SYLLABUS

IMMUNOLOGY

MEDICAL TECHNOLOGIST

Effective from Feb 2019 for exams from March 2021

Updated: March 2017
Approved by PBMT:
Nov 2017

Contents

1. INTRODUCTION

This Syllabus was compiled by:

Ms Anita Markotter
Ampath Laboratory

Dr Pieter W.A. Meyer
NHLS & University of Pretoria

Mr Andre Roux
NHLS

Acknowledgement:
All members of the Immunology Scientific Academic Committee (SAC) for their contributions.
Thanks to Noel Nel, Lebogang Mongongoa, Marcia Watkins, Hilary Dinnematin & Maria Vermeulen for your invaluable contribution.

Immunology is the branch of science that studies:

- All the aspects of the immune system,
- The disorders that are caused by dysfunction of the immune system,
- The laboratory tests that are used to diagnose disorders of the immune system.

The objective of this syllabus is to provide the intern/student with a guideline on the essential aspects that must be covered in order to adequately prepare for the HPCSA's Professional Board of Medical Technology examination. The examination is in the form of two three hour papers which will be based on the contents of this syllabus. Paper 1 will assess theoretical knowledge and Paper 2 will assess practical knowledge. Candidates are required to attain a minimum of 50% overall and a sub-minimum of 50% for each of the papers.
Laboratories must ensure that interns/students receive appropriate training in the laboratory tests that are referred to in this syllabus. The examination will place emphasis on the candidate's ability to relate practical and theoretical knowledge to the clinical conditions that are mentioned in the syllabus.

The syllabus denotes that the technologist must be able to 'demonstrate knowledge' in several specific aspects throughout the different modules. How this will be achieved is left to the specific institution to decide, as each will have their own teaching and training program and test modalities to assess the progress of their students. The aim of the syllabus is not to be prescriptive in this regard. However, it is essential that the student does have a comprehensive working knowledge applicable to each module in order to achieve a pass rate in the examination set by the board.
DIFFERENT LABORATORIES MAY BE USING DIFFERENT LABORATORY TESTS. THIS IS ACCEPTABLE.

2. STATUTORY REGULATIONS AND ETHICS

OBJECTIVE

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

SPECIFIED OUTCOMES

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.
- 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).

3. TOTAL QUALITY MANAGEMENT

3.1. LABORATORY SAFETY

OBJECTIVE

Provide the intern/student with information on the legal documents and safety standards which apply in the practice of medical technology.

SPECIFIED OUTCOMES

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

Demonstrate knowledge of the Occupational Health and Safety Act, Compensation for Occupational Injuries and Diseases Act and Hazardous Substances Act.

- Recognize the Safety symbols.
- Demonstrate knowledge of Good Laboratory Practice (GLP) in a medical laboratory.
- Demonstrate knowledge of the use of chemicals in a medical laboratory with reference to MSDS (Materials Safety Data Sheets), storage and cleaning of spills.
- Demonstrate knowledge of the use of PPE (Protective Personal Equipment).
- Demonstrate knowledge of the requirements regarding the following safety equipment: Eyewash station / Emergency showers / Fire hose / Fire extinguishers / First Aid Box / Safety blanket.
- Demonstrate knowledge of the following protocols:
    - Needle Stick injury.
    - Haemorrhagic fever specimen.
- Demonstrate knowledge of the procedures to follow in the event of a laboratory accident or emergency (e.g. a chemical or bio-hazardous spill, fire, flood, bomb threat)
- Describe the classification and disposal of the different types of waste.
- Define the role of the following designated safety personnel:
  First aid officer, Fire Marshal, Safety representative.

3.2. SPECIMENS, HANDLING & STORAGE

OBJECTIVE

Provide the intern/student with information on the Pre-analytic procedures applicable in an Immunology laboratory.

SPECIFIED OUTCOMES

On completion of this section the intern/student should be able to demonstrate knowledge of the following requirements regarding specimens:

- Demonstrate knowledge of the procedures that are to be followed to ensure continuous correct patient and specimen identification from collection site to analysis.
- Describe the colour codes that are used for the different types of blood samples.
- Describe the conditions under which different specimens must be transported to the laboratory.
- Demonstrate knowledge of the storage conditions that are required for different specimens.

3.3. LABORATORY EQUIPMENT

OBJECTIVE

Provide the intern/student with information regarding the validation of new equipment (or completion of verification), calibration, maintenance, safety precautions and completing of the documentation that is applicable to the following laboratory equipment:

- Pipettes (refer to "Forward" and "Reverse" pipetting / the making of "Serial" and "Series" dilutions).
- Balances.
- Hot plates.
- pH meter.
- Vortex mixer.
- Rotators & Shakers.
- Microscopes (refer to Bright field / Fluorescence / Inverted).
- Biological Safety Cabinets (refer to Class I / Class II / Class III).
- Centrifuges.
- Water baths and Incubators.
- Spectrophotometer.
- Thermometers (Min-Max / Temp Chex / Bulb).
- Fridges and freezers.
- Specialised equipment i.e. thermocyclers, sequencers etc.
- Analysers as applicable to each institution i.e. nephelometers, flow cytometers, luminex® etc.

SPECIFIED OUTCOMES

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

- Validate new equipment (or complete a verification).
- Perform calibrations and maintenance.
- Adhere to the required safety precautions when using equipment.
- Use the equipment correctly.
- Complete all the necessary documentation applicable to equipment.

3.4. LABORATORY REAGENTS

OBJECTIVE

Provide the intern/student with information and ensure proficiency in the correct storage and preparation of reagents for application in laboratory analyses.

SPECIFIED OUTCOMES

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

- Demonstrate an understanding of how to handle and prepare reagents used in the laboratory.
- Prepare reagents for use (i.e lyophilised reagents), formulate buffers (i.e. phosphate buffered saline, TE buffer etc.).
- Handle reagents and chemicals according to GLP.

3.5. STOCK CONTROL

OBJECTIVE

Provide the intern/student with information on:

The processes involved in good stock management with specific reference to:

- ordering of stock,
- receipt of stock,
- documentation regarding the condition of the stock at reception,
- noting the expiry dates and lot numbers,
- the retention of package inserts,
- the company policy regarding the use of expired reagents,
- counting and movement of stock according to GLP.

3.6. QUALITY ASSURANCE / ACCREDITATION

OBJECTIVE

Familiarize the intern/student to all aspects of comprehensive quality assurance (QA) management.

SPECIFIED OUTCOMES

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

- Differentiate between Quality Assurance and Quality Control.
- Understand QA in terms of the applicable ISO (International Organization for Standardization) guide (i.e. ISO 15189)
- Understand the implementation and continuous monitoring required for successful implementation and management of a QA program.

3.7. QUALITY CONTROL

OBJECTIVE

Familiarize the intern/student to all aspects of comprehensive quality control (QC) management.

SPECIFIED OUTCOMES

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

- Understand QC programs with reference to controls, calibrators, calibration curves, reference ranges
- Differentiate between:
    - External Quality Control vs. Inter-laboratory QC vs. Internal QC
    - Specificity, Sensitivity, Precision, Accuracy.
- Give interpretative comments on:
    - Levy Jennings Graph (refer to the terms Mean, Standard Deviation, % Coefficient of Variation, Trend, Shift, Outlier, Bias),
    - Westgard rules,
    - Measurement of Uncertainty,
    - Documents applicable to Quality Control.

3.8. METHOD VALIDATION

OBJECTIVE

Familiarize the intern/student with all aspects of method validations as required when introducing a new methodology.

SPECIFIED OUTCOMES

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

- Draft a method validation protocol stipulating:
    - The definition of criteria to be used to evaluate results obtained
    - The reviewing procedure for validating results and adjustment of experimental design if deemed necessary
    - How the data will be analysed
    - The acceptance criteria
    - The conclusions
- Demonstrate that the method is fit for purpose, performs correctly and achieves reliable results.

3.9. PERSONNEL MANAGEMENT

OBJECTIVE

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

Explain the use of job profiles, HPCSA registration certificates, training records, continuous competency testing and CPD (Continuous Professional Development).

