COVENANT UNIVERSITY

OMEGA SEMESTER TUTORIAL KIT
(VOL. 2)

PROGRAMME: MICROBIOLOGY

200 LEVEL

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LIST OF COURSES

*MCB221/BLY225: Introductory Parasitology
MCB222 :Techniques in Virology

***Not included**

COVENANT UNIVERSITY
CANNANLAND, KM 10 IDIROKO ROAD
P.M.B. 1023, OTA, OGUN STATE, NIGERIA

TITLE OF EXAMINATION: BSc. SEMESTER EXAMINATION
COLLEGE: SCIENCE & TECHNOLOGY
DEPARTMENT: BIOLOGICAL SCIENCES
SESSION: 2015/2016 **SEMESTER:** ALPHA
COURSE CODE: MCB 222 **CREDIT UNIT:** 3
COURSE TITLE: TECHNIQUES IN VIROLOGY
INSTRUCTION: Answer TWO questions from each section and FOUR questions in all.
TIME: 3 HOURS

SECTION A

1. (a) Discuss
   (i) A suitable method for determination of "Negri Bodies" 4.5 marks
   (ii) A suitable method for monoclonal antibody production 4.5 marks
   (iii) PCR for identification and quantification of a typical HIV virus 4.5marks

   (b) What are the uses of the following in virus identification? 4.0marks
   (i) Reporter molecule (ii) Reverse transcriptase (iii) Probe and (iv) Gel electrophoresis

2. Without repetitions between procedures, state four pieces of equipment and/or materials that you would require for the following procedures and in each case, state the function of the stated equipment or material;
   a. Virus cultivation in laboratory animal. 3.0 marks
   b. Virus cultivation in embryoneted egg. 3.0 marks
   c. Virus cultivation in cell/tissue culture. 2.5 marks
   d. Virus cultivation in whole plant. 3.0 marks
   e. Sample preparation for scanning electron microscopy. 3.0 marks
   f. Sample preparation for the transmission electron microscope 3.0 marks

3. (a) Compare and contrast the following:
   (i) Monoclonal and polyclonal antibody 2.0 marks
   (ii) Direct and indirect Enzyme Linked Immunosorbent Assay (ELISA) 2.0 marks
   (iii) Primary and secondary binding tests 2.0 marks
   (iv) Hemagglutination and hemagglutination inhibition 2.0 marks

   (b) Draw and label the structure of an antibody 5.5 marks
   (c) List two (2) enzymes and their respective substrates that are commonly used when carrying out ELISA 1.0 mark

(d) Explain the role of serological techniques in the diagnosis of viral infections

3.0 marks

SECTION B

4. (a) Define
   (i) *$TCID_{50}$* 1.0mark
   (ii) Primer 1.0mark
   (iii) CPE 1.0mark
   (iv) Apical meristem culture 1.0mark
   (v) Restriction endonuclease 1.0mark

   (b) List the steps involved in
   (i) ELISA (direct) 3.0marks
   (ii) Western blotting 3.0marks
   (iv) Southern blotting 3.0marks
   (v) Neutralization reaction 3.5marks

5. Briefly explain/state;
   a. Two major reasons the study of viruses require unique techniques 2.0 marks
   b. Five precautionary measures that you would take while working in the virology laboratory. 3.75 marks
   c. The five basic structural forms that a virus can take and give one example of each 3.75 marks
   d. Two essential components of an electron microscope and the major function of each component listed 2.0 marks
   e. Two function of the virus genome 1.5 marks
   f. Two function of the virus capsid 1.5 marks
   g. Two important reasons for virus cultivation 1.5 marks
   h. Two methods that are commonly used in the laboratory to achieve virus entry into plant cells 1.5 marks

6. a) Define the following:
   (i) Sensitized cells 1.0mark
   (ii) Antibody titer 1.0mark
   (iii) Anti-human antibody 1.0mark
   (iv) Epitope 1.0mark
   (v) Virus neutralization 1.0mark

   (b) Discuss the principle, procedure, advantages and disadvantages of any two (2) of these serological techniques: 10.0 marks
   (i) Fluorescent Antibody Test (FAT)
   (ii) Complement fixation
   (iii) Hemagglutination test
   (iv) Single radial haemolysis

   (c) Mention five (5) laboratory equipment that are used in a virology laboratory 2.5 marks

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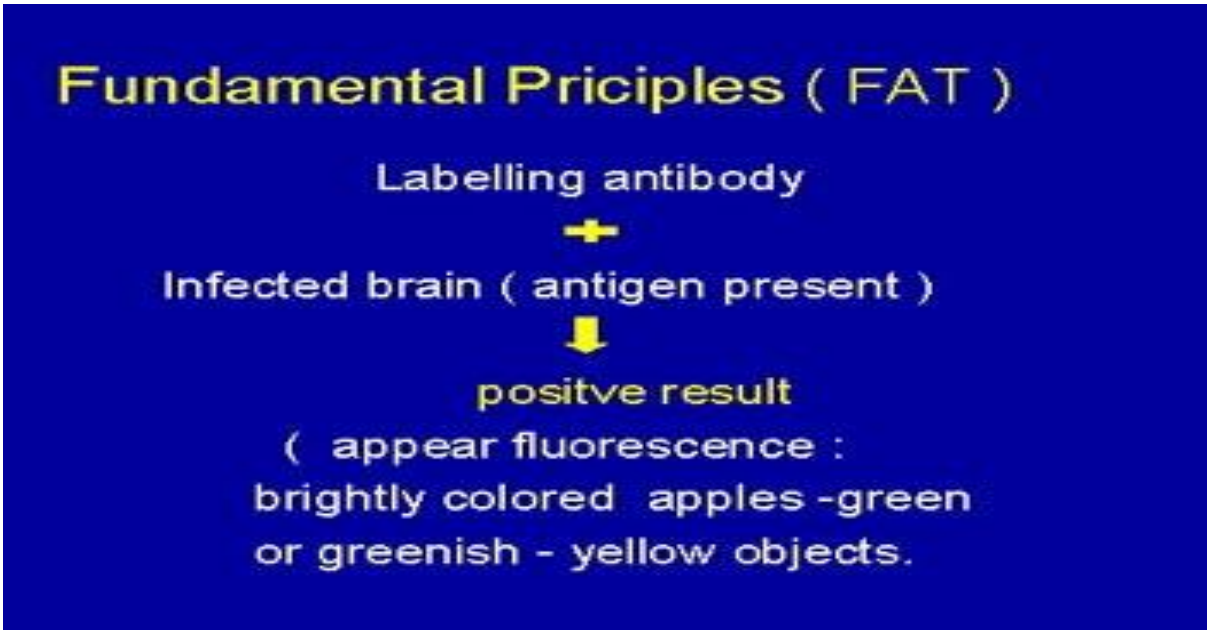
   (iii) PCR for identification and quantification of a typical HIV virus 4.5marks

