SYLLABUS

MICROBIOLOGY

MEDICAL TECHNICIAN

PBMT approved in September 2022 for training implementation in 2023 for students who write from October 2024 onwards

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CONTENTS

1. INTRODUCTION

The objective of this syllabus is to provide the student technicians 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 is in the form of two, two hours, written practical papers which will be based on the contents of this syllabus.

Candidates are required to attain a minimum of 50% overall and a sub-minimum of 50% for each of the papers.

Please refer to:

- 18. Reference material
- 19. Nomenclature / Acronyms

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/students 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 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 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 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).
- Demonstrate knowledge on the practice/ ethos of how confidentiality in the workplace is achieved and maintained.
- Demonstrate knowledge of No. 61 of 2003: National Health Act, 2004.

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 relating to laboratory safety procedures.

Specified outcomes

On completion of this section 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; 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.
- 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.
    - Range - *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.
- 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.
    - Range – *exits; assembly points; first aid; fire equipment; chemical and fire warnings*
- Demonstrate knowledge of all safety and emergency equipment.
    - Range - *Fire blankets; fire hose; fire extinguishers; first aid kit; eye wash bottle; emergency whistle/hor; emergency shower*

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 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 student should be able to:

- 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.

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 student should be able to:

- 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; Stains – refer to Section 8. Stains, Pg.14; Disinfectants – refer to Section 10 Sterilisation and Disinfection, Pg. 16; Media – refer to Section 11 Media, Pg. 17*

3.5 STOCK CONTROL

Objective

Outline the processes involved in good materials stock management.

Specified outcomes

On completion of this section the 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.
- Demonstrate knowledge of workplace policy with regard to the use of expired reagents, controls.
- Knowledge of the Maintenance of Reference Quality Control stock.

3.6 QUALITY ASSURANCE / ACCREDITATION

Objective

Expose the student to all aspects of quality assurance.

Specified outcomes

On completion of this section the 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 student to all aspects of quality control.
Specified outcomes

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

- Describe and apply the appropriate quality control processes which must be performed and applied to all the analytical procedures 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.

3.8 METHOD VALIDATION

Objective

Expose the 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).

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 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 Technicians.
- 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 Technicians.

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 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.
- Explanation/Description of the records needed to prove an audit trail on all procedures performed. This includes knowledge of the time, condition, as well as raw data (paper or electronic storage).
    - 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 student with instruction on the application of the correct mathematical formulae to relevant calculations.

Specified outcomes

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

- Describe workflow dynamics in a molecular biology 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*
- Demonstrate knowledge of the quality controls used in the assay procedure.
- Identify the potential causes of false positive and negative results.
- Discuss DNA extraction from biological samples.
- Describe the polymerase chain reaction (PCR) under the following headings:
    - Applications, advantages and disadvantages.
    - Understanding of the function of each component of a PCR mix.
    - Inhibiting factors.

- Discuss the applications of molecular probe assays.
- Demonstrate an understanding of agarose gel electrophoresis by discussing the following:
    - Procedure and applications.
    - Preparation and loading of a gel.
    - Quality control.

- Describe real-time PCR under the following headings:
    - Procedure and applications
    - Advantages and disadvantages

6. BASIC LABORATORY MANAGEMENT

Objectives:
To obtain a basic knowledge of the principles and practices involving laboratory safety, quality management systems and accreditation and basic laboratory administration.

Specified outcomes:
At the end of each section, the student should be able to:

6.1 LABORATORY SAFETY

-Describe the regulations relating to the transport of specimens referred to other centres,
-Describe the regulations relating to the handling of medico-legal specimens.
-Discuss laboratory safety in relation to the Occupational Health and Safety Act (1993)
-List the responsibilities of safety representatives and first aiders as required by the OHS Act (1993).
-Describe the procedures for the storage, handling and disposal of laboratory waste including chemicals, biohazardous waste, radioactive waste, human tissue, solid contaminated waste, liquid contaminated waste, sharps and gases.
-Describe/explain/point out the proper safety precautions while handling and disposing of infectious material including those potentially containing organisms like M. tuberculosis, HIV or Hepatitis virus.
-Explain the Safety protocols involved in event of a needle-stick injury and exposure to blood-borne pathogens.