SPECIFIED OUTCOMES

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

- Demonstrate an understanding of job profiles and HPCSA registration.
- Demonstrate an understanding of the terms 'competency' and 'on-going competency' in terms of the training of all laboratory personnel.
- Demonstrate an understanding of the CPD (Continuous Development Program) requirements.

3.10 DOCUMENT CONTROL

OBJECTIVE

Provide knowledge of basic requirements of document control in terms of the relevant ISO standards.

Refer to Policies, SOPs (Standard Operating Procedures), WIs (Working Instructions) and to the frequency of review, the process of making documents obsolete, retention and disposal of documents and to accreditation (refer to internal and external audits)

SPECIFIED OUTCOMES

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

- Differentiate between Policies, SOPs and WIs.
- Draft an SOP using the required format.
- Demonstrate knowledge of document control requirements regarding frequency of review, the process of making documents obsolete, document retention and disposal.
- Demonstrate understanding of the process of accreditation with reference to internal and external audits.

4. LABORATORY RELATED MATHEMATICS

OBJECTIVE

Student/interns must show to be competent to:

- Calculate molecular weights of chemicals for preparation of buffers.
- Calculate and convert all concentration units of analytes and buffers (i.e. calculate 1 Molar NaCl from the MW, convert mg/dl to ug/ml, etc).

SPECIFIED OUTCOMES

Students/interns must show fluency in laboratory related mathematics by calculating and converting concentrations of analytes, buffers and dilutions correctly.

5. MOLECULAR BIOLOGY

OBJECTIVE

Student/interns must be given the basic principles of the specific molecular biology application as applicable in the Immunology laboratory.

Emphasis must be placed on:

- Structure of Deoxyribonuclease acid (DNA).
- Different extraction methods of DNA.
- Requirements for long-term storage, and ethical considerations of working with DNA samples.
- Polymerase chain reaction (PCR).
- DNA Sequencing.
- GLP in the molecular laboratory (i.e. pre-and post PCR workspace, decontamination).
- Visualising and interpreting PCR products.

SPECIFIED OUTCOMES

Students/interns must be able to describe the above mentioned principles and applications as required in all the disciplines of Immunology and how the genetic/molecular testing relates to the theory of molecular biology and the human genome.

6. IMMUNOPHENOTYING

6.5.1. BASIC PRINCIPLES OF IMMUNOPHENOTING BY FLOW CYTOMETRY

BACKGROUND KNOWLEDGE

The analysis of heterogeneous populations of cells for the purpose of identifying the presence and proportions of the various cell populations of interest is utilised to assist in diagnosis of several disorders. The enumeration of the different cell populations contribute to the diagnosis of haematological malignancies, immune-deficiencies and HLA associated disorders.

OBJECTIVE

The student / intern must be instructed on the:

- Sample types that can be analysed.
- The preparation of samples for analysis.
- The setting of parameters for identifying and quantification of subpopulations of cells.
- The different monoclonal antibodies and associated stains that can be used to identify these subpopulations investigated.
- Interpretation and formulation of further steps in the analysis required to reach a conclusion. (i.e. to establish if a malignancy is myeloid or lymphoid in nature and to further assign lineage to determine acute or chronic disease).
- **SPECIFIED OUTCOMES**

The student / intern must be able to:

- Prepare different sample types correctly for analysis (i.e. bone marrow aspirates, whole blood etc.)
- Use the correct set of markers for the specific analysis (i.e. CD4/CD3/CD8/CD45 tetra-chrome for T- and B-cell enumeration).
- Set the instrument protocols according to the analysis required.
- Interpret the results and indicate if the cell populations acquired are indicative of the disorder investigated and if further investigations are required. (i.e. are the T- and B-lymphocyte cell population normal).

SECTION II: IMMUNO PATHOLOGY

The following RANGE STATEMENT applies to all DISORDERS:

- Explain the **aetiology**.
- Describe the **pathophysiology**.
- List the **treatment** options.
- List the **diagnostic laboratory tests** that can be used to diagnose the disorder.

The following RANGE STATEMENT applies to all LABORATORY TESTS:

- Explain the **principle**.
- List the steps in the **method**.
- List the **normal values**.
- Perform the **test**, where possible.
- **Interpret** the results of the test.
- List the **associated disorders** that can be diagnosed by this test.
- Analyse **case studies** that refer to the laboratory results and to diagnosis of a disorder.

6. MODULES

6.1. MODULE 1: PRIMARY IMMUNO-DEFICIENCIES (PID)

6.1.1. NEUTROPHILS

BACKGROUND KNOWLEDGE

OBJECTIVE

Provide interns/students with introductory knowledge of the innate immune response with regards to the following:

- The characteristics, components and functions of the innate immune response.
- The morphology and functions of the cells of the innate immune response.
- The receptors of neutrophils and monocytes.
- The cardinal signs of and the acute inflammatory response (AIR) cascade.
- The characteristics of CRP (C-reactive protein) as an example of an APP (acute phase protein).
- Nephelometry as the preferred method of testing for CRP.
- Interpretation of CRP results and analysis of case studies.

SPECIFIED OUTCOMES

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

- Describe the characteristics, components and functions of the innate immune response
- Illustrate (by labelled sketches) the morphology of the following cells of the innate immune response: neutrophils / monocytes / macrophages / dendritic cells (DCs) / eosinophils / basophils
- List the receptors of neutrophils and monocytes
- Describe the functions of the following cells: neutrophils / monocytes / macrophages / dendritic cells (DCs) / eosinophils / basophils
- Describe the cardinal signs of the AIR
- Give a detailed description of all the phases of AIR. Refer to the following steps:
  the cytokines that mediate the response / margination / pavementing / diapedesis / chemotaxis / phagocytosis / killing by OB (Oxidative Burst), MPO (Myelo Per Oxidase system) and NETs (Neutrophil Extracellular Traps)
- Describe the characteristics of CRP, as an example of an APP
- Describe the principle of Nephelometry as used to measure CRP
- List the steps in the method
- List the Normal ranges
- Interpret results and analyse case studies of CRP with bacterial and viral infections and as risk factor for Myocardial Infarction

6.1.1.1. THE PIDs ASSOCIATED WITH NEUTROPHILS

OBJECTIVE

Provide interns/students with knowledge of the following PIDs associated with neutrophils:

- Disorders of Phagocyte NUMBERS:
  SCID Reticular dysgenesis / Severe Congenital Neutropaenia / Cyclic Neutropaenia
- Disorders of Phagocyte FUNCTION:
  - Adhesion / Chemotaxis disorder: LAD 1 / LAD 2 (Leukocyte Adhesion Deficiency)
  - Phagocytosis disorders owing to abnormal Morphology:
    Chediak Higashi syndrome / SGD (Specific Granule Deficiency)
  - Killing disorders:
    Disorder of the OB (Oxidative Burst): CGD (Chronic Granulomatous Disease).
    Disorder of MPO (MyeloPerOxidase): Myeloperoxidase disorder
- Disorders associated with CYTOKINES:
  MSMD (Medelian Susceptibility to Mycobacterial Disease)
  CMC (Chronic Muco-cutaeous Candidiasis)
  AD (Autosomal Dominant) HIES (Hyper IgE Syndrome)

SPECIFIED OUTCOMES

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

- Explain the Aetiology of each disorder
- Describe the Pathophysiology of each disorder
- List the treatment options for each disorder
- List the laboratory tests that can be used to diagnose each disorder

6.1.1.2. LABORATORY TESTS THAT ARE USED TO DIAGNOSE DISORDERS ASSOCIATED WITH NEUTROPHILS

OBJECTIVE

Provide interns/students with knowledge and skills relating to the following laboratory tests:

- The Chemotaxis tests: Boyden Chamber and/or Migratest.
- The Phagotest .
- The NBT screening test (Nitro Blue Tetrazolium) and /or the Oxidative Burst test by Flow Cytometry and/or the Chemiluminescence test
- The Iodination test
- The Luminex method to determine Cytokine levels
- The FISH (fluorescence *in situ* hybridisation)test to detect the abnormal genes