   (b) What are the uses of the following in virus identification? 4.0marks
   (i) Reporter molecule (ii) Reverse transcriptase (iii) Probe and (iv) Gel electrophoresis

Topics covered by the question: ELISA, Cytopathatic effect, monoclonal antibody, Polymerase Chain Reaction, Gel electrophoresis

Answer hints/keywords for the question: FAT, Flow cytometer, Myeloma cells, Reverse transcriptase, DNA polymerase, annealing, HAT, clone

Full answer to the Question: 1(a)(i)

1. 

Direct fluorescent-antibody tests are used to identify "Negri bodies" (antigens).

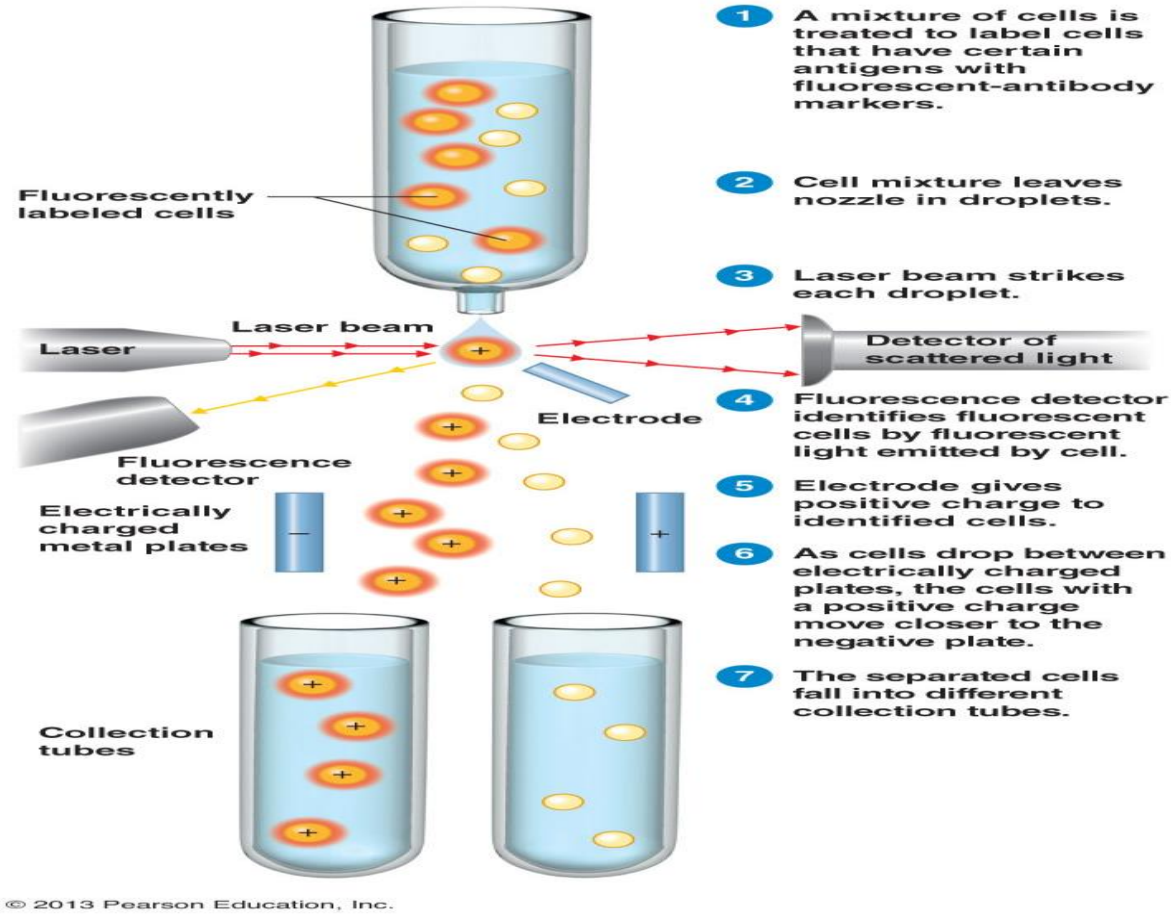
Antibodies directed against antigens on the surface of a specific microorganism are labeled with fluorescent dye. Fluorescent antibodies are incubated with the sample and antigen-specific binding allowed to occur. Excess and non-specifically attached antibodies are washed from the sample. The sample is viewed with a fluorescence viewer, whether a fluorescence microscope or plate reader or even fluorescence-activated flow cytometer **2.5marks**

