

Date : October 11, 2025
Total Marks : 100
Writing Time : 150 minutes (2.5 hours)
Reading Time : 15 Minutes (prior to writing time)

1. Write your Registration Number clearly and correctly on the Answer Booklet.
2. The first 15 minutes is to check the number of pages of Question Paper, printing errors, clarify doubts and to read the instructions. You are NOT permitted to write during this time.
3. This paper consists of **TWO SECTIONS**, namely SECTION A & SECTION B:
 - **SECTION A** has two parts:

Part I - 30 Multiple Choice Questions

Part II - 4 Short Answer Questions

All questions under SECTION A are **COMPULSORY**.
 - **SECTION B** consists of two Case Studies. Choose only **ONE** case study and answer the questions of your choice.
4. All answers should be written on the Answer Booklet provided to you. Candidates are not allowed to write anything on the question paper. If required, ask for additional Answer Booklet.
5. All answers should be written with correct numbering of Section, Part and Question Number in the Answer Booklet provided to you. Note that any answer written without indicating the Section, Part and Question Number will NOT be evaluated and no marks will be awarded.
6. Begin each Section and Part in a fresh page of the Answer Booklet.
7. You are not permitted to tear off any sheet(s) of the Answer Booklet as well as the Question Paper.
8. Use of any other paper including paper for rough work is not permitted.
9. **You must hand over the Answer Booklet to the Invigilator before leaving the examination hall.**
10. This paper has **9 printed pages**, including this instruction page.

SECTION A

PART I: Multiple Choice Questions [30 marks]

Choose the correct answer and write down the letter of your chosen answer in the Answer Booklet against the question number e.g. 31 (d). Each question carries ONE mark. Any double writing, smudgy answers or writing more than one choice shall not be evaluated.

1. Who postulates the “Germ theory of diseases”?
 - a) Robert Koch
 - b) Louis Pasteur
 - c) John Snow
 - d) Girolamo Fracastoro
2. Which group of organisms appears pink/red after gram staining?
 - a) Gram positive bacteria
 - b) Gram Negative bacteria
 - c) Acid fast bacteria
 - d) Spirochaete
3. Viruses which infect bacteria is called-
 - a) Prion
 - b) Bacteriophages
 - c) Plasmids
 - d) None of the above
4. Which of the following leukocytes is regarded as the **first line of defense** against invading pathogens and is often called the “*martyr of the immune system*”?
 - a) Eosinophils
 - b) Monocytes
 - c) Lymphocytes
 - d) Neutrophils
5. Which of the following is an **enriched medium** that supports the growth of most pathogenic bacteria and is commonly used for routine **primary isolation**?
 - a) Nutrient agar
 - b) Brilliant green agar
 - c) MacConkey agar
 - d) Blood agar
6. β -lactam antibiotics such as **penicillins** and **cephalosporins** exert their antibacterial action mainly by:
 - a) Inhibition of cell wall synthesis
 - b) Inhibition of cell membrane function
 - c) Inhibition of protein synthesis
 - d) Disruption of DNA structure and inhibition of DNA repair

7. Which of the following is **NOT** a biochemical test used for bacterial identification??
 - a) Indole test
 - b) Methyl red test
 - c) Catalase test
 - d) Bacterial culture

8. Which type of sample is **recommended** for the **laboratory detection/diagnosis of rabies in animals**?
 - a) Whole blood
 - b) Brain
 - c) Serum
 - d) Liver or lung tissues

9. For serological tests, blood samples are collected in which color-coded vacutainer tube?
 - a) Red
 - b) Purple
 - c) Green
 - d) Blue

10. All of the following are core biochemical components required for PCR, **EXCEPT** -
 - a) Template
 - b) Primer
 - c) DNA polymerase
 - d) All of the above

11. Penicillin, the first widely used antibiotic, was discovered by-
 - a) Alexander Fleming
 - b) Antony Van Leeuwenhoek
 - c) Louis Pasteur
 - d) Robert Koch

12. Which of the following organisms produces a characteristic metallic green sheen on Eosin Methylene Blue (EMB) agar?
 - a) Escherichia coli
 - b) Salmonella
 - c) Brucella
 - d) Campylobacter

13. Sabouraud's dextrose agar (SDA) is a selective culture medium primarily used for the growth of-
 - a) Bacteria
 - b) Fungi
 - c) Viruses
 - d) Parasites