SPECIFIED OUTCOMES

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

- Explain the Principle of the tests
- List the Steps in the method of the tests
- List the Normal values of the tests
- Perform the tests, where possible
- Interpret the results of the tests

- List the disorders that can be diagnosed by the tests
- Analyse case studies that refer to the laboratory results and to diagnosis of a disorder

6.1.2. NK (Natural Killer) -CELLS

BACKGROUND KNOWLEDGE

OBJECTIVE

Provide interns/students with introductory knowledge relating to NK-cells with regards to the following:

- The Morphology of the NK-cells
- The Subsets of NK-cell types
- The CD (Cluster of Differentiation) markers on NK-cells
- The Functions of NK-cells (with reference to Fas-Fas Ligand mediated and Perforin-Granzyme mediated Killing)
- Apoptosis and Necrosis

SPECIFIED OUTCOMES

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

- Illustrate (by labelled sketch) the morphology of an NK-cell
- Describe the subsets of NK-cells
- List the CD markers on NK-cells
- List the functions of NK-cells
- Describe Fas-Fas Ligand mediated and Perforin-Granzyme mediated killing
- Compare apoptosis and necrosis

6.1.2.1. THE PIDs ASSOCIATED WITH NK-CELLS

OBJECTIVE

Provide interns/students with knowledge of the following PIDs associated with NK-cells:
Infertility and Spontaneous abortion
Recurrent re-activation of the following Herpes infections:
HSV (Herpes Simplex Virus) and HVZV (Herpes Varicella Zoster Virus)

SPECIFIED OUTCOMES

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

- Explain the aetiology of each disorder
- Describe the pathophysiology of each disorder
- List the treatment options for each disorder
- List the laboratory tests that can be used to diagnose each disorder

6.1.2.2. THE LABORATORY TESTS THAT ARE USED TO DIAGNOSE DISORDERS ASSOCIATED WITH NK-CELLS

OBJECTIVE

Provide interns/students with knowledge and skills relating to the following laboratory tests:

- Phenotyping and Enumeration of NK-cells.
- NK-cell activation test.
- NK-cell killing test.

SPECIFIED OUTCOMES

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

- Explain the principle of the tests.
- List the steps in the method of the tests.
- List the normal values of the tests.
- Perform the tests, where possible.
- Interpret the results of the tests.
- List the disorders that can be diagnosed by the tests.
- Analyse case studies that refer to the laboratory results and to diagnosis of a disorder.

6.1.3. COMPLEMENT

BACKGROUND KNOWLEDGE

OBJECTIVE

Provide interns/students with introductory knowledge relating to complement with regards to the following:

- The characteristics and functions of complement.
- The 3 complement pathways and the regulators of the 3 complement pathways.

SPECIFIED OUTCOMES

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

- Describe the characteristics and functions of complement
- Describe the complement pathways and the regulators of complement

6.1.3.1. THE PIDs ASSOCIATED WITH COMPLEMENT

OBJECTIVE

Provide interns/students with knowledge of the following PIDs associated with complement:

- HAE (Hereditary angioedema)
- SLE (Systemic Lupus Erythematous)
- Hypo-Complementric MCGN (Mesangio capillary glomerulonephritis)
- HUS (Hereditary Uremic Syndrome)
- Recurrent pyogenic infections leading to sepsis
- PNH (Paroxysmal Nocturnal Haemoglobinuria)
- Neisserial infections (non-recurrent and recurrent)
- Anaphylaxis owing to excessive anaphylatoxins

SPECIFIED OUTCOMES

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

- Explain the aetiology of each disorder.
- Describe the pathophysiology of each disorder.
- List the treatment options for each disorder.
- List the laboratory tests that can be used to diagnose each disorder.

6.1.3.2. THE LABORATORY TESTS THAT ARE USED TO DIAGNOSE DISORDERS ASSOCIATED WITH COMPLEMENT

OBJECTIVE

Provide interns/students with knowledge and skills relating to the following laboratory tests:

The FUNCTIONAL SCREENING tests:

- The Classical Pathway Haemolytic Complement test (CPHC 100).
- The Alternative Pathway Haemolytic Complement test (APHC 100).

Tests to measure the LEVELS of Complement Components and Complement Regulators:

- Nephelometry.
- Single immunodiffusion and precipitation in gel.
- Rocket electrophoresis.

SPECIFIC TESTS to aid in the diagnosis of certain disorders:

- Crossed immunoelectrophoresis.
- Functional test for C1-inhibitor.
- Phenotyping and enumeration of CD55 and CD59.

SPECIFIED OUTCOMES

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

- Explain the principles of the tests.
- List the steps in the method of the tests.
- List the normal values of the tests.
- Perform the tests, where possible.
- Interpret the results of the tests.
- List the disorders that can be diagnosed by the tests.
- Analyse case studies that refer to the laboratory results and to diagnosis of a disorder.

6.1.4. T-CELLS

BACKGROUND KNOWLEDGE

OBJECTIVE

Provide interns/students with introductory knowledge relating to T-cells with regards to the following:

- The characteristics, components and functions of the adaptive immune response.
- The CD markers on T-cells.
- The structural composition of the thymus.
- The education of T-cells in the thymus.
- The structural composition of mucosal associated lymphoid tissue (MALT), lymph nodes and spleen.
- Lymphocyte trafficking.
- The activation of T-cells.

SPECIFIED OUTCOMES

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

- Describe the characteristics, components and functions of the adaptive immune response.
- List the CD markers of T-cells
- Illustrate (by labelled sketch) the structural composition of the thymus, MALT, lymph node, spleen.
- Describe the education of T-cells in the thymus.
- Describe lymphocyte trafficking
- Describe the activation of T-cells by exogenous antigen, endogenous antigen, co-stimulatory molecules and cytokines.

6.1.4.1 THE PIDs ASSOCIATED WITH T-CELLS

OBJECTIVE

Provide interns/students with knowledge of the following PIDs associated with T-cells:

- Disorders of decreased T-cell numbers:
  - SCID (Severe Combined Immunodeficiency) Reticular dysgenesis.
  - SCID ADA deficiency (Adenosine Deaminase Deficiency).
- Disorders associated with cytokine receptors or cytokine signalling:
  - SCID IL-2 RG (Interleukin-2 Receptor Gamma).
  - SCID IL-7 RA (Interleukin-7 Receptor Alpha).
  - SCID JAK3 (Janus Kinase 3).
  - Duncan's disease.
- Disorders associated with V(D)J recombination
  - SCID RAG1 / RAG2 (Recombinase Activating Genes).
  - SCID Omenn's syndrome.
  - SCID RS (Radiation Sensitivity).
  - Ataxia telangiectasia.
- Disorders associated with abnormalities of the thymus:
  - SCID Di George syndrome.
  - PNP deficiency (Purine Nucleoside Phosphorlyase enzyme deficiency)
- Disorders associated with abnormalities of MHC (Major Histocompatibility Complex).
  - Absence of MHC Class I.
  - SCID Absence of MHC Class II.
- Disorder associated with abnormalities of a co-stimulatory molecule:
  - Hyper IgM Type I.

SPECIFIED OUTCOMES

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

- Explain the aetiology of each disorder.
- Describe the pathophysiology of each disorder.
- List the treatment options for each disorder.
- List the laboratory tests that can be used to diagnose each disorder.

6.1.4.2. THE LABORATORY TESTS THAT ARE USED TO DIAGNOSE DISORDERS ASSOCIATED WITH T-CELLS

OBJECTIVE

Provide interns/students with knowledge and skills relating to the following laboratory tests:

- Phenotyping and Enumeration of T-cells.
- *In vivo* T-cell function test
- *In vitro* T-cell function test by measuring proliferation of cells
- The Luminex® method to determine cytokine levels
- The FISH test to detect the abnormal genes
- The TREC test to detect abnormal V(D)J recombination of T-cells

SPECIFIED OUTCOMES

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

Explain the principle of the tests.

List the steps in the method of the tests.