Indirect Fluorescent-Antibody Tests

Indirect fluorescent-antibody tests are used to demonstrate the presence of antibodies against a specific antigen in serum. Antigen or the microorganism itself is incubated with the patient's serum. Excess serum is washed away, leaving only antibodies specific for the antigen (or antigenic portion of the microorganism) present in the patient's serum bound

The sample (lab stock of antigen or organism) is then incubated with antibodies labeled with fluorescent dye that are specific for human antibodies (fluorescent aniti-human antibody antibodies)(inject human immunoglobulins into another species and it will produce anti-human immunoglobulin antibodies) **1.5marks**

The sample is viewed with a fluorescence viewer, exactly like the direct antibody tests **0.5marks**



Or

The viruses for studying negri bodies were obtained from dog brains sent to the Lab. for routine examination of rabies. The hippocampus was removed from each brain aseptically, ground with alundum in a mortar, and diluted with physiological saline to a 10% suspension. **1.0mark**

Three hundredth mls of this 10% suspension was injected intracerebrally into each of 6 4 week old Swiss albino mice.On the 14th day after injection of the first strain several mice showed tremors and paralysis and become moribund. The brains were removed aseptically from these moribund mice. Impressions made from the Ammon's horn of each mouse were stained with Sellers stain and under the light microscope showed numerous negri bodies throughout the tissue. **1.5marks**

The Ammon's horn from a mouse contained a great number of negri bodies was used for the electron microscopy.

The second strain of rabies was injected intracerebrally into 6 Swiss mice and on the seventh day after injection, several mice showed tremors and paralysis and become moribund. The Ammon's horn containing the greatest number of negri bodies was used for the electron microscope examination. **1.5marks**

Parlodion film supports were prepared 24 h before being used. The prepared screen was picked up with forceps and the parlodion surface was dipped in 10% formalin. The parlodion surface was then touched lightly to the same area of the Ammon's horn used for direct smear examination. These screens were left to dry for examination the following day. Several screens from each strain were examined in an EMU electron microscope at various magnifications for neurons and inclusion bodies. **0.5mark**

(ii) Antibody producing B lymphocytes normally die after several weeks in cell culture (in cell culture).Therefore antibody producing B lymphocytes are fused with B cell tumors called myelomas. These myelomas are capable of dividing indefinitely and are therefore often called immortal cell lines.

The immortal cell lines that result from the B cell –myeloma fusion are called hybridomas. The hybridoma cell lines share the properties of both fusion partners. They grow indefinitely in vitro and produce antibodies. To produce a monoclonal antibody, a mouse is immunized with the antigen of interest. During the next several weeks, antigen specific B cells proliferate and begin producing antibodies in the mouse. Spleen tissue, rich in B lymphocytes and begin producing antibodies in the mouse. **1.5marks**

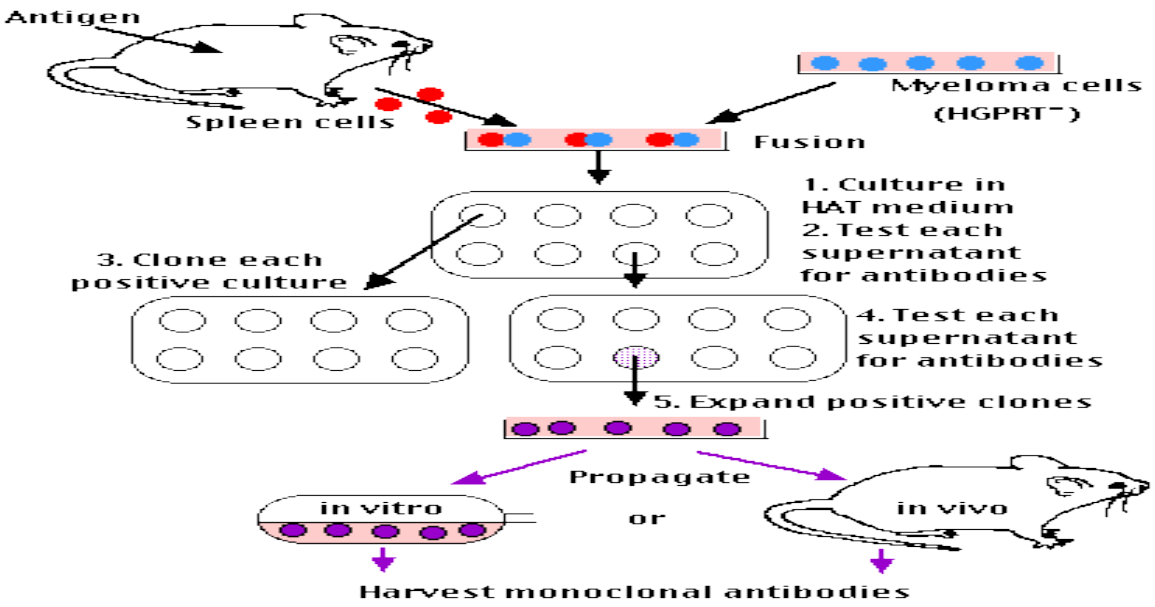
Spleen cells, rich in B lymphocytes, is then removed from the mouse and the B cells are fused with myeloma cells. Even though many cells fuse in culture and begin to grow, only a small fraction are viably antibody producing hybridomas. **1.5marks**

Hybridomas are first selected from other cells by addition of hypoxanthine, aminopterin and thymidine to the *in vitro* cell culture medium (HAT medium).

The HAT medium stops the growth of unfused myeloma cells , though able to grow indefinitely in cell culture, are unable to use the metabolites hypoxanthine and thymidine to bypass a metabolic attack caused by aminopterin, a cell poison. By contrast, fused hybridoma cells can use hypoxanthine and thymidine to bypass the aminopterin block and grow normally in HAT medium; they receive the genetic information for use of hypoxanthine and thymidine from the B-cell fusion partner. **1.0mark**

Any unfused B cells die in a few days because they can not grow in culture. Following fusion, the antibody producing hybridoma clones must be identified.

