NAMIBIA UNIVERSITY
OF SCIENCE AND TECHNOLOGY

FACULTY OF HEALTH, APPLIED SCIENCES AND NATURAL RESOURCES

DEPARTMENT OF HEALTH SCIENCES

QUALIFICATION: BACHELOR OF MEDICAL LABORATORY SCIENCES	
QUALIFICATION CODE: 08BMLS	**LEVEL: 6**
COURSE CODE: MOD621S	**COURSE NAME**: MOLECULAR DIAGNOSTICS
SESSION: NOVEMBER 2022	**PAPER:** THEORY
DURATION: 3 HOURS	**MARKS:** 108

FIRST OPPORTUNITY EXAMINATION PAPER	
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MODERATOR:	Dr A Shiningavamwe

INSTRUCTIONS
1. Answer ALL the questions. 2. Write clearly and neatly. 3. Number the answers clearly.

PERMISSIBLE MATERIALS

Scientific Calculator

THIS MEMORANDUM CONSISTS OF 6 PAGES (Including this front page)

SECTION A (10)

QUESTION 1 [10]

Choose the correct answer and report only the suitable letter next to the relevant Question: One (1) mark for each correct answer.

1.1 The following statements about the "Central Dogma of Molecular Biology" is correct except for:

A. Transfer of genetic information in a cell in one direction only
B. Replication
C. Reverse transcription
D. Transcription and translation

1.2 If a DNA is 30% cytosine, what is the percentage of thymine?

A. 30%
B. 35%
C. 60%
D. 70%

1.3 Goals of the 'Human Genome Project' include?

A. To identify all of the genes in a human DNA
B. To determine the sequence of the 3 billion bases that makes up human DNA.
C. To create a data base
D. all of the above

1.4 In preparing a 1/15 dilution of a DNA sample, which of the following volumes can be pipetted?

A. 10 µL DNA sample and 150 µL water
B. 15 µL DNA sample and 150 µL water
C. 30 µL DNA sample and 150 µL water
D. 50 µL DNA sample and 700 µL water

1.5 In preparing a 1.6 % agarose gel:

A. Dissolve 1.0 g agarose in 16 mL TAE buffer
B. Dissolve 1.6 g agarose in 10 mL TAE buffer
C. Dissolve 3.2 g agarose in 200 mL TAE buffer
D. Dissolve 3.2 g agarose in 160 mL TAE buffer

1.6 During Touch Down PCR

A. The annealing temparture is manipulated over the course of the PCR
B. Two primer sets are used during the PCR
C. Antibodies are used to inactivate Taq Polymerase during lower temperature.
D. Normal conditions of a PCR are followed

1.7 Which of the following do not form part a PCR ingredient mixture?

A. Forward and reverse primer
B. All four ddNTPs
C. cDNA
D. Mg^{++}

1.8 The following is true when choosing a restriction enzyme site for cloning purposes except for:

A. Restriction sites must only appear twice in the plasmid
B. They must not appear on your gene
C. Blunt sites are suboptimal for cloning
D. You must choose restriction sites that are available in the plasmid you are cloning into

1.9 Type II restriction endonucleases functions to:

A. Induce cleavage within or immediately outside their recognition Sequence
B. They cleave DNA in the immediate vicinity of their recognition sites
C. They cleave the DNA about 1000 bp away from the 5' end of the sequence "TCA" located within the recognition site
D. Recognize asymmetric target sites, and cleave the DNA duplex on one side of the recognition sequence up to 20 bp away

1.10 The following method is useful in identifying novel mutations:

A. Variable Number Tandem Repeats
B. Fluorescent In Situ Hybridisation
C. Sanger Sequencing
D. Microarray

SECTION B (47)

QUESTION 2 [20]

When designing oligonucleotide primers for a PCR reaction there are criteria to be considered. Answer the following questions on primer design.
Forward primer: 5'-CTGGGGTGAAGTCGTAACAAGG-3'
Reverse primer: 5'-GCGCCCTTTGTAGCTTGACC-3'

2.1 Calculate the annealing temperature for the primer pair (3)

2.1.1 Based on the characteristics that should be considered when designing primers, are these primers an appropriate set? Justify. (7)
Yes.