6.2 QUALITY MANAGEMENT SYSTEM

-Describe the components involved in a Quality Management System and Laboratory Accreditation (in keeping with the relevant ISO standards) under the following headings as listed in 3.1 -3.10

-Discuss the concept of laboratory accreditation as defined by the specific standards relevant to medical and public health laboratories.

6.3 LABORATORY ADMINISTRATION

-Describe/explain/motivate the components of a good Inventory/Stock control system
-Describe/explain/motivate the components of good data management system.

7. EQUIPMENT AND AUTOMATION

Objective: To obtain a basic understanding of laboratory equipment and automated systems

Specified outcomes:
At the end of this section, the student should be able to:

Describe the use of the following types of laboratory equipment / instrumentation under the following headings:

- Principles of use
- Operation and Maintenance
- Quality Control
- Record-keeping

a) Operation and maintenance of standard laboratory equipment:

- Centrifuges
- Biosafety cabinets
- Incubators (aerobic and CO2)
- Analytical balances
- Automated pipettes
- Water baths
- Anaerobic systems
- pH meters
- Microscopes
- Adjustable & fixed volume pipettes
- Plate pourer
- Thermometer

b) Basic operation and maintenance of automated equipment:

- Automated blood culture systems
- Automated TB culture and mycobacterial susceptibility systems
- Automated molecular amplification and detection instruments
- Automated and semi-automated bacterial identification and susceptibility testing systems
- Automated rapid detection of bacteria (including *Mycobacterium sp*.)

(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*).**

8. MICROSCOPY AND STAINING TECHNIQUES

Objective:

To describe the methods, use and application of microscopy techniques in a clinical laboratory setting.

Specified Outcomes: At the end of this section, the student should be able to:

a)

i) Know the component parts of the following microscopes and the paths of the optical rays.

Light
Phase-contrast
Fluorescent

ii) Describe with the aid of a diagram, the components and light path of each of these microscopes
iii) Discuss the use and application in a clinical laboratory setting of each of these microscopes
iv) Describe/discuss the maintenance of each type of microscope.

b) Describe the methods of preparation and use of wet preparations of faeces, urine and vaginal swabs in the identification of microorganisms.

c) Describe the concentration methods for parasites in faeces and urine specimens

d) Describe the following staining techniques (including quality control) commonly employed in a clinical laboratory:

- Cell counts on CSF specimens and other body fluids, e.g. pleural and synovial fluids
- Gram's stain
- Ziehl-Neelsen's stain (standard and modified).
- Auramine stain (TB and parasites)
- Acid-fast (Kinyoun) stain for *Cryptosporidium*.
- Capsular stain (e.g. India Ink)
- 10% KOH preparation for fungal elements.
- Lactophenol Cotton Blue mount (sticky tape mount) for microscopic morphology of fungi.

9. PROCESSING OF SPECIMENS

Objective:

1. Describe proper specimen collection, transportation, and processing of bacterial (aerobic, anaerobic, microphilic and capnophilic) cultures.
2. Select appropriate procedures for identifying the pathogens present in cultures, perform these procedures, identify and report the results accurately.