An ELISA test can be used to identify hybridomas that produce monoclonal antibodies. From a typical fusion, several distinct clones are isolated, each making a monoclonal antibody. Once the clones of interest are identified, they can be grown in vivo as an antibody producing tumor or in cell culture. **0.5mark**



(iii) In this method, plasma samples are treated to lyse HIV and release the RNA viral genome. The RNA, after precipitation with isopropanol, is reverse transcribed with reverse transcriptase enzyme to make a DNA copy.

The DNA copy then serves as the template in a PCR reaction with primers directed to a portion of the HIV gene. **0.5mark**

In PCR technique, to target a particular gene of interest, special primers need to be made. DNA polymerase can not start copying DNA unless it has a primer with a 3' end available for the attachment of new nucleotides. To start the process of copying the gene of interest, two single stranded primers that are complementary to the DNA are made on either side of the target DNA.

When the DNA polymerase begins making new DNA, it will begin at these primers and move toward the gene of interest. **1.0mark**

To begin the process of PCR, the DNA to be copied, lots of primers, nucleotides and DNA polymerase are combined in a tube. The thermocycler heats the tube at approximately 95°C for 1 minute. This disrupts the hydrogen bond holding the double-stranded together. The thermocycler then cools to an annealing temperature to 65°C, which is cool enough to allow hydrogen bonds to reform but high enough to avoid nonspecific hydrogen bonding. **1.0mark**

Because there is so much primer in the solution, it is very likely that primers will attach to the DNA that is being copied rather than the original DNA strands getting back together. The temperature is then raised to 72°C, which is the optimal temperature for DNA polymerase. The enzyme makes new DNA complementary to the original DNA strands beginning at the 3'ends of the primers. For every original strand of DNA that we wanted to copy, we now have two strands. **1.0mark**

The entire cycle is repeated and the two strands become four strands. When it is repeated again, the four become eight. The thermocycler is capable of automatically cycling multiple times until the desired numbers of copies are produced . Only thirty cycles are required to produce a billion copies of a single piece of DNA.

Using a standard DNA template of known quantity, the HIV signal present in the sample can be quantitated. By comparing viral load over time, a relatively accurate prognosis can be made for each patient. **1.0mark**

(b)

(i) Reporter molecule is used in ELISA and Western blot technique. To detect a reaction, the probe is labeled with a reporter molecule, a radioisotope, an enzyme or a fluorescent compound that can be detected following hybridization. **1.0mark**

(ii) One of the most useful applications of the PCR method is the test for viral load in individuals infected with HIV. Viral load is the number of HIV RNA strands in the plasma or serum of an HIV infected person. In this method, plasma samples are treated to lyse HIV and release the RNA viral genome. The RNA, after precipitation with isopropanol, is reverse transcribed with reverse transcriptase enzyme to make a DNA copy. **1.0mark**

The DNA copy then serves as the template in a PCR reaction with primers directed to a portion of the HIV gene.

(iii) The probe is a single stranded piece of nucleic acid that has been tagged or labeled, with a detectable marker such as a radioactive isotope, a fluorescent dye or biotin; the probe is complementary to either of the two strands being detected using Southern blot technique.

1.0mark

(iv) The first step of the Southern Blot technique is the isolation and restriction enzyme digestion of the DNA to be studied. The DNA fragments are then separated according to size using gel electrophoresis.

To perform the immunoblot, a purified preparation of HIV is treated with sodium dodecyl sulfate (SDS), a detergent that solubilizes HIV proteins and also inactivates the virus. The HIV proteins are then resolved by polyacrylamide gel electrophoresis and blotted from the gel onto membranes. **1.0mark**

2. Without repetitions between procedures, state four pieces of equipment and/or materials that you would require for the following procedures and in each case, state the function of the stated equipment or material;
    a. Virus cultivation in laboratory animal. 3.0 marks
    b. Virus cultivation in embryoneted egg. 3.0 marks
    c. Virus cultivation in cell/tissue culture. 2.5 marks
    d. Virus cultivation in whole plant. 3.0 marks
    e. Sample preparation for scanning electron microscopy. 3.0 marks
    f. Sample preparation for the transmission electron microscope 3.0 marks

Guide: 0.75 marks for every correct equipment and/or materials listed and not repeated with correct function including but not limited to the following

1. Laboratory animal
2. cotton wool
3. Syringe and needle
4. Virus inoculum
5. Embryonated egg
6. Punch
7. Candler
8. Iodine
9. Discard tray
10. Culture plates
11. Cell line
12. Inverted microscope
13. Micro pipette
14. Pipette tips
15. Micro-centrifuge
16. Healthy uninfected plants
17. Carborundum powder
18. Wash bottle
19. Tap water
20. Mortar and pestle
21. Hand gloves

3. (a) Compare and contrast the following:
   (i) Monoclonal and polyclonal antibody 2.0 marks
   (½ mark each = 2 marks)

Comparison

- They are both produced by B-cells
- They are both used in carrying out ELISA

Contrast

- Monoclonal antibody is derived from a single B cell line while polyclonal antibody is derived from different B cell lines
- Monoclonal antibody is specific for a single epitope while polyclonal antibody can recognise and bind to several epitopes

   (ii) Direct and indirect Enzyme Linked Immunosorbent Assay (ELISA) 2.0 marks
   (1 mark each = 2 marks)

Contrast

- Direct ELISA involves the direct detection of an analyte by an enzyme-labelled specific antibody

- Indirect ELISA involves the detection of an analyte by a specific antigen or antibody, which is then detected by an enzyme-labelled, secondary anti-immunoglobulin molecule.