List the normal values of the tests.

Perform the tests, where possible.

Interpret the results of the tests.

List the disorders that can be diagnosed by the tests.

Analyse case studies that refer to the laboratory results and to diagnosis of a disorder.

6.1.4.3. ACQUIRED DISORDER OF T-CELLS

HIV (Human Immunodeficiency Virus) and AIDS (Acquired Immunodeficiency Disorder)

OBJECTIVE

Provide interns/students with knowledge of HIV and AIDS with regards to the following:

- The types and sub-types of HIV.
- The life cycle of HIV.
- The phases of HIV infection.

SPECIFIED OUTCOMES

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

- Explain the aetiology of HIV with reference to the types and sub-types of HIV.
- Describe the pathophysiology of HIV / AIDS with reference to the lifecycle of HIV and the phases of the infection.
- List the treatment options.
- List the laboratory tests that can be used to diagnose HIV and to monitor progression of the disease.

6.1.4.4. THE LABORATORY TESTS THAT ARE USED FOR HIV / AIDS

OBJECTIVE

Provide interns/students with knowledge and skills relating to the following laboratory tests:

Laboratory tests to DIAGNOSE HIV infection:

- Screening test: 4th generation chemiluminescence
- Confirmatory tests: HIV Western blot and qualitative PCR

Laboratory tests to MONITOR progression of the infection:

- Phenotyping, enumeration and ratio of CD4 / CD8
- Quantitative PCR

SPECIFIED OUTCOMES

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

Explain the principle of the tests.

List the steps in the method of the tests.

List the normal values of the tests.

Perform the tests, where possible.

Interpret the results of the tests.

Analyse case studies that refer to the laboratory results and to diagnosis of HIV / AIDS

6.1.5. B-CELLS

BACKGROUND KNOWLEDGE

OBJECTIVE

Provide interns/students with introductory knowledge relating to B-cells with regards to the following:

- The CD markers of B-cells.
- The functions of B-cells.
- The development of B-cells (Primary and Secondary Phase).
- The activation of B-cells (refer to Thymus dependent, Thymus-independent, co-stimulatory molecules and cytokines).
- Class-switching (refer to the structure and functions of the different isotypes).
- Somatic hyper-mutation.
- The theories of Somatic recombination and Clonal selection.
- Definitions associated with antigens. Refer to the following terms: immunogen / antigen / requirements for immunogenicity / hapten / super antigen / naive antigen / epitope / allelic exclusion / isotypes / allotypes / idiotypes.
- Definitions associated with antibodies. Refer to the following terms: antibody / primary and secondary response / affinity and avidity / lock and key metaphor / specificity / cross-reactivity / associative recognition / monoclonal and polyclonal / natural antibodies (active and passive) and artificial antibodies (active and passive).
- Vaccines. (refer to the following types of vaccines: live attenuated / inactivated / sub-unit / toxoid / conjugated / and to the use of adjuvants)

SPECIFIED OUTCOMES

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

- List the CD markers of B-cells.
- Describe the functions of B-cells.
- Describe the development of B-cells (primary and secondary phase).
- Describe the activation of B-cells. (Thymus dependent and Thymus Independent, Co-stimulatory molecules and Cytokines)
- Describe class-switching.
- Describe and illustrate (by labelled sketch) the structure of the different Isotypes.
- Describe the functions of the different Isotypes and subtypes.

- Describe Somatic hyper-mutation.
- Describe the theories of Somatic recombination and Clonal selection.
- Explain the meaning of the following terms: antibody / primary and secondary response / affinity and avidity / lock and key metaphor / specificity / cross-reactivity / associative recognition / monoclonal and polyclonal / natural antibodies (active and passive) and artificial antibodies (active and passive).
- Describe the following types of vaccines: live attenuated / inactivated / sub-unit / toxoid / conjugated / and the use of adjuvants.

6.1.5.1. THE PIDs ASSOCIATED WITH B-CELLS

OBJECTIVE

Provide interns/students with knowledge of the following PIDs associated with B-cells:

- Disorders of decreased B-cell numbers:
  - SCID Reticular dysgenesis.
  - SCID ADA deficiency (Adenosine Deaminase Deficiency).
  - A-gammaglobulinemia.
- Disorders associated with V(D)J recombination:
  - SCID RAG1 / RAG2.
  - SCID Omenn's syndrome.
  - SCID RS (Radiation sensitivity).
  - Ataxia telangiectasia.
- Disorders associated with abnormalities of Co-stimulatory molecules:
  - Hyper IgM.
  - CVID (Common variable immune deficiency).
- Disorder associated with slow development of antibody synthesis:
  Transient hypo-gammaglobulinemia.
- Disorders associated with selective antibody deficiency.
  - Selective IgA deficiency.
  - Selective IgG subclass deficiency.

SPECIFIED OUTCOMES

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

- Explain the aetiology of each disorder.
- Describe the pathophysiology of each disorder.
- List the treatment options for each disorder.
- List the laboratory tests that can be used to diagnose each disorder.

6.1.5.2. THE LABORATORY TESTS THAT ARE USED TO DIAGNOSE DISORDERS ASSOCIATED WITH B-CELLS

OBJECTIVE

Provide interns/students with knowledge and skills relating to the following laboratory tests:

- Nephelometry for quantitation of immunoglobulins and sub-classes.
- Radial immunodiffusion and precipitation in gel (or ELISA) for quantitation of secretary IgA.
- Quantitation of Serum proteins by Immunofixation electrophoresis.
- Vaccination response tests by ELISA.
- Phenotyping and enumeration of B-cells.
- *In vitro* B-cell function test by measuring proliferation of cells.
- The FISH test to detect the abnormal gene.
- The KREC test to detect abnormal V(D)J recombination of B-cells.

SPECIFIED OUTCOMES

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

- Explain the principle of the tests.
- List the steps in the method of the tests.
- List the normal values of the tests.
- Perform the tests, where possible.
- Interpret the results of the tests.
- List the disorders that can be diagnosed by the tests.
- Analyse case studies that refer to the laboratory results and to diagnosis of a disorder.

6.1.5.3. ACQUIRED DISORDER OF B-CELLS: MULTIPLE MYELOMA

OBJECTIVE

Provide interns/students with knowledge of Multiple Myeloma.

SPECIFIED OUTCOMES

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

- Explain the aetiology of multiple myeloma.
- Describe the pathophysiology of multiple myeloma.
- List the treatment options for multiple myeloma.
- List the laboratory tests that can be used to diagnose multiple myeloma.

6.1.5.4. THE LABORATORY TESTS THAT ARE USED TO DIAGNOSE MULTIPLE MYELOMA

OBJECTIVE

Provide interns/students with knowledge and skills relating to the following laboratory tests:

- Nephelometry for quantitative determination of immunoglobulins and Kappa / Lambda serum free light chains (SFLC).
- Immunofixation electrophoresis.

SPECIFIED OUTCOMES

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

- Explain the principle of the tests.
- List the steps in the method of the tests.
- List the normal values of the tests.
- Perform the tests, where possible.
- Interpret the results of the tests.
- Analyse case studies that refer to the laboratory results and to diagnosis of Multiple myeloma.

6.2. MODULE 2: AUTOIMMUNITY

BACKGROUND KNOWLEDGE

OBJECTIVE

Provide interns/students with introductory knowledge relating to Auto-immunity with regards to the following:

- The principle of fluorescent microscopy.
- ANA(anti-nuclear antigen) and HEp-2 (Human epithelium carcinoma)pattern recognition.
- Tolerance and breakdown of tolerance.
- Protection against tumours and immunological escape by tumour cells.

SPECIFIED OUTCOMES

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

- Describe and illustrate (by labelled sketch) the principle of fluorescent microscopy
- Be proficient in ANA(anti-nuclear antigen) and HEp-2 pattern recognition
- Describe tolerance and breakdown of tolerance
- Describe protection against tumours and immunological escape by tumour cells

6.2.1. DISORDERS ASSOCIATED WITH AUTOIMMUNITY

OBJECTIVE

Provide interns/students with knowledge of the following autoimmune disorders:

PRIMARY IMMUNE DEFICIENCY associated autoimmune disorders.

