Name:
Student number:

UNIVERSITY OF JOHANNESBURG

FACULTY	: Science
DEPARTMENT	: Biochemistry
CAMPUS	: APK
MODULE	: BICX04 CURRENT ADVANCES IN BIOTECHNOLOGY
SEMESTER	: Second
EXAM	: SSA January 2021

DATE : January 2021 **SESSION** : 08:00-11:00

ASSESSOR(S) : PROF LA PIATER & Guest Lecturers

MODERATOR : DR L STEENKAMP

DURATION : 3 HOURS **MARKS** : 100

NUMBER OF PAGES: 5 PAGES (including this page)

INSTRUCTIONS:

1. Answer ALL THE QUESTIONS.
2. Number your answers clearly
3. *"I certify that my submitted answers are entirely my own work and that I have neither given nor received any unauthorized assistance on this assessment item".*

QUESTION 1: Genes and Genomes **[10]**

Humans have tens of thousands of genes, and the development of DNA microarrays by Patrick O. Brown, Joseph DeRisi, David Botstein, and colleagues in the mid-1990s has led to many applications. Describe how microarrays work, and discuss how it has been used in cancer diagnosis and treatment thus far? (10)

QUESTION 2: Yeast Biotechnology **[10]**

2.1 Briefly discuss how a yeast cell may be engineered into (i) producing useful plant secondary metabolites and (ii) "humanizing" (authentic glycosylation) proteins. (7)

2.2 Briefly discuss the application of each of the following techniques:

2.2.1 TAR (1)

2.2.2 Yeast-two hybrid system (2)

QUESTION 3: Plant Biotechnology **[15]**

3.1 What are the genetic engineering strategies to create the following traits in transgenic crops. Where applicable, give an example of each.

3.1.1 Insect resistance (5)

3.1.2 Virus resistance (5)

3.2 How are the use of refuge areas expected to slow the development of BT-resistant European Corn Borers? (3)

3.3 Give one example of the genetic engineering of a plant for improved (a) nutritional properties and (b) storage life or improved quality of the final product. (2)

QUESTION 4: Molecular Diagnostics **[15]**

You are part of a research team currently investigating the gene expression changes of the p53 gene in response to a new potential anticancer drug X458 in MCF-7 breast cancer cell lines in comparison to normal cells. You carry out a preliminary study to measure the gene expression changes of p53 and obtain the following data.

Samples	Raw Ct	
	GAPDH	p53
Tumor cells 1	21.00	23.00
Tumor cells 2	20.50	22.00
Tumor cells 3	20.60	22.50
Normal cells 1	20.00	26.00
Normal cells 2	20.50	26.20
Normal cells 3	20.30	26.40

4.1. Which molecular technique was most likely used to generate this data? (2)

4.2. What does the “Raw Ct” represent? (3)

4.3. What does GAPDH represent in the table provided? (1)

4.4. Which calculation would you use for your downstream data analysis to determine if p53 expression changed in the X458-treated MCF-7 cell lines? (1)

4.5. Using the table provided, calculate the changes in the p53 gene and indicate if the p53 was upregulated or downregulated. (8)

QUESTION 5: Biofuels **[15]**

5.1. Various feedstocks are used to produce biofuels. Using corn as a feedstock name and explain in detail the two processes employed for corn processing. (5)

5.2. Elaborate on the differences, advantaged and disadvantages of SHF and SSCF approaches to biofuel production. (6)

5.3. Name and define the 2 categories of biofuels. (4)

QUESTION 6: Biotechnology-based Drug Discovery [15]

6.1. The binding energy of a drug to a protein is given by the change in Gibbs Free Energy, which has an enthalpic and an entropic contribution:
$\Delta G = \Delta H - T\Delta S$
Name any 2 types of protein-drug interaction, and state whether they are enthalphic or entropic. (3)

6.2. For assessing a drug's ADME properties, logP measures the partition coefficient and logD the distribution constant, with $logD = logP - | 7.4 - pK_a |$

6.2.1 What behaviour of the drug does the partition coefficient quantify? (1)

6.2.2 How it is measured? (1)

6.2.3 Given the equation above, which of the two quantities (logP or logD) is affected by the charge state of the molecule, and why? (1)

6.3. Once a drug is absorbed in the small intestine, it is carried straight to the liver by the hepatic portal vein. What is the role of the liver in determining drug bioavailability, what are some of the general mechanisms it employs to affect this, and what other organ works in tandem with the liver to eliminate drugs? (3)

6.4. Given what you know about the process of drug discovery, discuss why it has been broadly assumed that it was not possible to develop a novel antiviral in time to affect the COVID-19 pandemic. (2)

6.5. Discuss exactly why an effective antiviral drug would help quell the ongoing pandemic, and why it might be more effective than a vaccine. (4)

QUESTION 7: Vaccines [15]

7.1. Explain eradication and give an example of an eradicated disease. (5)

7.2.1 Describe the two types of polysaccharide vaccines. (2)

7.2.2 Give their differences and explain why the conjugate polysaccharide vaccine was developed. (8)

QUESTION 8: Application of knowledge in project [5]

During your individual BICX00 research project, you made use of “omics” (genomics, proteomic, metabolomics, *etc.*) method(s) or aspects thereof (genetics using qPCR, *etc.*). Suggest an alternative approach (one not used in your project) to answer your scientific research question, and briefly give an outline of the experimental design you will follow.