Specified Outcomes:

At the end of this section, the student should be able to:

a) Describe/discuss the standard operating procedures (SOPs) used for the examination of the following types of specimens for the presence of pathogenic organisms:

   i) Aspirates from normally sterile sites
   ii) Blood cultures
   iii) CSF
   iv) Pus/pus swabs (eye, ear, nose, throat, genital, wound, burn)
   v) Respiratory samples
   vi) Stool
   vii) Tissue
   viii) Urine
   ix) Skin, hair and nails
   x) Catheter tips
   xi) IUCD (Intra-uterine contraceptive device)

b) State the organisms commonly implicated in each of these specimen types
c) Determine the suitability of specimens for processing
d) Discuss sterile techniques that are employed
e) Explain the safety precautions to be observed
f) Collect specimens as defined within current statutory requirements and limitations

10. STERILIZATION AND DISINFECTION

Objective: To obtain a basic knowledge of the principles and applications of sterilization and disinfection procedures in a clinical laboratory setting

Specified outcomes: At the end of this section, the student should be able to:

- Discuss the principles and applications of sterilization used in a laboratory under the following headings:
    - Autoclaves
    - Hot-air oven
    - Steam
    - Filtration
    - Tyndallisation
    - Inspissation

- Describe the use and action of disinfectants commonly employed in a laboratory. The following list of disinfectants is compulsory.

    - Alcohols
    - Phenolics
    - Peroxygen compounds (oxidising agents)
    - Aldehydes
    - Quartenary ammonium compounds

- Describe the methods employed when testing the bactericidal or bacteriostatic activity of disinfectants

11. MEDIA

Objective: To enable the student to select appropriate media for isolation of various pathogenic microorganisms from various body areas.

Specified Outcomes:

At the end of this section, the student should be able to:

a) Discuss the preparation and sterilization of solid, semi-solid and liquid culture media necessary for the isolation, identification and sensitivity testing of all the micro-organisms listed in this study guide. This will vary from laboratory to laboratory.

Basic microbiology media to be included for study:

- Primary isolation media:
  Blood agar
  Chocolate agar (Boiled Blood agar)
  MacConkey agar & MacConkey with crystal violet
  Salmonella Shigella agar/ XLD
  Sabouraud dextrose agar & Sabouraud dextrose agar with Chloramphenicol/Gentamycin
  New York City/Thayer Martin agar
  Lowenstein & Jensen medium / Middlebrooks
  Thiosulfate-Citrate-Bile Salts-Sucrose TCBS agar
  Egg yolk agar (EYA)
  Sorbitol MacConkey agar
  Cooked meat broth/ Thioglycolate broth
  The principle and one example of a chromogenic agar used for urine culture

- Identification media:
  Glucose
  Lactose/ONPG
  Urea
  Decarboxylases (e.g. lysine, ornithine)
  H2S
  Citrate
  DNAse
  Oxidation/Fermentation (OF) media
  Mannitol salt agar
  Bile aesculin agar
  5%NaCl
  CTA sugars
  Motility
  Nitrate
  Arginine

- Susceptibility testing media:
  Mueller Hinton agar
  Haemophilus test medium

Gonococcus testing Media
MHA with sheep blood/MHF with horse blood
MHA with laked blood

- Isolation of Anaerobic organisms: e.g. Brucella Agar + Vitamin K and Haemin / Anaerobic Colstin Nalidixic Acid Agar (selective agar) / Nalidixic Acid Agar (selective)

- Isolation of *Campylobacter spp.*

- Media used in Public health testing:
  Baird Parker agar (BPA)
  Tryptone Glucose Extract agar (TGEA).
  Peptone water

b) Discuss the media relevant to the organisms listed in this study guide with regard to:
   - Application/Use
   - Describe the main ingredients and their function.
   - Discuss/describe the quality control procedures and selection of the correct organisms for testing.

c) Differentiate between selective, non-selective, enriched, indicator and differential (including chromogenic) culture media.

12. SYSTEMATIC BACTERIOLOGY

Objective:
To provide the student with a systematic study of the bacteria that are parasitic to man with special reference to:

Classification
Isolation
Identification- phenotypic
Type of disease caused

Many of the species given in each genus are merely the most common representative organisms in that genus. In most cases there are many more. However, for examination purposes, only the species listed in this syllabus need be studied.

- The following codes have been assigned to represent the extent of knowledge required for each organism:

B - *basic* knowledge

S - knowledge of the *serological confirmation* of the identity of the organisms.