Refer to the following disorders:

- APECED (Auto-immune Poly-Endocrinopathy Candidiasis Ectodermal Dystrophy)
- IPEX (Immune dysregulation Poly-Endocrinopathy Enteropathy X-linked syndrome)
- ALPS (Auto-immune Lympho-Proliferative Syndrome)
- Defective CTLA-4 (Cytotoxic T-Lymphocyte Antigen 4)

NON-ORGAN SPECIFIC autoimmune disorders:

Refer to the following disorders:

- Sjorgen's syndrome
- Scleroderma (Scl 70 and CREST)
- MCTD (Mixed Connective Tissue Disorder)
- Polymyositis
- RA (Rheumatoid Arthritis)
- SLE (Systemic Lupus Erythematosus)
- APLS (Anti Phospholipid Syndrome)
- Churg-Strauss Syndrome
- Wegener's Granulomatosis
- IBD (Inflammatory Bowel Disease): Ulcerative Colitis and Crohn's disease

ORGAN SPECIFIC Autoimmune disorders:

Refer to the following disorders:

- PBC (Primary Biliary Cirrhosis)
- Pernicious anaemia
- Celiac disease
- Goodpasture's syndrome

- IDDM Type I (Insulin Dependent Diabetes Mellitus Type I)
- Hashimoto's Thyroiditis
- Graves' disease
- Myasthenia Gravis
- Multiple Sclerosis
- Auto-immune Haemolytic anaemia (Warm and Cold)
- Rheumatic Fever

SPECIFIED OUTCOMES

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

- Explain the aetiology of each disorder.
- Describe the pathophysiology of each disorder.
- List the treatment options for each disorder.
- List the laboratory tests that can be used to diagnose each disorder.

6.2.2. THE LABORATORY TESTS THAT ARE USED TO DIAGNOSE AUTO-IMMUNE DISORDERS

OBJECTIVE

Provide interns/students with knowledge and skills relating to the following laboratory tests:

Laboratory tests for NON-ORGAN SPECIFIC disorders:

- The ANA test (anti-nuclear antibodies) – utilising both non-species tissue as well as Hep-2.
  Refer to the following patterns: Homogenous / Speckled / Scl-70 / Centromere / Nucleolar /Jo-1.
- ENAs (extractable nuclear antigens), cytoplasmic antibodies and non-soluble antigens.
  Refer to the following antibodies:
  - ENAs: SS-A (Ro) / SS-B (Ra) / Sm / RNP / Scl-70 / CENP-B / Ribosomal P / Mi-2 / PM-Scl / Fibrillarin / PCNA.
  - Cytoplasmic antibody: Jo-1
  - Non-soluble antigen: dsDNA
- Tests to detect free CIC (Circulating Immune Complexes)
  Refer to precipitation and solubility in PEG (Polyethylene Glycol) and Affinity for C1q by ELISA
- ANCA (Anti Neutrophil Cytoplasmic Antibodies)
  Refer to the following patterns: Anti-MPO / Anti-PR3 / Atypical
- Specific tissue IFA
  Refer to the following tissue: Parotid / Oesophagus / Umbilical vein / Pancreas / Colonic Goblet cells / *Crithidia luciliae* / ASCA (Anti *Sacchromyces Cerevisiae* Antibody).
- Nephelometry for RF (Rheumatoid Factor).
- FEIA /ELISA for CCP (Cyclic-citrullinated peptide)
- ELIA (automated ELISA) for Cardiolipin and β2 Glycoprotein.

Laboratory tests for ORGAN SPECIFIC disorders:

- The CB test (Composite Block)
  Refer to the following patterns: Anti-smooth muscle antibody (ab) / Anti-parietal ab /Anti-ribosomal ab / Anti-mitochondrial ab / Anti-microsomal ab.
- Specific tissue IFA
  Refer to the following tissues: Intestine / Pancreas / Kidney / Thyroid / Skeletal muscle / Optic nerve

- ECL (Electro Chemiluminescence) for anti TSH-receptor.
- ELISA for anti-acetylcholine receptor ab.
- Direct Coombs.
- Nephelometry of ASL and anti-DNAse to aid in the diagnosis of rheumatic fever.

Laboratory tests to investigate a HEREDITARY CAUSE for autoimmunity:

- FISH to detect the abnormal gene (if applicable).
- HLA typing to detect HLA alleles that are associated with certain Auto-immune disorders.

SPECIFIED OUTCOMES

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

- Explain the principle of the tests.
- List the steps in the method of the tests.
- List the normal values of the tests (if applicable).
- Perform the tests, where possible.
- Interpret the results of the tests.
- List the disorders that can be diagnosed by the tests.
- Analyse case studies that refer to the laboratory results and to diagnosis of a disorder.

6.3. MODULE 3: HYPERSENSITIVITY

6.3.1. HYPERSENSITIVITY TYPE I (ALLERGY)

BACKGROUND KNOWLEDGE

OBJECTIVE

Provide interns/students with introductory knowledge of Hypersensitivity Type 1 (Allergy), with reference to the following:

- The Coombs and Gell Classification of hypersensitivities.
- The causes of allergy (include atopy, the hygiene hypothesis and environmental factors).
- The definition, characteristics and routes of exposure of allergens.
- The most common food allergens and inhalant allergens in adults and infants.
- The different phases (i.e. induction and effector: early, late, secondary) and mechanism of damage of hypersensitivity type I (allergy).
- The structure of IgE.

SPECIFIED OUTCOMES

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

- Describe the Coombs and Gell Classification of Hypersensitivities.
- Explain the following causes of allergy: atopy, the hygiene hypothesis, and environment.
- Describe the definition, characteristics and routes of exposure of allergens.
- List the most common food allergens and inhalant allergens in adults and infants.
- Describe the different phases (i.e. induction and effector: early, late, secondary) and mechanism of damage of Hypersensitivity Type I (Allergy)
- Describe and illustrate (by labelled sketch) the structure of IgE

6.3.1.1. DISORDERS ASSOCIATED WITH HYPERSENSITIVITY TYPE I (ALLERGY)

OBJECTIVE

Provide interns/students with knowledge of the following Hypersensitivity Type I disorders (Allergy):

Localized allergic reactions to food.

Refer to the following:

- Allergic gastro-intestinal reactions.
- Allergic urticaria.
- Allergic angioedema.
- Allergic eczema.

Localized allergic reactions to inhalants.

Refer to the following:

- Allergic rhinitis.
- Allergic asthma.
- Systemic allergic reaction: allergic anaphylaxis

IgE Negative allergic disorders.

Refer to the following:

- Allergy mediated by Basophils
- Allergy mediated by T-cells

Non-Allergy disorders, associated with a very high IgE.

Refer to the following:

- Multiple Myeloma
- Autosomal Dominant Hyper IgE Syndrome
- Omenn's syndrome
- Wiskott-Aldrich syndrome
- IPEX (Immunodysregulation polyendocrinopathy enteropathy X-linked syndrome)

Non-Allergy disorders, associated with increased tryptase or increased eosinophils.
Refer to the following:

- Mastocytosis
- MCAS (Mast Cell Activation Syndrome)
- HES (Hyper Eosinophilic Syndrome)

SPECIFIED OUTCOMES

On completion of this section the intern/student should be able to:
Explain the aetiology of each disorder.
Describe the pathophysiology of each disorder.
List the treatment options for each disorder.
List the laboratory tests that can be used to diagnose each disorder.