(iii) Primary and secondary binding tests 2.0 marks

(Any two, 1 mark each = 2 marks)

Contrast

- Primary binding tests directly measure the binding of antigen to antibody while secondary binding tests measure the effects of the interaction between antigen and antibody.
- Primary binding tests are more sensitive than secondary binding tests
- Primary binding tests usually involve the use of radioisotopes, fluorescent dyes, or enzymes as labels to identify one of the reactants.

(iv) Hemagglutination and hemagglutination inhibition 2.0 marks

(Any two, 1 mark each = 2 marks)

Comparison

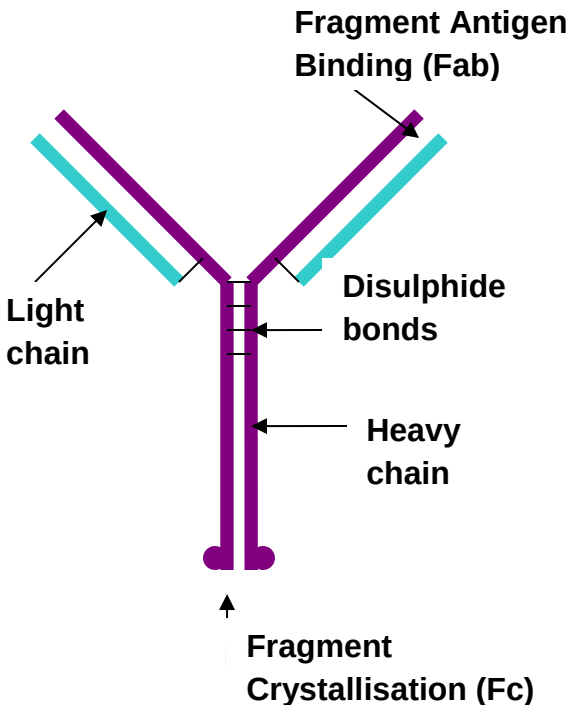
- Both tests measure the effect of virus on agglutination/clumping of red blood cells

Contrast

- A positive hemagglutination test is evidenced by agglutination/clumping of red blood cells while a positive hemagglutination inhibition shows a lack of agglutination activity.
- Hemagglutination test makes use of sensitized red blood cells while hemagglutination inhibition does not.

(b) Draw and label the structure of an antibody 5.5 marks

(1 mark each for correct labelling and 0.5 marks for correct diagram = 5.5 marks)



(c) List two (2) enzymes and their respective substrates that are commonly used when carrying out ELISA 1.0 mark

(0.5 marks for each correct enzyme and substrate = 1 mark)

Enzyme	Substrate
Horseradish peroxidase	Phenylenediamine dihydrochloride
Alkaline phosphatase	p-nitro phenyl phosphate

(d) Explain the role of serological techniques in the diagnosis of viral infections 3.0 marks

- Viruses are not easy to culture and isolate as they do not grow on culture media like bacteria **-1 mark**
- During viral infections, the virus are present as antigens within the host and are able to stimulate the production of antibodies that are specific for the virus- **1 mark**
- The viral antigen and antibody are able to interact and form immune complexes, and serological techniques detect and measure these interactions- **1 mark**

SECTION B

4. (a) Define

(i)	$TCID_{50}$	1.0mark
(ii)	Primer	1.0mark
(iii)	CPE	1.0mark
(iv)	Apical meristem culture	1.0mark
(v)	Restriction endonuclease	1.0mark

(b) List the steps involved in

(i) ELISA (direct)	3.0marks
(ii) Western blotting	3.0marks
(iv) Southern blotting	3.0marks
(v) Neutralization reaction	3.5marks

Topics covered by the question: Neutralization reaction, Apical meristem culture, Southern blotting, Western Blotting, ELISA, Cytopathic effect

Answer hints/keywords for the question: Immunoblot, microtitre plate, antigen-antibody complex, gel electrophoresis, restriction endonuclease, nylon membrane, reporter molecule

Full answer to the question:

(a) $(i)TCID_{50}$ - Tissue culture infective dose, that amount of a pathogenic agent that will produce pathological change in 50% of cell cultures inoculated expressed as $TCID_{50}$/ml. **1.0mark**

(ii) A primer is a short chain of nucleotides which is complementary to the DNA to be made. DNA polymerase can not start copying DNA unless it has a primer with a 3' end available for the attachment of new nucleotides. To start the process of copying the gene of interest, two single stranded primers that are complementary to the DNA are made on either side of the target DNA. **1.0mark**

(iii) When a virus is propagated in tissue culture cells, it often changes the cells' appearance. Often, these changes are characteristic for a particular virus and are referred to as the cytopathic effect of the virus. So the visible effect viruses have on cells is called the cytopathic effect (CPE). **1.0mark**

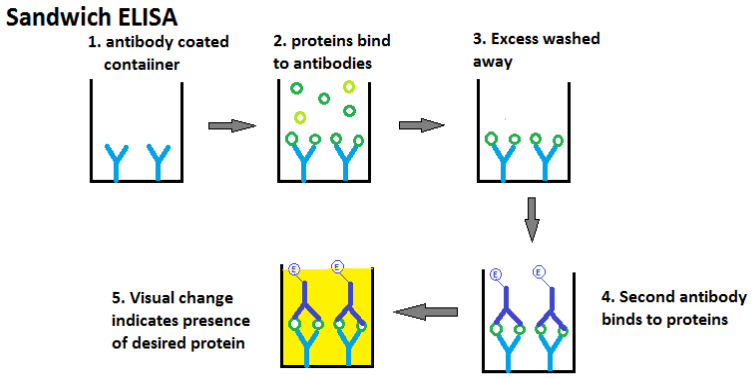
(iv) It has been observed that plants raised in culture using apical meristem, a localized group of actively dividing cells, from which permanent tissue systems (root, shoot, leaf, flower) are derived, of infected plants are free of pathogens. The development of pathogen free plants using meristem culture is known as apical meristem culture. **1.0mark**