G - knowledge to *genus level only*

Specified outcomes:

Upon completion of this section, candidates should be able to:

i) Have knowledge of the isolation, Gram morphology and biochemical identification of the organism and demonstration of presence of toxins and/or enzymes – including colonial morphology, growth requirements, incubation and temperature.**(B)**
ii) Describe the methods used for serological confirmation of the identity of the organism.**(S)**
iii) Describe the isolation, Gram morphology and biochemical identification of the organism to genus level only – including colonial morphology, growth requirements, incubation and temperature. **(G)**

12.1 AEROBIC AND FACULTATIVE ANAEROBIC ORGANISMS

12.1.1 GRAM NEGATIVE ORGANISMS:

- **ENTEROBACTERIACEAE**
    - Fermenters:

Tests: oxidase, glucose, ONPG, urea, H2S, citrate, motility, indole, Methyl Red, Voges-Proskauer (VP test), decarboxylase tests, Nitrate

Citrobacter freundii **(B)**
Enterobacter cloacae **(B)**
Escherichia coli **(B & S)**
Klebsiella pneumoniae **(B)**
Morganella morganii **(B)**
Proteus (P. mirabilis and *P. vulgaris)* **(B)**
Providencia rettgeri **(B)**
Serratia marcescens **(B)**
Shigella (S. flexneri, S. boydii, S. sonnei and *S. dysenteriae)* **(B & S)**
Salmonella enterica subsp*. enterica* ser Typhi **(B & S)**
Salmonella spp **(G, & S)**

- Non-fermenters:

Tests: oxidase, citrate, O/F media, motility, pigment production, growth temperature requirements, Nitrate

Acinetobacter baumannii **(B)**
Pseudomonas (P. aeruginosa) **(B)**

- **MISCELLANEOUS GRAM NEGATIVE ORGANISMS:**

NB*: The organisms below have been grouped according to the approach to identification. This was done for the sake of convenience and is not a formally accepted system.*

Group 1
Tests: oxidase, glucose, ONPG, urea, H2S, citrate, motility, indole, mannite, DNAse, Arginine, Nitrate

Aeromonas hydrophila **(B)**
Vibrio cholerae **(B &S)**

Group 2
Tests: oxidase, CTA sugars (glucose, lactose, maltose, sucrose), DNAse, nitrate reduction, growth factor and special media requirements.

Haemophilus (H. influenzae, H. parainfluenzae) **(B)**
Moraxella (M. catarrhalis) **(B)**
Neisseria gonorrhoeae **(B)**
Neisseria meningitidis **(B & S)**

Group 3
Tests: growth requirements, temperature, atmospheric conditions, oxidase, hippurate, catalase,

Campylobacter jejuni **(B)**

12.1.2 GRAM POSITIVE COCCI:

- ***Staphylococcus sp.*** **(B)**

  Tests: catalase, DNAse, coagulase, mannitol salt agar, novobiocin susceptibility
  Staphylococcus aureus
  Coagulase negative staphylococci (unnecessary to speciate)
  Staphylococcus saprophyticus

- **Beta Haemolytic Streptococci (B&S)**

  Tests: **catalase , bacitracin and trimethoprim susceptibility, latex test for Lancefield grouping, CAMP**

  Lancefield groups:
  Group A - *Streptocoocus pyogenes*
  Group B - *Streptococcus agalactiae*
  Group D - *Streptococcus bovis* and *Streptococcus. equinus (non-enterococcal)*

- **Alpha Haemolytic and Non-haemolytic Streptococci**

  Tests: growth requirements, colony morphology, Optochin susceptibility, bile solubility

  - *Streptococcus pneumoniae* **(B&S)**

  Tests: growth requirements, colony morphology, Optochin susceptibility

  - Viridans Group Streptococci **(B)** (group level only)

  Tests: bile aesculin agar, salt (6,5% NaCl) tolerance, tellurite agar, aesculin hydrodysis.