6.3.1.2. THE LABORATORY TESTS THAT ARE USED TO DIAGNOSE HYPERSENSITIVITY TYPE I DISORDERS

OBJECTIVE

Provide interns/students with knowledge and skills relating to the following clinical and laboratory investigations:

- Patient history.
- Clinical examination.
- The oral food challenge test.
  Also refer to the use of this test to desensitize a patient
- The skin prick test.
- Testing of Total IgE, allergen mixes and specific IgE
  using EIA (FEIA vs. ELISA), Immunoblots or CLA (Chemiluminescent assay)
- Additional tests for allergic rhinitis and allergic asthma: Nasal and bronchial mucus smear / ECP (Eosinophil cationic protein).
- Additional tests for anaphylaxis: Tryptase / ISAC® (Immuno solid-phase allergen chip) test for essential confirmatory components.
- Additional tests for IgE Negative allergies: The CAST (cellular allergen stimulation test) test for basophil activation / The MELISA (Memory Lymphocyte Immunostimulation Assay) for T-cell activation.

SPECIFIED OUTCOMES

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

- Explain the principle of the tests.
- List the steps in the method of the tests.
- List the normal values of the tests (if applicable).
- Perform the tests, where possible.

- Interpret the results of the tests.
- List the disorders that can be diagnosed by the tests.
- Analyse case studies that refer to the laboratory results and to diagnosis of a disorder.

6.3.2. HYPERSENSITIVITY TYPE II

BACKGROUND KNOWLEDGE

OBJECTIVE

Provide interns/students with introductory knowledge of Hypersensitivity Type II as well as the subtype sometimes referred to as Hypersensitivity Type V.

Refer to the definition, time to onset and mechanism of damage of Hypersensitivity Type II and distinguishing traits of Type V (i.e. ab. Bind to cell surface receptors instead of cell surface components thus blocking or impairing cell signalling).

SPECIFIED OUTCOMES

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

- Describe the definition, time to onset and mechanism of damage of Hypersensitivity Type II.

6.3.2.1. DISORDERS ASSOCIATED WITH HYPERSENSITIVITY TYPE II

OBJECTIVE

Provide interns/students with knowledge of the following Hypersensitivity Type II disorders:

Transfusion reactions

Refer to the following:

- Intra-vascular haemolysis (The ABO System)
- Extra-vascular haemolysis (Other Systems e.g. Kidd / Kell / Duffy)
- HDNB (Haemolytic Disease of the New Born)

Drug-induced haemolytic anaemia.

Type V: Graves' disease and Myasthenia gravis

SPECIFIED OUTCOMES

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

- Explain the aetiology of each disorder.
- Describe the pathophysiology of each disorder.
- List the treatment options for each disorder.
- List the laboratory tests that can be used to diagnose each disorder.

6.3.2.2. THE LABORATORY TESTS THAT ARE USED TO DIAGNOSE HYPERSENSITIVITY TYPE II DISORDERS

OBJECTIVE

Provide interns/students with knowledge and skills relating to the following laboratory test:
The Coombs test (Direct and Indirect)

SPECIFIED OUTCOMES

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

- Explain the principle of the test.
- List the steps in the method of the test.
- List the normal value of the test (if applicable).
- Perform the test, where possible.
- Interpret the results of the test.
- List the disorders that can be diagnosed by the test.
- Analyse case studies that refer to the laboratory results and to diagnosis of a disorder.

6.3.3. HYPERSENSITIVITY TYPE III

BACKGROUND KNOWLEDGE

OBJECTIVE

Provide interns/students with introductory knowledge of Hypersensitivity Type III.
Refer to the following: Clearance of immune complexes, definition, time to onset and mechanism of damage of Hypersensitivity Type III.

SPECIFIED OUTCOMES

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

- Describe the clearance of immune complexes and the factors that influence the process.
- Describe the definition, time it takes to occur and mechanism of damage of hypersensitivity Type III.

6.3.3.1. DISORDERS ASSOCIATED WITH HYPERSENSITIVITY TYPE III

OBJECTIVE

Provide interns/students with knowledge of the following Hypersensitivity Type III disorders:

- Serum Sickness.
- The Arthus Reaction.
  Refer to repeated injections of therapeutic drugs or vaccinations subcutaneously.
- <u>Persistent infections</u>
  Refer to the following infections:
  - Syphilis (refer to the different stages of the disease).
  - APSGN (Acute Post Streptococcal Glomerulonephritis).
  - Vasculitis.
  - EAA (Extrinsic “Allergic” Alveolitis). Refer to Bird Fancier’s disease and Farmer’s lung disease.

Refer to the initial uncomplicated disease and the later Hyper Type III complications.

SPECIFIED OUTCOMES

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

- Explain the aetiology of each disorder.
- Describe the pathophysiology of each disorder.
- List the treatment options for each disorder.
- List the laboratory tests that can be used to diagnose each disorder.

6.3.3.2. THE LABORATORY TESTS THAT ARE USED TO DIAGNOSE HYPERSENSITIVITY TYPE III DISORDERS

OBJECTIVE

Provide interns/students with knowledge and skills relating to the following laboratory tests:

- Tests to detect CICs (Circulating Immune Complexes).
  Refer to the following tests:
  - PEG (Polyethylene glycol) solubility and precipitation.
  - ELISA to detect CICs affinity for the C1q complement component.
- Specific tests to diagnose EAA.
  Refer to the following tests:
  - Ouchterlony (Double Immunodiffusion and precipitation in gel).
  - FEIA (Fluorescent Enzyme Immunoassay) on the Immunocap.
- Specific tests to diagnose Syphilis.
  Non Treponemal Screening tests. Refer to the following tests:
  - RPR (Rapid Plasma Reagin) test.
  - VDRL (Venereal disease Research Laboratory) test.
  Treponemal Confirmatory tests. Refer to the following tests:
  - TPHA (Treponema Pallidum Haemagglutination) test.
  - TPA Poly (Treponema Pallidum Antibody) by Chemiluminescence.
  - FTA-Abs (Fluorescence Treponemal Assay-Adsorbent) test for IgM and IgG.
  - ELISA test for IgM and IgG.
- Specific test to diagnose APSGN.
  Refer to the following test: Nephelometry for ASL and Anti-DNAse B.

SPECIFIED OUTCOMES

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

- Explain the principle of the tests.
- List the steps in the method of the tests.
- List the normal values of the tests (if applicable).
- Perform the tests, where possible.
- Interpret the results of the tests.
- List the disorders that can be diagnosed by the tests.
- Analyse case studies that refer to the laboratory results and to diagnosis of a disorder.

6.3.4. HYPERSENSITIVITY TYPE IV

BACKGROUND KNOWLEDGE

OBJECTIVE

Provide interns/students with introductory knowledge of Hypersensitivity Type IV and the subtype VI known as ADCC (Antibody Dependant Cell Mediated Cytotoxicity).

Refer to the definition, time to onset and mechanism of damage of Hypersensitivity Type IV and subtype VI.

SPECIFIED OUTCOMES

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

- Describe the definition, time to onset and mechanism of damage of Hypersensitivity Type IV and subtype VI.

6.3.4.1. DISORDERS ASSOCIATED WITH HYPERSENSITIVITY TYPE IV & TYPE VI

OBJECTIVE

Provide interns/students with knowledge of the following Hypersensitivity Type IV associated tissue damage/ disorders:

- Granulomatous lesions in leprosy.
- Cavitation and caseation (in the lung) in tuberculosis.
- The rash found in measles and smallpox.
- Skin damage in contact hypersensitivity reactions to dyes, metals and chemicals.
- Bronchial obstruction in asthmatic individuals (in this condition TH_2 and eosinophils dominate).

Type VI

- Poison Ivy Contact Dermatitis
- Parasitic larvae infections (Eosinophils kill helminths)

SPECIFIED OUTCOMES

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

- Explain the aetiology of each disorder.
- Describe the pathophysiology of each disorder.
- List the treatment options for each disorder.
- List the laboratory tests that can be used to diagnose each disorder.

6.3.4.2. THE LABORATORY TESTS THAT ARE USED TO DIAGNOSE HYPERSENSITIVITY TYPE IV DISORDERS

OBJECTIVE

Provide interns/students with knowledge and skills relating to the following laboratory tests:

- The patch test to diagnose contact dermatitis.
- The tuberculin test to demonstrate the existing immunity.
- IFN-γ tests (interferon gamma) to diagnose tuberculosis.
  Refer to the following tests: the TB spot test / Quantiferon TB gold in-tube test

SPECIFIED OUTCOMES

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

- Explain the principle of the tests.
- List the steps in the method of the tests.
- List the normal values of the tests (if applicable).
- Perform the tests, where possible.
- Interpret the results of the tests.
- List the disorders that can be diagnosed by the tests.
- Analyse case studies that refer to the laboratory results and to diagnosis of a disorder.

