SECTION A

QUESTION ONE

In an attempt to identify an unknown virus, you are required to perform direct-double antibody sandwich ELISA (DAS-ELISA) test on an infected plant sample. You are testing for the presence of Cucumber mosaic virus (CMV). The test has been planned out using the ELISA map shown below.

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The following information is provided:

- A volume of 100ul will be used for each well.
- A total of 27 samples will be tested using duplicate wells for each sample as shown in the ELISA map.
- A positive, negative and buffer control will be used as shown in the ELISA map.
- Coating antibodies and antibody-enzyme conjugate will be used at a dilution of 1/100.
The substrate will be used at a concentration of 1mg/ml and substrate tablets are provided at a concentration of 5mg/tablet. **You can only use whole tablets**

Allow 1000ul volume for pipetting errors in all your calculations

i. Using a well labelled diagram, show all the components/reagents that would be used in detecting CMV using direct DAS-ELISA **(5)**

ii. Show detailed calculations of the volumes of the coating antibodies and the antibody-enzyme conjugate needed for this test virus. The buffers used for the dilutions must be stated **(8)**

iii. Name the enzyme that is attached to the antibody and is commonly used in ELISA. What substrate does this enzyme act on? **(2)**

iv. Using the information provided, calculate the number of substrate tablets you would need for your test. **Use whole tablets only.** **(3)**

v. Clearly explain how you would be able to tell the difference between a positive and a negative test result for CMV. **(2)**

**QUESTION TWO**

a) Describe the concept of pathogen-derived resistance in relation to transgenic plants with virus resistance. **(2)**

b) Describe in detail all the stages that are undertaken to transfer plant virus genes into plants during the production of transgenic plants with virus resistance. **(10)**

c) Explain why *Agrobacterium tumefaciens* is used as a vector during genetic transformation of plants. **(3)**

d) State any five (5) tests that can be used to check the success of a plant transformation experiment. What does each test check for **(5)**
QUESTION THREE

a) Define local and systemic plant virus symptoms. Briefly explain how each type of symptom develops. (10)

b) Explain the following in detail:
   i) Plant virus movement within an infected cell to the plasmodesmata (5)
   ii) The source-sink relationship as it relates to plant virus movement. (5)

QUESTION FOUR

a) Outline the steps you would follow to control Citrus tristeza virus (CTV) in a citrus orchard using cross-protection. (10)

b) Describe the proposed mechanism of how cross-protection works, i.e., how does the cross-protected plant become resistant to infection by the challenging virus strain. (10)

SECTION B (ANSWER QUESTION ONE AND ANY OTHER QUESTION)

QUESTION ONE

Crop adaptation can be improved by breeding for host plant resistance using marker-assisted selection. In the process, plant breeders use specific DNA marker alleles as diagnostic tools to identify plants carrying the resistant genes.

Compare and contrast the features, advantages and disadvantages of any four commonly used DNA molecular markers. (20)
QUESTION TWO

a) Describe the general procedures for cloning using plasmid vectors. (10)

b) What is a DNA genomic library? Describe the steps you would follow to construct a DNA genomic library? (10)

QUESTION THREE

Using concrete examples, discuss how the agriculture industry can benefit/is benefiting from modern day biotechnology. (20)