2.2 After 6 cycles of a PCR, how many copies are produced? Show your calculations. (2)

2.3 Discuss the differences between SYBR Green and Taq man probe in qPCR (3)

2.4 Discuss in detail the steps involved in reverse transcription until before PCR. (5)

QUESTION 3 [17]

3.1 When collecting a blood specimen, the addition of heparin as an anticoagulant should be avoided for the purpose of some downstream molecular analysis. WHY should it be avoided? Suggest a more appropriate anticoagulant. (3)

3.2 Suggest ways by which RNA specimens can be protected against RNases. (3)

3.3 You are a research student in the Postgraduate Laboratory at the Namibia University of Science and Technology. Your research group is interested in assessing the presence of a certain gene in a mouse. Discuss in details how you would go about extracting DNA using the phenol-chloroform method. (11)

QUESTION 4 [10]

4.1 Tabulate the main differences between Gel electrophoresis and SDS-PAGE.

SECTION C (51)

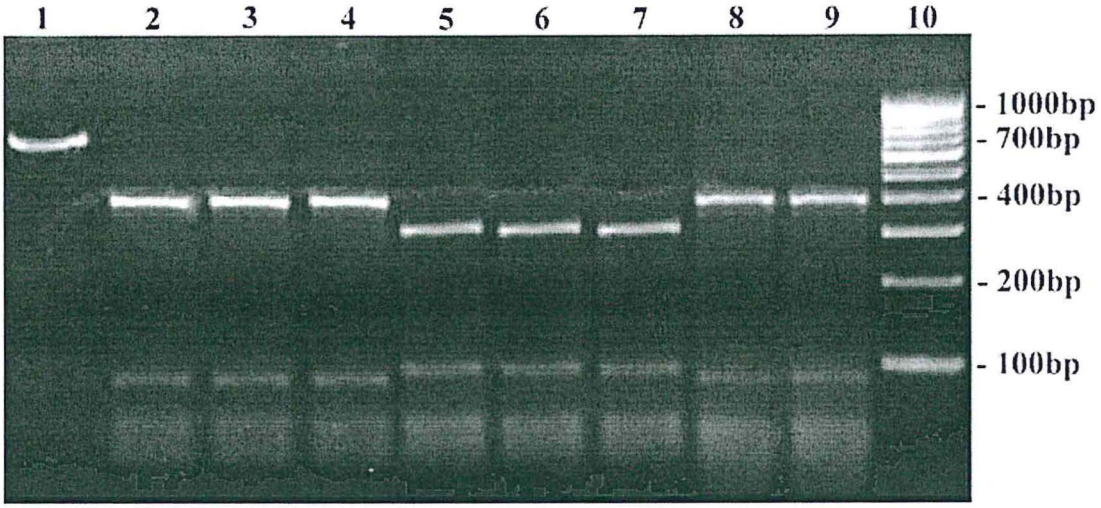
QUESTION 5 [21]

5.1 Outline the differences between a Northern blot and Southern blot analyses starting from the nucleic acid preparation (after nucleic acid extraction) until the visualisation of the target sequences. (11)

5.2 Study the case study below and answer the question.

"On August 28, 2022, the Ministry of Health and Social Services was notified by a private hospital emergency department of a suspected foodborne outbreak. On the previous day, 4 members of a sports team were admitted to the emergency room with complaints of abdominal pain, nausea, vomiting, and diarrhoea; all patients reported to have had the same dinner at the same local restaurant 3 h before. They did not eat together at any other restaurant on the day they became unwell. Two of these patients needed medical care. From 8.30 to 9.30 p.m., a total of 42 meals were served and at 11.30 p.m., the first set of customers started manifesting gastrointestinal symptoms. Microbiological analyses of biological, hand swabs from the food handlers, and food samples were performed, and an epidemiological investigation was conducted to characterize the outbreak."

Results obtained from the samples analysed from NIP molecular unit are shown below:



Lane 1 is the positive control; **Lane 2** is from one of the food handlers sample; **lane 3 & 4** is dessert (cream cheesecake) eaten by the team members; **lane 5-7** is from the environmental surfaces; **lane 8 & 9** is from the stool samples from the two team members. **Lane 10** is the DNA ladder.

5.2.1 What tests were performed? (2)

5.2.3 Discuss the results from the laboratory in relation to the report above. (8)

QUESTION 6 [30]

6.1 Explain the steps involved in Pyrosequencing. (10)

6.2 You are interested in finding out the mis-regulated genes responsible for cancerous cells in a certain tissue of a patient. Using comparative hybridization techniques, explain step-by-step how you will achieve this. (9)

6.3 The following sequence was obtained from the Chain Termination Method. Generate the profile on the gel electrophoresis or write it out. Show all the steps involved. (11)
5' TTCAGCCGAT 3'

END OF EXAMINATION!