6.4. MODULE 4: TRANSPLANT IMMUNOLOGY

6.4.1. RED BLOOD CELL ANTIGENS

BACKGROUND KNOWLEDGE

OBJECTIVE

Provide interns/students with introductory knowledge of the red blood cell antigens.

Include the following:

- Characteristics of the ABO blood group system.
  Refer to preformed antibodies / the Landsteiner rule / universal donor and universal recipient.
- Characteristics of the Rhesus blood group system.
  Refer to development of specific antibodies after sensitisation.

SPECIFIED OUTCOMES

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

- Describe the characteristics of the ABO Blood group system with reference to preformed antibodies, the Landsteiner rule and universal donor and universal recipient.
- Describe the characteristics of the Rhesus group system with reference to development specific antibodies after sensitisation.

APPLICATION OF ABO AND RHESUS GROUPING

OBJECTIVE

Provide interns/students with knowledge of where ABO and Rhesus grouping is applied e.g.:

- Blood transfusion.
- Organ transplants.
- Pre and post natal care (to prevent and diagnose HDNB).
- Paternity testing (would include grouping of the "other systems" as well).
- Forensic investigations (would include grouping of the "other systems" as well).

SPECIFIED OUTCOMES

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

- List situations where ABO and Rhesus grouping is applied.

6.4.1.1. THE LABORATORY TESTS THAT ARE USED TO PERFORM RED BLOOD CELL GROUPING

OBJECTIVE

Provide interns/students with knowledge and skills relating to the following laboratory tests:

- Forward and reverse grouping for ABO and Rh. Refer to the following methods:
  Slide Agglutination / Tube Agglutination.
- Additional test for Discrepancies. Refer to the following: Anti-H / Anti-A1 or Anti-A2.
- Cross-matching of Recipient and Donor.

SPECIFIED OUTCOMES

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

- Explain the principle of the tests.
- List the steps in the method of the tests.
- List the normal values of the tests (if applicable).
- Perform the tests, where possible.
- Interpret the results of the tests.
- Analyse case studies that refer to the laboratory results and an application of the test.

6.4.2. WHITE BLOOD CELL (HLA) ANTIGENS

BACKGROUND KNOWLEDGE

OBJECTIVE

Provide interns/students with introductory knowledge of the white blood cell (HLA) antigens.

Include the following:

- Composition of the MHC System (Major Histocompatibility Complex).
- The structure of MHC Class I and MHC Class II (refer to the heavy and light chains and the peptide grooves).
- Cells on which MHC Class I and MHC Class II are expressed.
- Inheritance of the MHC with reference to co-dominance.
- The functions of the MHC in the immune system.

Include the following definitions:

- Gene polymorphism / Phenotype / Genotype / Haplotype / Heterozygous / Homozygous / Linkage disequilibrium.

SPECIFIED OUTCOMES

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

- Describe the composition of the of the MHC system.
- Illustrate (by labelled sketches) the structure of MHC Class I and MHC Class II.
- List the immune cells on which MHC Class I and MHC Class II are expressed.

- Describe the functions of the MHC in the immune system

Explain the following definitions:

- Gene polymorphism / Phenotype / Genotype / Haplotype / Heterozygous / Homozygous / Linkage disequilibrium

APPLICATION OF THE HLA CLASS I & II TYPING.

OBJECTIVE

Provide interns/students with knowledge of application of HLA typing e.g.:

- Organ transplants.
- Paternity testing.
- Forensic investigations.
- Disease associations with HLA alleles.

SPECIFIED OUTCOMES

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

- Understand and explain the applications of HLA Class I & II typing.

6.4.2.1 THE LABORATORY TESTS THAT ARE USED TO PERFORM HLA CLASS I & CLASS II TYPING

OBJECTIVE

Provide interns/students with knowledge and skills relating to the following laboratory tests:

<u>HLA Class I and Class II typing tests.</u>

Refer to the following methods:

1. <u>Serological Method</u>

- CDC (Complement dependent cytotoxicity) HLA antigen test with reference to:
  - Preparation of a lymphocyte suspension utilising ficoll density gradient separation.
  - Separation of cells using nylon wool / immunomagnetic beads (IMB).
  - Operation of an inverted microscope.
  - The use of light microscopy stains (eosin, bromothymol blue) vs. fluorescent microscopy stains (Acridin Orange, Ethidium bromide).

2. <u>Molecular methods</u>

Include the following methods:

- SSP (Sequence specific primers)
- SSOP (Sequence specific oligonucleotide probes)
- rSSOP (reverse Sequence specific oligonucleotide probes)

- SBT (Sequence Based Typing) and New generation SBT

3. PRA (Panel Reactive Antibodies) methods

Refer to the following methods:

- CDC
- Solid-phase bead arrays (i.e. Luminex®) HLA Class I & II screening and LSA (Luminex-Single Antigen).

4. DSA (Dobor Specific Antibodies) methods

Refer to the following methods:

- CDC
- MLC (mixed lymphocyte culture)
- Solid-phase bead arrays (i.e. Luminex®)

SPECIFIED OUTCOMES

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

- Describe the preparation of a lymphocyte suspension using ficoll density gradient centrifugation.
- Describe the separation of T & B lymphocytes using nylon wool / IMB.
- Illustrate (by labelled sketch) an Inverted Microscope.
- Explain the principle of the tests.
- List the steps in the method of the tests.
- Perform the tests, where possible.
- Interpret the results of the tests.
- Describe the advantages and shortcomings of each method.
- Differentiate between: low-, medium- and high-resolution typing of HLA alleles.
- Analyse case studies that refer to the laboratory results of transplant patients

6.4.3. REJECTION OF TRANSPLANTED TISSUE

OBJECTIVE

Provide interns/students with knowledge relating to the rejection of transplanted tissue and underlying immunological pathways with reference to:

- Types of rejection: Hyper-acute / Acute / Chronic / GVH (Graft Versus Host).
- Strategies to prevent rejection pre- and post-transplantation.

SPECIFIED OUTCOMES

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

- Describe the types of rejection with reference to the underlying immunological pathways causing the rejection.
- Describe strategies to prevent rejection pre- and post-transplantation with reference to post treatment modalities.

6.4.4. AUTOIMMUNE DISORDERS ASSOCIATED WITH HLA CLASS I & II

OBJECTIVE

Provide interns/students with knowledge relating to the application of HLA typing to determine genetic predisposition to certain autoimmune diseases with reference:

- Application of serological HLA antigen typing compared to high-resolution HLA specific alleles typing to identify disease association. (e.g. HLA-B*27 vs. HLA-B*27:xx)

Refer to the following diseases:

- Insulin Dependent Diabetes Mellitus Type 1.
- Ankylosing spondylitis.
- Rheumatoid arthritis.
- Celiac disease.

SPECIFIED OUTCOMES

On completion of this section the intern/student should be able to list autoimmune disorders and the predisposing HLA high-resolution alleles or HLA serological antigen and how it contributes to the diagnosis of the disease.

6.4.4.1. LABORATORY TESTS FOR SELECTED HLA ALLELES / ANTIGENS

OBJECTIVE

Provide interns/students with the knowledge and skills relating to the following laboratory tests to identify specific HLA alleles or antigens:

- CDC HLA antigen typing.
- Flow Cytometry.
- High-resolution HLA allele typing (i.e. rSSOP microarray).

SPECIFIED OUTCOMES

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

- Interpret the results of the tests.
- Analyse case studies that refer to the laboratory results and diagnosis of an HLA-associated disorder.

