VIROLOGY

TECHNICIAN EXAMINATION

STUDY GUIDE

1. Examination Information

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

Both papers will be broadly based and will cover the entire field under study. Details relating to specific times and quantities involved in methods will not be asked. Candidates will however be expected to know the principles on which the tests are based and how to interpret the results obtained.

The final percentage will be based on the combined mark obtained in both papers. Candidates must achieve a minimum of 50% to pass the examination.

SYLLABUS

2. General

A basic knowledge of the syllabus is required: i.e.

Laboratory safety including the Occupational Health and Safety Act and the responsibilities of safety and first aid representatives.

Procedures for the storage, handling and disposal of hazardous chemicals and laboratory waste.

Basic understanding of the quality control and practical application according to relevant SOP’s.

The ethical rules relating to reporting of results and confidentiality.

Stock control and processing.

The preparation and use of disinfectants including cleaning of glassware and sterilisation techniques.

The operation and maintenance of standard laboratory equipment.

VIROLOGY

Study Guide – Technician Training

General

The aim of this syllabus guide is to serve as an up-to-date record of all work that must be performed by the student.

To the Supervisor

Please ensure that your laboratory is registered with the Health Professionals Council of South Africa as a Virology training laboratory.

To the Student

Please ensure that your laboratory is registered as a Virology training laboratory with Health Professionals Council of South Africa.

Section One: Laboratory Safety

Aim: To ensure that the student medical technician has a sound knowledge of all the safety procedures used in the specific laboratory in which he/she is employed.

Objectives and Exercises:
The student medical technician must be able to:-

1. List the procedures done in the diagnostic virology laboratory, such as pipetting, centrifugation, etc, which may be potentially hazardous to oneself and others;
2. Itemise and discuss safety precautions that should be taken to minimise infection or injury when carrying out the procedures and include existing laboratory safety rules;
3. Investigate the safety conditions in the virology laboratory by:-

    3.1 Drawing a floor plan of the laboratory to scale,
    3.2 Marking the position of emergency exits, fire extinguishers and hoses, safety blankets, showers, keys to locked exits, etc,
    3.3 Make practical suggestions, if necessary, on how these safety features could be improved;

4. Detail the official plan of action in the events of:-

    4.1 Fire
    4.2 Bomb threat
    4.3 Minor injury such as a cut or needle stick injury,
    4.4 Screening of laboratory staff for immunity,
    4.5 Protection of pregnant staff who are immunocompromised;

5. Describe how the following are decontaminated and/or disposed of in the virology laboratory

    5.1 Decontamination of working surfaces, such as benches or hoods;
    5.2 Used glassware, such as glass bottles, volumetric flasks, syringes, etc;
    5.3 Used plastic ware, such as tissue culture flasks, micro titre plates, graduated pipettes, etc;
    5.4 Tissue biopsies, stools, etc;
    5.5 Waste water from ELISA washings;
    5.6 Used syringe needles, scalpels, and disposable glass Pasteur pipettes;

    Describe the action and chemical base of any disinfectants, soaps, etc.

6. Name the vaccines currently commercially available which should be given to susceptible staff members, and state your reasons for recommending them.

Section Two: Laboratory Apparatus

Aim: To ensure that the student medical technician understands the functioning of the laboratory apparatus, and how to maintain and use them correctly.

Objectives:
The student medical technician must be able to:-

1. Explain the basic operational principles of the apparatus listed below;
2. Demonstrate practically how the apparatus should be used and maintained.
3. Describe briefly how the apparatus should be tested or checked to ensure that it is operating efficiently and accurately

The Apparatus
(This list should be updated regularly as the diagnostic virology laboratory modernises)

1. Autoclaves
2. Balances (including single pan and top pan analytical balances)
3. Laminar flow cabinets (including vertical and horizontal)
4. Centrifuges and microfuges
5. Incubators (including CO2 incubators)
6. Membrane filter systems
7. Microscopes (including light and fluorescent microscopes)
8. pH meters
9. Photometers
10. Pipettes and pipette aids
11. Water baths
12. Water purification systems

Section Three: Cell Culture

Aim: To ensure that the student medical technician has the technical competence and theoretical knowledge to prepare and maintain a range of cell cultures for use in the diagnostic virology laboratory.