MEMO

QUESTION 1: Genes and Genomes [10]

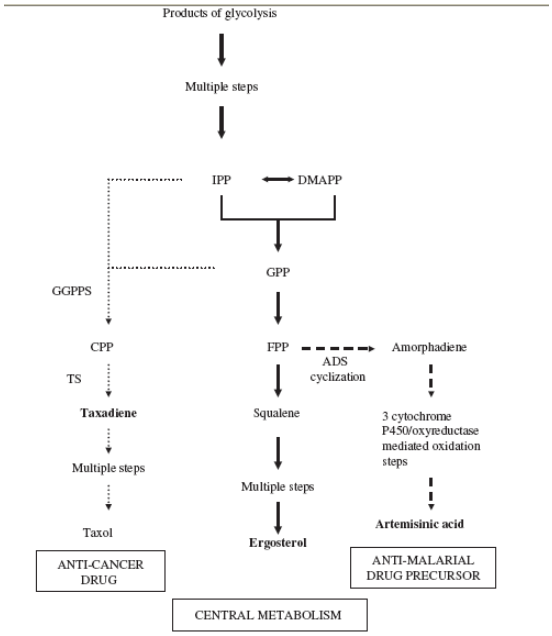
Humans have tens of thousands of genes, and the development of DNA microarrays by Patrick O. Brown, Joseph DeRisi, David Botstein, and colleagues in the mid-1990s has led to many applications. Describe how microarrays work, and discuss how it has been used in cancer diagnosis and treatment thus far? (10)

DNA microarrays exploit the ability of complementary strands of nucleic acids to base-pair with each other and bind (hybridise). Developers of the DNA microarray dot an array of DNA copies (cDNAs) corresponding to a large number of different mRNAs of known sequence onto a glass slide. In early microarray experiments, mRNA from one cell type was made into cDNA labeled with a red fluorescent dye, and mRNA from another cell type was made into cDNA labeled with a green fluorescent dye. The two cDNAs were then mixed, denatured and hybridized to the same DNA microarray. After washing away the unbound molecules, bound fluorescent nucleic acid samples were identified by laser microscopy. Fluorescent dots indicated expressed genes, and differences in microarray patterns between normal and cancerous cells could be quickly identified for comparative gene expression during diagnosis and treatment.

QUESTION 2: Yeast Biotechnology [10]

2.1 Briefly discuss how a yeast cell may be engineered into (i) producing useful plant secondary metabolites and (ii) “humanizing” (authentic glycosylation) proteins. (7)

Students choice of hijacking central metabolism Taxol or Artemisinic acid, and glycosylation of *P. pastoris*



Glycosylation:

- Knockout of *OCH1* (mannose transferring enzyme)
- Transporter genes isolated, transformed & targeted into Golgi membrane
- N-term localization signals fused in frame with mannosidae/transferase
- Expression of human genes that modify sugars in CMP-sialic acid

2.2 Briefly discuss the application of each of the following techniques:

2.2.1 TAR (1)

Isolation of specific chromosomal sections with the need to generate an entire library.

2.2.2 Yeast-two hybrid system (2)

Transcription factor DBD and AD fused to two different proteins for studying protein-protein interactions

QUESTION 3: Plant Biotechnology [15]

3.1 What are the genetic engineering strategies to create the following traits in transgenic crops. Where applicable, give an example of each.

3.1.1 Insect resistance (5)

They have been transgenically engineered to express *Bacillus thuringiensis* (*Bt*) toxins that confers resistance to chewing insects. These toxins are expressed by the *Cry* genes, and upon ingestion, are cleaved in the alkaline midgut to forn active toxins which results in the formation of pores in the insect gut and eventual death.

3.1.2 Virus resistance (5)

- Exploitation of **pathogen-derived resistance (PDR).**
- Initial mild infection of virus confers protection against subsequent inoculation.
- **Host resistance strategies** have 2 mechanisms:
  - Protection by expression of native/modified viral proteins that interfere with viral replication cycle.
  - Protection at transcriptional level (gene silencing / sequence-specific RNA breakdown)

Transgenic resistance to Geminiviruses

(Shephard *et al.* 2009)

- PDR (pathogen derived resistance) based approaches:
  - Coat protein mediated resistance (CPMR)
    - tomato plants expressing the CP of TYLCV exhibited delayed symptom development that was dependent on the expression levels of the transgenic CP (Kunik *et al.*, 1994)
  - Rep protein (viral replication and transcription) tolerance or strain specific immunity to ACMV, TYLCV-SV, ToLCNDV, BGMV, MSV - broader resistance.