7. THE IMMUNE RESPONSE TO SOME OTHER INFECTIONS

7.1. SOME OTHER INFECTIONS THAT ARE VERY RELIANT ON IMMUNOLOGICAL INVESTIGATIONS

OBJECTIVE

Provide interns/students with knowledge of the following disorders:

- Typhoid fever
- Brucellosis
- Tick bite fever
- Yersinia enterocolitica and Yersinia pseudotuberculosis
- Cryptococcus infection
- Toxoplasmosis
- Amoebiasis
- Echinococcus granulosus infection
- Taenia solium infection
- Pneumocystis jiroveci infection

- Chlamydia trachomatis infection
- Hepatitis A / B / C infections.

SPECIFIED OUTCOMES

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

- Explain the aetiology of each disorder.
- Describe the pathophysiology of each disorder.
- List the treatment options for each disorder.
- List the laboratory tests that can be used to diagnose each disorder.

7.2. THE IMMUNOLOGICAL LABORATORY TESTS THAT AID IN THE DIAGNOSIS OF THESE INFECTIONS

OBJECTIVE

Provide interns/students with knowledge and skills relating to the following laboratory tests:

- The TMX test to aid in screening for Typhoid fever, Brucellosis and Tick Bite fever.
- The Tube agglutination or ELISA tests to aid in the diagnosis of Yersinia enterocolitica or Yersinia pseudo tuberculosis infection.
- The agglutination test to detect Cryptococcus in CSF.
- Specific tests to diagnose Toxoplasmosis.
  Refer to the following tests:
  - ELISA for IgM and IgG.
  - ELISA for Avidity / Affinity.
- Haemagglutination to diagnose Entamoeba histolytica, Echinochoccus granulosis and Taenia solium.
- DFA to diagnose Pneumocystis jiroveci and Chlamydia trachomatis.

- Specific test to diagnose Hepatitis A / B / C.
  Refer to the following tests:
  - Rapid Lateral flow Screening tests.
  - Chemiluminescence tests.

SPECIFIED OUTCOMES

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

- Explain the principle of the tests.
- List the steps in the method of the tests.
- List the normal values of the tests (if applicable).
- Perform the tests, where possible.
- Interpret the results of the tests.
- List the disorders that can be diagnosed by the tests.
- Analyse case studies that refer to the laboratory results and to diagnosis of a disorder.

8. CLINICAL APPLICATIONS

Each module of the Immunology syllabus requires case studies and mentions clinical application of diseases and disorders. The student/intern must study these as per the required section and must be tested to ensure understanding of the clinical application of the results produced with each test performed.

TRAINERS HAVE THE RESPONSIBILITY TO ENSURE THAT THIS IS TAUGHT AND TESTED.

9. REFERENCE MATERIAL

The following books are recommended:

- Immunology, Latest edition. Roitt.
- Immunobiology, Latest edition. Janeway.
- Cellular and Molecular Immunology, Latest Edition. Abbas
- Good Laboratory Practice (GLP) Handbook, Latest edition. WHO
- Occupational Health and Safety Act, 1993 (Act No. 85 of 1993)

10. NOMENCLATURE / ACRONYMS

Acronym	Meaning
ABO	Red blood cell groups A, B and O
AD	Autosomal dominant
ADA	Adenosine deaminase deficiency
ADCC	Antibody Dependant Cell Mediated Cytotoxicity
AIDS	Acquired immunodeficiency disorder
AIR	Acute inflammatory response
ALPS	Autoimmune lymphoproliferative syndrome
ANA	Anti-nuclear antigen
ANCA	Anti-neutrophil cytoplasmic antibodies
APECED	Autoimmune polyendocrinopathy candidiasis ectodermal dystrophy

APHC 100	Alternative pathway haemolytic complement test
APLS	Anti-phospholipid syndrome
APP	Acute phase protein
APSGN	Acute post-streptococcal glomerulonephritis
ASL	Anti-streptolysin o
CAST	Cellular allergen stimulation test
CB	Composite block
CCP	Cyclic-citrullinated peptide
CD	Cluster of differentiation
CDC	Complement dependent cytotoxicity
CEUs	Continual education units
CGD	Chronic granulomatous disease
CIC	Circulating immune complexes
CLA	Chemiluminescent assay
CMC	Chronic mucocutaeous candidiasis
CPD	Continuous professional development
CPHC 100	Classical pathway haemolytic complement test
CREST	Calcinosis, Raynaud phenomenon, Esophageal dysmotility, Sclerodactyly, and Telangiectasia
CRP	C-reactive protein
CTLA-4	Cytotoxic T-lymphocyte antigen 4
CVID	Common variable immunodeficiency
DCs	Dendritic cells
DNA	Deoxyribonucleic acid
DNAse	Deoxyribonuclease
DSA	Donor specific antibodies
EAA	Extrinsic “Allergic” Alveolitis
ECL	Electro-chemiluminescence
ECP	Eosinophil cationic protein
EDTA	Ethylenediaminetetraacetic acid
ELIA	Automated ELISA
ELISA	Enzyme-linked immunosorbent assay
ENAs	Extractable nuclear antigens
FEIA	Fluorescence enzyme immunoassay
FISH	Fluorescence *in situ* hybridisation
FTA-Abs	Fluorescence Treponemal Assay-Adsorbent
GLP	Good laboratory practice
GVH	Graft-versus-Host
HAE	Hereditary angioedema
HEp-2	Human epithelium carcinoma-2
HIES	Hyper IgE syndrome
HIV	Human immunodeficiency virus
HLA	Human leucocyte antigen

HPCSA	Health professions council of South Africa
HSV	Herpes simplex virus
HUS	Hereditary uremic syndrome
HVZV	Herpes varicella zoster virus
IBD	Inflammatory bowel disease
IDDM1	Insulin dependent diabetes mellitus type i
IFN-γ	Interferon-gamma
IL	Interleukin
IL-2 RG	Interleukin-2 receptor gamma
IL-7 RA	Interleukin-7 receptor alpha
IMB	Immunomagnetic beads
IPEX	Immune dysregulation polyendocrinopathy enteropathy x-linked syndrome
ISAC®	Immuno solid-phase allergen chip
ISO	International organization for standardization
JAK3	Janus kinase 3
LAD-1	Leukocyte adhesion deficiency-1
LAD-2	Leukocyte adhesion deficiency-2
MALT	Mucosal associated lymphoid tissue
MCAS	Mast cell activation syndrome
MCGN	Mesangio capillary glomerulonephritis
MCTD	Mixed connective tissue disorder
MELISA	Memory lymphocyte immune-stimulation assay
MHC	Major histocompatibility complex
MLC	Mixed lymphocyte culture
MPO	Myeloperoxidase
MSDS	Materials safety data sheets
MSMD	Medelian susceptibility to mycobacterial disease
MW	Molecular weight
NBT	Nitro blue tetrazolium
NETs	Neutrophil extracellular traps
NK	Natural killer
OB	Oxidative burst
PBC	Primary biliary cirrhosis
PCR	Polymerase chain reaction
PEG	Polyethylene glycol
PIDs	Primary immunodeficiencies
PM	Polymyositis
PNH	Paroxysmal nocturnal haemoglobinuria
PNP	Purine nucleoside phosphorylase enzyme
PPE	Protective personal equipment
QA	Quality assurance
QC	Quality control

RA	Rheumatoid arthritis
RAG1/2	Recombination activating gene 1 & 2
RF	Rheumatoid factor
RPR	Rapid Plasma Reagin
RS	Radiation sensitivity
rSSOP	Reverse sequence specific oligonucleotide probes
SBT	Sequence Based Typing
SCID	Severe combined immunodeficiency
Scl-70	Scleroderma-70
SFLC	Serum free light chains
SGD	Specific granule deficiency
SLE	Systemic lupus erythematosus
SOPs	Standard operating procedures
SSOP	Sequence specific oligonucleotide probes
SSP	Sequence specific primers
TB	Tuberculosis
TE	Triss EDTA
TPA	Treponema Pallidum Antibody
TPHA	Treponema Pallidum Haemagglutination
TREC	T-cell receptor excision circles
UV	Ultra-violet
VDRL	Venereal disease Research Laboratory
WIs	Working instructions

APPENDICES

None