(b) (i) For detecting antigens such as virus particles from a blood or fecal sample, the direct ELISA method is used. In this procedure the antigen is trapped between two layers of antibodies. Thus, this method is sometimes called the "sandwich ELISA". **0.5mark**

The specimen is added to the wells of a microtiter plate previously coated with antibodies specific for the antigen to be detected. If the antigen (virus particle) is present in the sample, it will be trapped by the antigen binding sites on the antibodies. **1.0mark**

After washing unbound material away, a second antibody containing a conjugated enzyme is added. The second antibody is also specific for the antigen, and so it binds to any remaining exposed determinants. 1.0mark

Following a wash, the enzyme activity of the bound material in each microtiter well is determined by adding the substrate of the enzyme. The color formed is proportional to the amount of antigen present. 0.5mark



(ii)

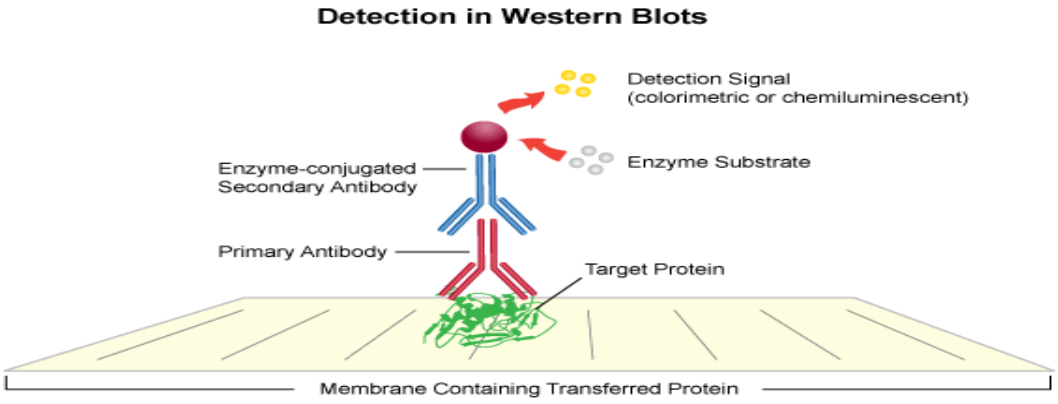


Diagram 2: *Illustration of detection in Western Blots.*

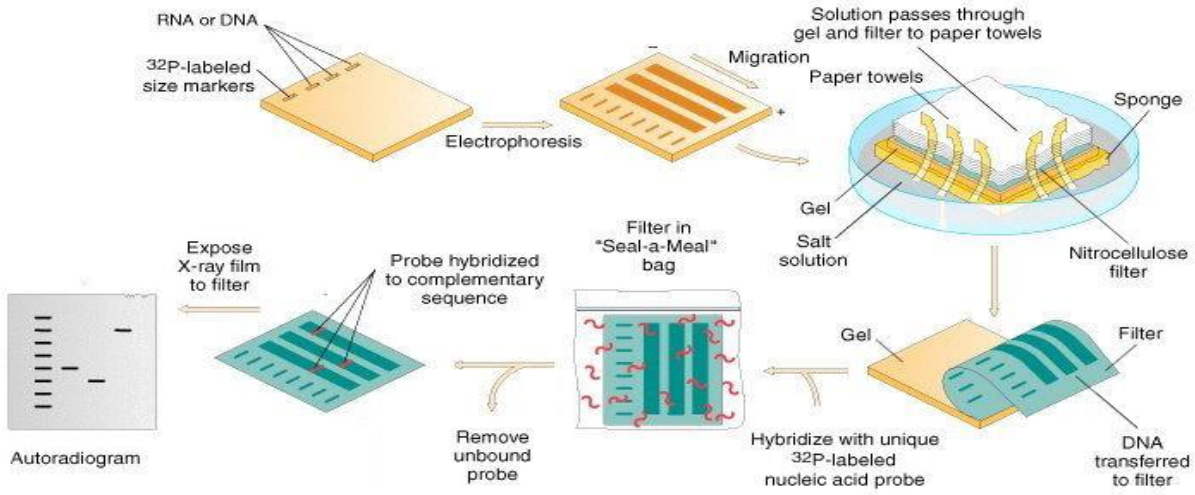
To perform the immunoblot, a purified preparation of HIV is treated with sodium dodecyl sulfate (SDS), a detergent that solubilizes HIV proteins and also inactivates the virus. 0.5mark

The HIV proteins are then resolved by polyacrylamide gel electrophoresis and blotted from the gel onto membranes. At least seven major HIV proteins are resolved by electrophoresis, and two of them, designated , designated P24 and GP41-45, are used as specific diagnostic proteins in the HIV immunoblot. Protein P24 is the HIV core protein, and proteins GP41-45 are HIV coat proteins. 0.5mark

Following blotting of the proteins, the membrane strips are incubated with the test serum sample. If the sample is HIV positive, antibodies against HIV proteins will be present and will bind to the HIV proteins on the membrane. 1.0mark

To detect whether antibodies from the serum sample have bound to HIV antigens, a detecting antibody , anti-human IgG conjugated to the enzyme peroxidase, is added to the strips. If the detecting antibody binds, the activity of the conjugated enzyme, after addition of the substrate, will form a brown band on the strip at the site of antibody binding. The patient is confirmed as HIV positive if the position of the bands in the patient serum and a positive control serum are identical; negative control serum is also analyzed in parallel and must show no bands. 1.0mark

(iii)



(1) Digestion and Gel Electrophoresis of DNA

The first step of the Southern Blot technique is the isolation and restriction enzyme digestion of the DNA to be studied. The DNA fragments are then separated according to size using gel electrophoresis.