Objectives and exercises:
The student medical technician must be able to:-

1. Prepare all the media and reagents required. Details of the formula and the methods of preparation, sterilisation and quality control must be included in section three as an appendix;
2. Name the basic constitutions used in cell culture medium, and discuss their action and reasons for inclusion in the medium;
3. Name the various cell types used for diagnostic virology, and state which are used in your laboratory;
4. Illustrate the morphology of the normal cells in labels , diagrams and drawings;
5. Discuss the types, quality and treatment of the glass and plastic ware used in the cell culture laboratory;
6. Manipulate a continuous cell line:-

    6.1 Produce a flask of Hela cells (or other continuous cell line) from A frozen-down ampoule;
    6.2 Passage these cells at least four times and use one flask to produce some tube cultures containing coverslips; (these cultures may be used for the isolation of adenovirus, see section four);
    6.3 Freeze and store the cells contained in the remaining flasks for one or two months;
    6.4 Use one of these frozen ampoules and repeat step 6.1

7. Fully describe the procedure used to initiate a primary cell culture of monkey or rabbit kidney, human amnion, or similar cells;
8. Discuss the possible ways in which contaminations could be introduced into cell cultures, and name the contaminants commonly occurring in your laboratory;
9. Discuss the precautions taken and aseptic techniques used to prevent contamination of these, and other cell cultures.

Section Four: Viral Isolation

Aim: To ensure that the student medical technician can isolate culture and identify commonly encountered viruses, from the appropriate specimens.

Objectives and Exercises:
The student medical technician must be able to:-

1. Tabulate the main virologically-associated clinical syndromes (such as respiratory, congenital, etc.) and the appropriate specimens which should be collected in each case;
2. Describe how these specimens, named above, are obtained and transported to the laboratory;
3. Compile a protocol for each of the following viruses which includes:-

    3.1 A labelled diagram of the virus showing its morphological features,
    3.2 A brief description of the properties and classification of the virus,
    3.3 The type of specimens required for the isolation of each virus and describe how they are processed for isolation,
    3.4 A detailed description of how the virus is isolated, passages harvested and stored.
    3.5 The identification of the virus detection including traditional cell culture and rapid procedures.

4. Adenoviruses

    4.1 Compile a protocol for adenoviruses as outlined in 3;
    4.2 Fix coverslips obtained upon culturing the adenovirus in cell cultures for identification by immunofluorescence;
    4.3 In each case; tabulate the different illness caused by the specimens adenovirus serotype in man and name the appropriate specimens which should be collected for isolation.

5. Mumps virus

    5.1 Compile a protocol for the mumps virus as outlined in 3;
    5.2 Isolate a mumps virus in cell cultures, stain coverslips with Haematoxylin and Eosin, and fix coverslips for immunofluorescence identification as well.

6. Parainfluenza virus

    6.1 Compile a protocol for the parainfluenza virus as outlined in 3;
    6.2 Inoculate types 1,2 and 3 into cell cultures and perform haemadsorption assays;
    6.3 Stain coverslips to demonstrate cytopathic effects;
    6.4 Fix coverslips for immunoflourescence identification.

7. Measles virus

7.1 Compile a protocol for the measles virus as outlined in 3;
7.2 Isolate a measles virus in cell culture and stain coverslips to demonstrate cytopathic effects;
7.3 fix coverslips for immunoflourescence identification;
7.4 Discuss the clinical disease and post-infectious complications that may occur, and how the laboratory can assist in the diagnosis of these conditions.

8. Respiratory syncytial virus

8.1 Compile a protocol for the respiratory syncytial virus as outlined in 3;
8.2 Isolate a measles virus in cell culture and stain coverslips to demonstrate cytopathic effects;
8.3 Fix coverslips for immunoflourescence identification;
8.4 Discuss the importance of this disease in infants.