  - *Enterococcus faecalis*

12.1.3 GRAM POSITIVE BACILLI:

- **Spore-forming gram positive bacilli:**
  Tests: catalase, haemolysis on sheep blood agar, lecithinase, motility

B. cereus **(B)**
Bacillus cereus
Bacillus anthracis

- **Non spore-forming gram postive bacilli:**
  Tests: catalase, haemolysis, Bile esculin agar, tumbling motility, cold enrichment, CAMP test, growth on MCC

  Listeria monocytogenes **(B)**

  Tests: catalase, motility, Tellurite

  Corynebacterium species **(B)**

12.2 ANAEROBIC ORGANISMS

- **Non-pigmented gram negative bacilli:**

  Tests: identification using special potency disks-

  Colistin; erythromycin; vancomycin; rifampicin; penicillin G and kanamycin

  Bacteroides (B. fragilis group only) **(B)**

- **Gram positive cocci:**

  Tests: Sodium polyanethol sulphate (SPS), urea, nitrate reduction, aesculin for anaerobes, indole for anaerobes.

  Peptostreptococcus anaerobius **(G)**

- **Spore-forming gram positive bacilli:**

  Test: reverse CAMP test, lecithinase

  Clostridium perfringens **(B)**

12.3 MYCOBACTERIA

Objective: To enable the student to acquire a basic knowledge of the microscopy, culture and identification and susceptibility testing of Mycobacteria.

Learning Outcomes:
On completion of this section, the student will be able to discuss the following:

-Decontamination-liquefaction of specimens before TB culture.

Reasons for the procedure and selection of specimens for decontamination.

Principle and one method for decontamination-liquefaction of sputa.

-Staining.

Methods (see section on MICROSCOPY AND STAINING TECHNIQUES)
The International Union Against Tuberculosis and Lung Disease (IUATLD) and World Health Organization (WHO) guidelines for the reading and reporting of TB sputum direct smears.

-Isolation (culture) of Mycobacteria.

Conventional (solid + liquid media)

Automated systems (e.g. Mycobacteria Growth Indicator Tube - MGIT)

-Mycobacterium tuberculosis complex.

Identification: conventional, Niacin & real time PCR

Be able to list the species within the complex.

Theoretical difference between *M. tuberculosis* complex and the Bacillus Calmette-Guerin (BCG) (*M. bovis*) intended as vaccine strain.

NTM's (Non-tuberculous Mycobacteria), previously known as MOTT's (Mycobacteria Other Than Tuberculosis)

Basic identification of MOTTS: conventional

Classification (Runyon classification) of MOTTS and differentiation using biochemical tests, growth temperature and other growth requirements (one example from each group):

Nonchromogens: *M. avium*- intracellulare group

Photochromogens: *M. kansasii*

Rapid growers: *M. fortuitum* group and *M. chelonei*
Scrotochromogens: *M. scrofulaceum*

Niacin, PNBA (para-nitro-benzoic acid), ZN stain

- Have knowledge of the South African national protocol for diagnosis and treatment of Mycobacterium tuberculosis, with specific reference to section 4.1 <u>XPERT diagnostic algorithm.</u>

13. MYCOLOGY

Objective: To enable the student to understand microscopy and culture methods used for the detection and identification of yeasts.

Specified Outcomes:
At the end of this section, the student should be able to discuss the following:

- Selection of appropriate microscopy and culture methods (staining, media, incubation conditions) for detection of yeasts in the following specimens:
    - Blood
    - CSF and body fluids
    - Skin, hair and nails
    - Sputum, and tracheal aspirates
    - Tissue and pus
    - Urine and stool
- Identification of the following yeasts using microscopic and colonial morphology, Germ Tube test, urea, commercial identification methods as well as media and growth temperature requirements:

Yeasts

Candida albicans
Cryptococcus neoformans

14. ANTIMICROBIAL SUSCEPTIBILITY TESTING

Objective:

To obtain an understanding of antimicrobial susceptibility testing principles and procedures in a clinical setting.