1.0mark

(2) Transferring the DNA to a solid membrane support

In order to immobilize the DNA on a solid support, the DNA fragments are transferred to a nylon membrane. To do this, the gel is first soaked in an alkaline solution, which denatures the DNA. Then, by placing the membrane directly on the gel in a special set up, the DNA transfers to the membrane in the same relative position as it occupied in the gel. 1.0mark

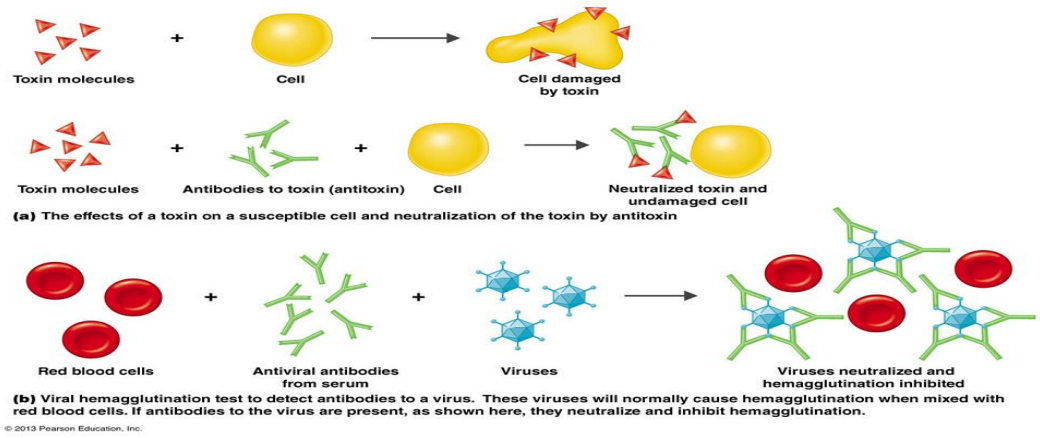
(3) Detecting specific DNA sequences

A liquid solution containing the labeled probe is added to the membrane and incubated under conditions that allow the probe to hybridize to complementary sequences. Any probe that has not bound is then washed off, and the location of the bound probe is detected. To detect a reaction, the probe is labeled with a reporter molecule, a radioisotope, an enzyme or a fluorescent compound that can be detected following hybridization. 1.0mark

(iv) In this procedure, patient serum is mixed with a suspension of infectious virus particles of the same type as those suspected of causing disease in the patient. **1.0mark**

A control suspension of virus is mixed with normal serum and is then inoculated into an appropriate cell culture. **1.0mark**

If the patient serum contains antibody to the virus, the antibody will bind to the virus particles and prevent them from invading the cells in culture, thereby neutralizing the infectivity of the virus. **1.0mark**



5. Briefly explain/state;
   a. Two major reasons the study of viruses require unique techniques 2.0 marks

Guide: 1 mark each

1. Viruses are very minute in size and cannot be seen with the normal light microscope there for a unique microscopy techniques (electron microscopy) had to be developed for the studies of viruses.

2. Viruses are obligate intercellular parasites that can only thrive and replicate inside a living cell, therefore they cannot be cultures in synthetic media like bacteria and fungi rather they can only be cultured within living cells. This necessitated the need for specialised techniques for studying viruses.

   b. Five precautionary measures that you would take while working in the virology laboratory. 3.75 marks

Guide: 1 mark each for any of the following or any other useful precautionary measure.

1. Use of personal protective clothing such as lab coat, gloves, nose mask etc depending on the virus being handled, to avoid personal contamination.
2. No hand to mouth activities allowed, such as eating and making up, to avoid ingestion of pathogenic virus or harmful chemicals.
3. Ensure to complete all necessary paper work including sample tagging, reagent calculations etc, before commencing any lab work. This is to avoid experimental error.

4. Avoid unnecessary conversations in the lab, particularly when working with nucleic acids. This is to avoid degradation of the nucleic acid by nucleases that maybe present in saliva.
5. Read instruction and caution on all reagent bottles before use. This is to avoid exposure to dangerous substances.
6. Ensure sufficient knowledge of every virus is obtained before use

c. The five basic structural forms that a virus can take and give one example of each 3.75 marks

Guide: 0.75 mark for every correct structural forms with example
1. Naked icosahedral e.g. poliovirus, adenovirus, hepatitis A virus
2. Naked helical e.g. tobacco mosaic virus. So far no human viruses with this structure are known
3. Enveloped icosahedral e.g. herpes virus, yellow fever virus, rubella virus
4. Enveloped helical e.g. rabies virus, influenza virus, parainfluenza virus, mumps virus, measles virus
5. Complex e.g. poxvirus

d. Two essential components of an electron microscope and the major function of each component listed 2.0 marks

Guide: 1 mark each for any two correct essential components listed along with their uses.
1. Power Supply
2. Vacuum System
3. Cooling system
4. Vibration-free floor
5. Room free of ambient magnetic and electric fields.