9. Rubella virus

9.1 Compile a protocol for the rubella virus as outlined in 3;
9.2 Inoculate a rubella virus into suitable cell cultures to demonstrate:-

9.2.1 The cytopathic effects of the rubella virus,
9.2.2 How the rubella virus may interfere with the growth of a cytopathic virus such as echovirus type 11.

10. Herpes simplex virus

10.1 Compile a protocol for herpes simplex viruses as outlined in 3;
10.2 Inoculate the isolated virus into a monkey cell line.

11. Varicella virus

11.1 Compile a protocol for varicella virus as outlined in 3;
11.2 Isolate a varicella virus in an appropriate cell culture;
11.3 Fix coverslips containing foci of infection for identification by immunofluorescence.

12. Cytomegalovirus

12.1 Compile a protocol for cytomegalovirus as outlined in 3
12.2 Isolate a cytomegalovirus in cell cultures describing both the roller tube and shell vial techniques;
12.3 Fix coverslips containing foci of infection for identification by immunofluorescence.

13. Enteroviruses

13.1 Compile a protocol and isolate the following enteroviruses in primary, continuous and diploid cell cultures:-

13.1.1 Poliovirus
13.1.2 Echovirus type 9
13.1.3 Coxsackie virus type A
13.1.4 Coxsackie virus type B

13.2 Titrate and then type the poliovirus by performing a neutralisation test (use the Reed and
Meunch, or the Karber method for endpoint calculations);

14. Influenza virus

14.1 compile a protocol for the influenza virus as outlined in 3,
14.2 isolate an influenza virus in cell cultures and perform a haemadsorption assay with the cell culture findings,
14.3 Identify the isolated influenza virus by specific immunofluorescence assays.

15. Hepatitides

Discuss the hepatitis viruses A – E in terms of:

15.1 Their clinical differentiation;
15.2 Their diagnostic markers;
15.3 Diagnostic tests available.

16. Enzyme-Linked-Immunosorbent Assays : ELISA

16.1 Discuss and illustrate the principles involved in the direct, indirect, competitive and µ-capture ELISA assays. Give examples of these assays which are used in diagnostic virology;
16.2 Discuss the various types of antigens which are used in ELISA assays, such as viral lysates, recombinant and synthetic antigens; give examples of commercial assays which use these antigens;
16.3 List the ELISA assays performed in your diagnostic virology laboratory;
16.4 Perform an ELISA assay of your own choice on a selected group of sera. Calculate your results graphically and interpret your results;
16.5 Discuss the advantages and disadvantages of the ELISA assays.

17. Human Immunodeficiency Virus

    17.1 Illustrate with a labelled diagram the morphology of HIV
    17.2 Briefly discuss the properties, characteristics and classification of HIV
    17.3 Outline the precautions taken in your laboratory to minimise HIV infection
    17.4 Describe and perform the screening and confirmatory tests done in your laboratory for HIV and explain their significance in the diagnosis of AIDS / HIV infection.

18. Direct Viral Antigen Detection

    Describe the diagnostic assays done in your laboratory which can detect viral antigens directly in the clinical specimen.

19. Haemagglutination Inhibition Assay

    19.1 Tabulate the haemagglutinating viruses, the type of red blood cells they agglutinate and the conditions required for haemagglutination such as pH and temperature;
    19.2 Detail how non-specific inhibitors and agglutinins are removed from the patient's serum prior to testing in the rubella haemagglutination inhibition (HAI) assay;
    19.3 Draw the antibody response curves for man following primary, re-infection and congenital rubella infection.

20. Immunofluoresence

    20.1 Discuss the principles involved in the direct and indirect immunoflourescence assays;
    20.2 Discuss the use of immunofluorescence as a method for:-

        20.2.1 Rapid viral diagnosis;
        20.2.2 Typing viruses such as the parainfluenza viruses;
        20.2.3 Identifying antibodies in the sera of patients

Suggested Reading

1. Diagnostic Procedures for Viral, Rickettsial and Chlamydial Infection - By N. Schmidt and R. Emmons.

2. Diagnostic Virology - By Hsiung

3. Medical Virology - By Fenner and White

4. Latest Edition of Introduction to Medical Laboratory Technology - By Baker, Silverton, Luckcock

5. Biology for Senior Certificate