Specified Outcomes:
At the end of this section, the student should be able to:

- Describe the methods and report the results involved in:
    - Disk (Kirby Bauer)
    - Minimum Inhibitory Concentration (MIC) testing by broth dilution and ETEST.
    - Testing for the presence of the ß-lactamase enzyme (chromogenic cephalosporin test)
- Discuss quality control of antibiotic susceptibility testing, in keeping with the accepted international standards (Clinical Laboratory Standards institute (CLSI) and/or - European Committee on Antimicrobial Susceptibility Testing (EUCAST)
    - Procedures/protocols.
    - Media (application only).
    - Recording of results and reporting.
    - Use and storage of control strains of organisms.
    - Use and storage of antibiotic discs/solutions/ETEST.

15. PARASITOLOGY

Objective: To enable the student to recognize and identify parasites at various stages of their life-cycles through the selection of appropriate microscopy and staining techniques

Specified Outcomes:
At the end of this section, the student should be able to:

- Describe the life cycles and epidemiology of the parasites listed below.
- Discuss sample preparation to optimize detection e.g. stool concentration (see section on MICROSCOPY AND STAINING)
- Select and describe appropriate microscopy and staining techniques. (see section on MICROSCOPY AND STAINING)
- Perform visual (micro – and macroscopic) recognition and identification of the parasites at various stages of their life-cycles, as well as demonstration of the most important morphological features of each, with an aid of a simple, labelled sketch.

Use the following as a key:
ova **(O),** oocysts **(OO),** cysts **(C),** larvae **(L),** trophozoites **(T)** and adult forms **(A).**

Cestodes:

- *Hymenolepis nana* **(O)**
- *Taenia sp.* **(O & A)**
- *Echinococcus granulosus* **(O), Hooklets**

Nematodes:

- *Ascaris lumbricoides* ***(O & A)***
- *Enterobius vermicularis* ***(O & A)***
- *Strongyloides stercoralis* ***(L)***
- *Trichuris trichiura* **(O & A)**
- *Ancylostoma duodenale* **(O)**
- *Necator americanus* **(O)**

Trematodes:

Schistosoma haematobium **(O),** *Schistosoma. mansonii* **(O)**

Protozoa:

- *Entamoeba spp.* **(C & T)**
- *Giardia lamblia* **(C & T)**
- *Trichomonas vaginalis* **(T)**

Coccidia:

- *Cryptosporidium parvum* ***(OO)***

16. PUBLIC HEALTH

Objective:

To obtain comprehensive knowledge of testing procedures and their application in a Public health laboratory.

Specified Outcomes:

Upon completion of this section, candidates should be able to describe the following tests:

- **POTABLE (DRINKING) WATER**
  - Filtration procedure for presence of coliform organisms.
  - Confirmatory procedure for presence of *E. coli*.
  - Interpretation and reporting of above results according to the SABS standards.

- **MILK**

  - Method for determining the mesophilic aerobic count.
  - Interpretation and reporting of above results according to the milk regulations.
  - Methods to detect the presence of coliform organisms and *E. coli* in pasteurised milk.
  - Method of the phosphatase test and the interpretation of results.
  - Brucella ring test
  - Detection of pathogenic organisms i.e. *Staphylococcus aureus* and *Salmonellae*.

17. SEROLOGY

Objective:

To obtain knowledge and understanding of serological tests employed by the bacteriology laboratory in the diagnosis of disease.