14. *Leptospira*, the causative agent of leptospirosis, is classified as-
- Bacteria
 - Spirocheate
 - Virus
 - Fungus
15. The ability of a microbial species to cause disease in a host is termed -
- Pathogenicity
 - Virulence
 - Infectivity
 - Parasitism
16. Cefotaxime belongs to which generation of cephalosporin antibiotics?
- First-generation cephalosporin
 - Second-generation cephalosporin
 - Third-generation cephalosporin
 - Fourth-generation cephalosporin
17. Which of the following statements about bacterial cell walls is correct?
- The cell wall of Gram positive bacteria has a higher amount of peptidoglycan than gram negative bacteria.
 - The cell wall of Gram negative bacteria has a higher amount of peptidoglycan than gram positive bacteria.
 - Gram positive bacteria have more lipid content in their cell wall than gram negative bacteria.
 - All of the above.
18. During routine Hematoxylin & Eosin (H&E) slide preparation, which step preserves the tissue's structure and prevents degradation?
- Fixation
 - Embedding in paraffin
 - Staining
 - Slicing
19. Bacteria primarily multiply by which of the following processes?
- Sexual reproduction
 - Budding
 - Binary fission
 - Replication
20. Which method is commonly used to sterilize culture media in microbiology?
- Autoclave
 - Hot air oven
 - Pasteurization
 - None of the above

21. Organisms that obtain energy from chemical compounds is called-
- a) Phototrophs.
 - b) Autotrophs
 - c) Heterotrophs
 - d) Chemotrophs.
22. Multidrug-resistant (MDR) organisms usually refer to bacteria that are:
- a) Resistant to at least one agent in two or more antimicrobial categories
 - b) Resistant to at least one agent in three or more antimicrobial categories
 - c) Resistant to at least one agent in four or more antimicrobial categories
 - d) Resistant to at least one agent in five or more antimicrobial categories
23. The ability of a diagnostic test to correctly identify samples that are truly positive is called:
- a) Specificity
 - b) Sensitivity
 - c) Precision
 - d) Accuracy
24. What happens during the denaturation in the PCR steps?
- a) DNA breaks down into two single strands
 - b) Primers bind to the complementary sequences on the single-stranded DNA
 - c) DNA polymerase attaches to the primer and synthesizes a new DNA strand by adding dNTPs
 - d) All of the above
25. The best phase for collection of samples for microbiology or pathogen detection is -
- a) Incubation period
 - b) Prodromal phase
 - c) Early illness (acute) phase)
 - d) Convalescence (recovery) phase
26. Genetic recombination in bacteria can occur through which of the following mechanisms?
- a) Transformation
 - b) Transduction
 - c) Conjugation
 - d) All of the above
27. ELISA is primarily used to detect:
- a) Whole organisms
 - b) Antibodies
 - c) DNA
 - d) RNA

28. Which of the following bacteria is acid-fast?
- a) Bacillus anthracis
 - b) Mycobacterium tuberculosis
 - c) Staphylococcus aureus
 - d) Salmonella Typhi
29. Which bacterial structure helps in attachment to host cells?
- a) Capsule
 - b) Flagella
 - c) Pili/Fimbriae
 - d) Endospore
30. Which immunoglobulin is first produced in a primary immune response?
- a) IgG
 - b) IgM
 - c) IgA
 - d) IgE

PART II – Short Answer Questions [20 marks]

This part has 4 Short Answer Questions. Answer ALL the questions. Each question carries 5 marks.

1. Staining techniques in microbiology are broadly classified into **Simple, Differential, and Special** stains. For each category, list at least two examples in each category and explain their principles and specific uses in microbial identification.
 - a. Simple stains
 - b. Differential stain
 - c. Special stains
2. Define the following terms and give at least one example of each:
 - a) Sterilization
 - b) Disinfection
 - c) Antiseptics
 - d) Degerming
 - e) Sanitation
3. Using a standard 2×2 diagnostic test evaluation table, define and explain the following terms:
 - a) Sensitivity
 - b) Specificity
4. Classifying viruses according to **Baltimore classification** and give one example each.

SECTION B: Case Study [50 marks]

Choose either Case I OR Case II from this section. Each case study carries 50 marks. Mark for each sub-question is indicated in the brackets.

