal cases when using the different probe types,
    9.1. To be able to recognize colocalization and split signals.
11. Be able to accurately report the findings – understand and be able to use short term ISCN accurately.
12. Understand the quality control and quality assurance procedures required,
13. Understand and be able to use short form ISCN accurately.

Evaluation

1. Give an example of when FISH would be used to diagnose, and then follow minimal residual disease in haematological cancer.
2. How many cells should be counted for a result?
3. What is a fusion signal? Give an example and describe the process of its formation and how it causes cancer.
4. Is the white cell count important in the sample preparation for FISH and why?

16. Report writing

Objective

Writing accurate reports, both normal and abnormal, according to international guidelines

Specified Outcomes

Upon completion of this course the student should be able to write accurate reports according to international guidelines which should include the following:

1. Karyotype designation using correct ISCN nomenclature, including karyotypes, FISH, QF, CMA and MLPA.
2. A clear written description of the abnormality – include whether the abnormality is balanced / reciprocal or unbalanced.
3. Correct ISCN nomenclature for all tests: Karyotypes, FISH, QF, CMA and MLPA.
4. The name of any associated syndrome
5. Methods used in obtaining the result

6. Clinical interpretation whether the result is consistent with the clinical findings, if follow-up studies are needed on family members, an assessment of risk/occurrence, recommendations for the referral for prenatal diagnosis in future pregnancies and onward referral for genetic counselling
7. G-band resolution
8. Laboratory contact details
9. Laboratory details where test was performed
10. Limitations of the test

Evaluation

1. Evidence of report writing.

17. Related topics

Objective
Pedigrees, inheritance patterns, cell cycle including mitosis and meiosis, chromosome recombination, common recurrent translocations, principles and use of relevant antenatal biochemical tests e.g. AFP and Triple test, In vitro fertilisation and pre-implantation genetics, history of Cytogenetics techniques

Specified Outcomes

Upon completion of this course the student should have reviewed and discussed the following important elements of Medical Genetics:

1. Family history

2. Usefulness of chromosome studies

3. Gene function

4. Use of DNA for studies

5. Gene mutation detection

6. DNA mutations

7. Epigenetics

8. Effects of metabolism, drug responses, and immune system

9. Rare vs. common conditions

10. Cancer and genetics – Background knowledge required,

11. Cytogenetics and molecular genetics of solid tumours ie breast cancer – background knowledge required.

12. Susceptibility,

13. Evaluate pedigree analysis and determine pattern of inheritance – Inheritance and pedigrees – At the completion of this module, the student will be able to:

13.1 Understand, describe and give examples of the different types of inheritance patterns:

Understand, describe and give examples of the different types of inheritance patterns:

- Autosomal dominant
- Autosomal recessive
- X-linked dominant
- X-linked recessive

13.2 Interpret and draw a pedigree.

14. **Cell Cycle**

Objective:

A comprehensive understanding of the cell cycle and how these stages give rise to chromosome abnormalities.

Specified outcomes:

At the completion of this module, the student will be able to:

- describe the different phases of the cell cycle and know the times for each stage,
- describe and illustrate how these stages produce trisomies, monosomies, mosaicism and balanced and unbalanced translocations.
- Describe the stages of meiosis and mitosis

15 Compare and contrast medical terminology as it relates to Mendelian Genetics

16. Describe the circumstances in which a DNA test involves scanning a gene for mutations or checking for a specific change.

17. Discuss how genotype-phenotype correlations can be established.

18. Describe the basic principles of inborn errors of metabolism and give examples of disease caused by metabolic blocks.

19. Describe the technical, social and ethical requirements that a screening program should fulfill.

20. Chromosome recombination

21. Common recurrent translocations

22. History of cytogenetic techniques

Evaluation

Knowledge of the above theory

19. REFERENCE MATERIAL

The following list of books is included merely as a guide; there are many other suitable textbooks available.

i) Association for Clinical Cytogenetics Guidelines
ii) American Board of Medical Genetics, Cytogenetic Learning Guide, February 2009
iii) Shaffer, L.G., Slovak, M.L., Campbell, L.J., ISCN 2016, An International System of Human Cytogenetic Nomenclature
iv) SANAS website with special reference to Vertical Assessment document (F95-05) and Witnessing of Activity document (F15-04)
v) Barch, M.J., Knutsen, T., Spurbeck, J.L. The Association of Genetic Technologists Cytogenetics Laboratory Manual, 3rd ed, 1997
vi) Gersen, Steven L. and Keagle, Martha B., The Principles of Clinical Cytogenetics, 2nd ed, 2005

vii) Bain, Barbara, Leukaemia Diagnosis, 4th ed, 2010
viii) Czepulkowski, B., Analyzing Chromosomes, 2001
ix) Occupational Health and Safety Act 85 of 1993
x) Dunn, B, Monchrani, P and Keagle, M. Association of Genetic Technologists: The Cytogenetic Symposia 2nd ed.
xi) Heim, S and Mitelman, F. Cancer Cytogenetics 3rd ed, 2009
xii) Gardner, RJM and Sutherland, GR. Chromosome Abnormalities and Genetic Counseling, 3rd ed, 2004
xiii) Firth, HV and Hurst, JA. Oxford Desk Reference Clinical Genetics, 2008
xiv) Harper, J. Preimplantation Genetic Diagnosis. 2009.
ISCN 2020. An International System for Human Cytogenomic Nomenclature (2020). Editors: Jean McGowan-Jordan, Ros J Hastings, Susan Moore.
xv) Guidelines: European Guidelines f or Cytogenetics Quality Assurance
xvi) ISCN 2020: An International System for Human Cytogenetic Nomenclature (2020) Editors: Jean McGowan-Jordaan, Ros J Hastings, Sarah Moore.

20. ACRONYMS

For student completion:

CVS

AF

BM

POC

ISCN

SANAS

PCR

PUBS

MTX

FISH

CGH

MLPA

NOR

PPE

AFP

QF-PCR

CML

AML

MDS

ALL

CLL