Specified Outcomes:

Upon completion of this section, candidates should be able to:

- Describe the procedure for the listed tests
- Demonstrate a basic knowledge of the application of the tests within the range
- Apply the appropriate methodology to the correct test

    - Agglutination
    - Enzyme Linked Immunosorbent Assay (ELISA)
    - Haemagglutination
    - Immunofluorescence, direct antigen detection
    - Immunofluorescence, indirect antibody detection
    - Neutralization reactions
    - Precipitation
    - Lateral Flow immunoassay

  Range of tests
    - RPR, CLAT
    - TPHA and Amoeba
    - Pneumocystis and/or Chlamydia
    - FTA
    - ASOT
    - VDRL
    - Widal
    - Weil Felix
    - Brucella
    - HIV
    - Toxoplasma

18. REFERENCE MATERIAL

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

Bailey & Scott's Diagnostic Microbiology , Betty A Forbes Daniel F Sahm and Alice S Weissfeld

Colour Atlas Textbook of Diagnostic Microbiology, 5th Edition-1997. (Eds.) E.W. Koneman, S.B.Allen *et al*. Lippencott, New York.

Medical Microbiology (Eds) David Greenwood and Richard Slack

Manual of Clinical Microbiology. (Eds) Albert Balows, William Hausler *et al*. American Society of Microbiology.

Medical Mycology-The Pathogenic Fungi and the Pathogenic Actinomycetes. (Eds) John Willard Rippon.

Parasites: A guide to Laboratory procedures and identification. (Eds) Lawrence R. Ash and Thomas C. Onhel.

Atlas of Medical Helminthology and Protozoology. (Eds) Jeffrey and Leach.

Quality control and accreditation references: www.iso.org , www.clsi.org, www.sanas.co.za.

Health Professions Council of South Africa (HPCSA): www.hpcsa.co.za

National TB Management Guidelines 2014
https://www.tbonline.info/media/uploads/documents/national_tuberculosis_management_guidelines_%282014%29.pdf

19. NOMENCLATURE/ACRONYMS

AIDS	Acquired Immune Deficiency Syndrome
BPA	Baird Parker Agar
CLSI	Clinical and Laboratory Standards Institute
CO_2	Carbon Dioxide
CPE	*Clostridium perfringens* enterotoxin
CSF	Cerebrospinal Fluid
CTA	Cystine Tryptic Agar
CVP	Central Venous Pressure
DFAT	Direct Fluorescent Antibody Test
DNA	Deoxyribonucleic Acid
ELISA	Enzyme Linked Immunosorbent Assay
ETH	Ethambutol
EUCAST	European Committee on Antimicrobial Susceptibility Testing
EQC	External quality control
H_2S	Hydrogen Sulphide
HIV	Human Immunodeficiency Virus
HPCSA	Health Professions Council of South Africa
INH	Isoniazid
ISO	International Organisation for Standardisation
IUATLD	The International Union Against Tuberculosis and Lung Disease
IQC	Internal quality control
KOH	Potassium Hydroxide
LFA	Lateral Flow Assay
MBC	Minimum Bactericidal Concentration
MDR	Multi Drug Resistant
MGIT	Mycobacteria Growth Indicator Tube
MIC	Minimum Inhibitory Concentration
MOTT	Mycobacteria Other Than Tuberculosis
MRS	Man-Rogosa-Sharpe broth
MRSA	Methicillin-resistant *Staphylococcus aureus*
NaCl	Sodium Chloride
NTM	Non-tuberculous Mycobacterium
OF	Oxidation/Fermentation
OHSA	Occupational Health and Safety Act
ONPG	ortho-Nitrophenyl -β-galactoside
PCR	Polymerase Chain Reaction
PUO	Pyrexia of Unknown Origin
PZA	Pyrazinamide
RFLP	Restriction Fragment Length Polymorphism
RIF	Rifampicin
RNA	Ribonucleic Acid
SABS	South African Bureau of Standards

SOP	Standard Operating Procedure
SPS	Sodium Polyanethol Sulphonate
STI	Sexually Transmitted Infection
STR	Streptomycin
TB	Tuberculosis
TCBS	Thiosulphate Citrate Bile Salts Sucrose agar
TGEA	Tryptone Glucose Extract Agar
UTI	Urinary Tract Infections
VP	Voges-Proskauer
WHO	World Health Organisation
XDR	Extensively Drug Resistant
XLD	Xylose-Lysine-Deoxycholate Agar
ZN	Ziehl Neelsen