e. Two function of the virus genome 1.5 marks

Guide: 0.75 mark each for any two correct functions
1. The viral nucleic acid contains genes that code for the proteins of the viral coat
2. The viral nucleic acid contains genes that code for the enzymes required for genome replication

f. Two function of the virus capsid 1.5 marks

Guide: 0.75 mark each for any two correct functions
1. The protein coat protects the nucleic acid from extracellular environmental insults such as nucleases
2. It permits attachment of the virion to the membrane of the host cell

g. Two important reasons for virus cultivation 1.5 marks

Guide: 0.75 mark each for any two correct functions
1. For isolation (from specimen)
2. For identification (Diagnostics)
3. For vaccine production
4. For research

h. Two methods that are commonly used in the laboratory to achieve virus entry into plant cells 1.5 marks

Guide: 0.75 mark each for any two correct functions
1. Mechanical sap inoculation

2. Grafting
3. Use of vectors such as insects, mealybugs etc
4. Microinjection

6. a) Define the following:
   (i) Sensitized cells: antigen coated red blood cells **1.0 mark**
   (ii) Antibody titer: the highest dilution of serum in which agglutination can be detected **1.0mark**
   (iii) Anti-human antibody: antibody gotten from other mammals (e.g sheep, horse, rat) that are able to bind to human antibody **1.0mark**
   (iv) Epitope: the particular site on an antigen that an antibody will specifically recognize and interact with **1.0mark**
   (v) Virus neutralization: the binding of a virus by a specific antibody preventing the virus from infecting susceptible cells **1.0mark**

   (b) Discuss the principle, procedure, advantages and disadvantages of any two (2) of these serological techniques: 10.0 marks

   (i) Fluorescent Antibody Test (FAT)
   Principle

   Antigen-antibody complexes are shown by fluorescent dyes (flourochromes) which are illuminated by UV light fluorescing against a dark background. **1 mark**

Procedure (Direct FAT) **(1 mark each = 3 marks)**

- A tissue or smear containing the antigen is fixed to a glass slide and incubated with the fluorescent antibody
- It is then washed to remove the unbound antibody.
- Subsequently, it is examined by under a microscope with UV light source. The antigen that has bound by the labelled antibodies are seen to fluoresce brightly.

OR

Procedure (Indirect FAT) **(1 mark each = 3 marks)**

- A slide preparation of the specimen is made and an unlabelled specific antibody is added. After an appropriate incubation time, the preparation is washed.

- A fluorescent labelled anti- species globulin is added and allowed to combine with the antibody. The excess is washed from the slide.
- The preparation is examined by fluorescence microscopy

Advantages of Fluorescent antibody test **(¼ marks each = ½ mark)**

1. sensitive
2. Reproducible.

Disadvantages of Fluorescent antibody test **(¼ marks each = ½ mark)**

1. Special training is needed to perform and interpret.
2. Fluorescence microscope and other high quality reagents required are expensive.

(ii) Complement fixation

Principle **(1 mark)**

Antigen-antibody reactions lead to immune complex formation. When complement takes part in antigen antibody reactions; it is bound or fixed to the antigen antibody complexes, and cannot causes lysis of red blood cells.

Procedure **(1 mark each = 3 marks)**

- Patient serum is first added to a standard concentration of a known antigen, and complement is added to the solution
- Sheep red blood cell and hemolysin are then added.
- The result is read as positive (no lysis) or negative (lysis).

Advantages of complement fixation test **(Any two ¼ mark each = ½ mark)**

1. Specific.
2. It does not require expensive equipment or reagents to carry out.
3. Large number of samples can be screened at the same time.

Disadvantages of complement fixation test **(Any two ¼ mark each = ½ mark)**

1. Not very sensitive
2. Takes a considerable time to perform.
3. May gives inconclusive results when the test serum contains anti-complementary substances.

(iii) Hemagglutination test

Principle **(1 mark)**

Sensitized red cells are added to dilutions of the patient's serum. If the serum contains the corresponding antibody in sufficient concentration, the red cells will be agglutinated and settle to form an even covering in the bottom of the well.

Procedure **(1 mark each = 3 marks)**

- Red blood cells are coated with the antigen of interest (sensitized cells).
- The sensitized red cells are added to dilutions of the patient's serum.
- If the serum contains the corresponding antibody in sufficient concentration, the red cells will be agglutinated and settle to form an even covering in the bottom of the well. If the sensitized cells are not agglutinated they will settle and form a red button in the bottom of the well.

Advantages of agglutination test **(Any two ¼ mark each = ½ mark)**

1. It is simple to perform
2. It does not require any special equipment
3. It is inexpensive.

Disadvantages of agglutination test **(Any two ¼ mark each = ½ mark)**

1. The specificity and sensitivity of the test depends on the antigen used and how the cells are prepared.
2. Some hemagglutination test may give non-specific results due to heterophil antibodies present in the patient's serum.

(iv) Single radial haemolysis

Principle

The presence of serum antibody against an antigen is detected by zone of lysis on agar plate.

Procedure **(1 mark each = 3 marks)**

- Viral antigen and complement are added to molten agar and allowed to set in the test plate. Positive and negative control plates are also prepared.
- The test serum is then placed in the wells in the agar of the test plate. The plates are incubated at 37°C overnight.
- The presence of antibodies specific to the virus of interest is detected by the lysis of the antigen, which is shown as a zone of lysis surrounding the wells in the test plate.

Advantages of single radial hemolysis **(Any two ¼ mark each = ½ mark)**

1. Sensitive.
2. Cheap and easy to carry out.
3. The results are easy to interpret.

Disadvantage of single radial hemolysis **(Any two ¼ mark each = ½ mark)**

1. Result is prone to human errors.
2. Cross reactivity between antibodies may be observed.

(c) Mention five (5) laboratory equipment that are used in a virology laboratory 2.5 marks

(½ mark each = 2.5 marks)

- Electron microscope
- PCR thermocycler
- Laminar flow
- Vortex mixer
- Centrifuge
- Spectrophotometer
- UV transilluminator
- ELISA plate reader
- CO_2 incubator