CASE I

In mid-August 2025, a dairy cluster (5 animals in the household) in Samtse Dzongkhag reported a Lumpy skin disease (LSD) outbreak, detected through a rapid diagnostic kit.

LSD is caused by various strains of Capri poxvirus genus within the family Poxviridae, disease of cattle characterized by fever, nodules on the skin, mucous membranes and internal organs, emaciation, enlarged lymph nodes, and oedema of the skin. The disease is of economic importance as it can cause a temporary reduction in milk production, temporary or permanent sterility in bulls, damage to hides and, occasionally, death.

Transmission of LSD virus is thought to be predominantly by arthropods, natural contact transmission in the absence of vectors is minimal. Vector pressure (biting flies) is high after monsoon rains. National referral lab, NCAH was requested for sampling and confirmatory testing.

Note: LSDV is not transmissible to humans.

Answer the following questions-

1. The following questions are related to sampling during LSD outbreaks. Answer it accordingly.
(9 marks)
 - a) State the main objectives of the field sampling during LSD outbreaks.
 - b) List the types of samples suitable for laboratory diagnosis of LSD, ranking them by expected diagnostic yield.
 - c) Explain how animals should be selected and prioritized for sampling during LSD outbreaks, and give reasons for this approach.
 - d) Describe the essential biosafety and biosecurity measures to be followed in the field while collecting LSD samples.
 - e) Outline the correct packaging and transport procedure for LSD samples from the field to the laboratory.
2. Explain the principle, components and procedures of Polymerase Chain Reaction (PCR).
(5 marks)
3. Outline a **CONCEPTUAL** detection workflow for LSDV through real time PCR including sample processing and nucleic acid extraction, PCR workflow, machine run, data analysis (real time-read out and cycle threshold), and result interpretation.

You need not mention the name of commercial kits during the workflow.

The primer and probe available for LSDV at NCAH are as follows:

Probe – 5' TGGCTCATAGATTTCCT3'

Primer forward - 5' AAA ACGGTATATGCAATAGAATGGAA 3'

Prime Reverse - 5' AAATGAAACCAATGGATCGGATA 3'

(8 marks)

4. Why is RT-PCR preferred over conventional PCR, list the differences, and advantages/disadvantages? **(3 marks)**
5. Define Quality control (QC) and Quality assurance (QA) and list at least one QC tool of quality control followings and its significance in any laboratory setting? **(5 marks)**
6. The following questions related to other tests options and differential diagnosis of LSD. Answer it accordingly. **(12 marks)**
- a) Beyond PCR, list other test/ methods that can be adopted for LSDV detection.
 - b) In terms of causative agent, list differential diagnoses to consider/rule out.
 - c) Outline the laboratory biosafety and biosecurity while dealing with LSDV.
 - d) Would you consider collecting the arthropods surrounding the outbreak area, and if so why and what are the test(s) that can be conducted and explain?
7. These are 5 sample results of RT PCR from different outbreak regions using skin biopsy samples, interpreting the results as asked below **(8 marks)**

Samples	Results
A	Ct=22
B	Ct=36
C	Ct=39 (repeated once, similar result)
D	No amplification, but internal control amplified normally
E	No amplification, internal control failed

- a) Which samples are positive for LSDV?
- b) Which sample is indeterminate and what would you do next?
- c) What does sample E indicate and what corrective step is required?
- d) Which Samples are negative for LSDV?

CASE II

National Centre for Animal Health (NCAH), Serbithang had received a report of a suspected rabid dog biting the humans and animals in Samtenthang village, Samtenling geog under Sarpang geog. As per the report from the dzongkhag veterinary hospitals, the suspected dog had bitten 10 cattles, 4 dogs, 1 goat, 2 cats and 1 child before it disappeared into the forest. The dzongkhag veterinary hospitals along with other relevant stakeholders are implementing the preventive and control measures to prevent the further spread of the infection.

Taking the above scenario into consideration, answer the following questions:

- a) Describe the structure and genome organization of the virus suspected in this outbreak?
(10 marks)
- b) Outline the steps of rabies virus replication cycle in host cells?
(10 marks)
- c) Explain the mechanism of viral entry into the nervous system and spread to the CNS.
(10 marks)
- d) List direct and indirect laboratory diagnostic methods for rabies available to microbiologists.
(10 marks)
- e) What preventive measures and post-exposure prophylaxis (PEP) should be recommended for this scenario?
(10 marks)

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