Post-transcriptional gene silencing

- PTGS = RNA silencing or RNA interference (RNAi).
- Seq-specific breakdown mechanism in plants & eukaryotes which represents a natural antiviral defense mechanism.
- Involves cleavage of dsRNA to 21-25 nt siRNAs by Dicers.
- siRNAs interact with host proteins to form RNA-induced silencing complexes (RISC) - ds siRNAs are unwound and used as guides in the specific binding and destruction of targeted mRNA molecules.
- PTGS activated in transgenic plants by the introduction of dsRNAs homologous to viral sequences.
- Several studies reported RNAi resistance against geminiviruses:
  - ACMV -Vanderschuren *et al.*, 2009;

3.2 How are the use of refuge areas expected to slow the development of BT-resistant European Corn Borers? (3)

a. Resistant insects do not compete successfully against susceptible individuals on non-BT corn.

b. Rare resistant insects are likely to mate with more numerous susceptible insects that survive on non-BT corn.

c. Increased populations of Monarch butterflies that survive on non-BT corn refuges compete against BT-resistant ECB and reduce their populations.

d. Most scientists think that refuges will fail to slow the development of BTresistant ECB.

3.3 Give one example of the genetic engineering of a plant for improved (a) nutritional properties and (b) storage life or improved quality of the final product. (2)

- Delay in ripening (climatic fruit) & longer storage life.
- Flavr-Savr tomatoes (switch of polygalacturonase gene - pectin)
- Flavr-Savr puree first sold in European Union (not since 2002)
- Transgenic long vase-life carnations in Aus (inhibit ethylene production, down regulate ACC synthase gene (ACC=precursor of ethylene)
- Golden rice – enriched with Vit A
- Proteins
- Animals cannot synthesize 10 of 20 a.a.
- Possible to over-express genes encoding proteins with limited a.a.
- (S) Met in grain legumes (lupin) from sunflower seed albumin.

nature biotechnology

Enrichment of tomato fruit with health-promoting anthocyanins by expression of select transcription factors

QUESTION 4: Molecular Diagnostics **[15]**

You are part of a research team currently investigating the gene expression changes of the p53 gene in response to a new potential anticancer drug X458 in MCF-7 breast cancer cell lines in comparison to normal cells. You carry out a preliminary study to measure the gene expression changes of p53 and obtain the following data.

4.1. Which molecular technique was most likely used to generate this data? (2)

RT-qPCR → (Ct values) Gene expression measures changes in RNA transcripts therefore RT needs to be conducted first followed by relative quantification qPCR where p53 transcripts are measured in X458-treated cells tumour cells vs. normal cells

4.2. What does the "Raw Ct" represent? (3)

- In a real time PCR assay a positive reaction i detected by accumulation of a fluorescent signal.
- The Ct (cycle threshold) is defined as the number of cycles required for the fluorescent signal to cross the threshold (ie exceeds background level).
- Ct levels are inversely proportional to the amount of target nucleic acid in the sample (i.e. the lower the Ct level the greater the amount of target nucleic acid in the sample).

4.3. What does GAPDH represent in the table provided? (1)

Expression of a housekeeping/constitutively expressed gene. (i.e. control)

4.4. Which calculation would you use for your downstream data analysis to determine if p53 epression changed in the X458-treated MCF-7 cell lines? (1)

Delta-delta Ct method (comparative method)

4.5. Using the table provided, calculate the changes in the p53 gene and indicate if the p53 was upregulated or downregulated. (8)

- Relative quantification (ie GOI vs HKG) therefore Delta-delta CT calculation needs to be used to measure the changes in p53 expression.
- Students to show calculations (2 marks per step)
- Students can be given half the marks if the steps are correct but the values are not correct. Sometimes students incorrectly start by averaging in the beginning and not at the end.

Step 1: Normalization

First, you will need calculate relative difference between the gene of interest (p53) and the house keeping gene (GAPDH).

ΔCt = Ct (gene of interest) – Ct (housekeeping gene)

Samples	Raw Ct		Delta Ct
	GAPDH	p53	
Tumor cells 1	21.00	23.00	=C3-B3
Tumor cells 2	20.50	22.00	
Tumor cells 3	20.60	22.50	
Normal cells 1	20.00	26.00	
Normal cells 2	20.50	26.20	
Normal cells 3	20.30	26.40	

Step 2: Average of the control samples (normal cells)

To compare tumor (treatment) to control (normal cells), need to average the ΔCt for the 3 control (normal) samples.

Samples	Raw Ct		Delta Ct	Del
	GAPDH	p53		
Tumor cells 1	21.00	23.00	2.00	
Tumor cells 2	20.50	22.00	1.50	
Tumor cells 3	20.60	22.50	1.90	
Normal cells 1	20.00	26.00	6.00	
Normal cells 2	20.50	26.20	5.70	
Normal cells 3	20.30	26.40	6.10	
Avg delta Ct			=average(E6:E8)	

Step 3: Calculate the ΔΔCt relative to the average of ΔCt normal cells

ΔΔCt = ΔCt (Tumor sample) – ΔCt (normal average)

Samples	Raw Ct		Delta Ct	Delta Delta ct	2
	GAPDH	p53			
Tumor cells 1	21.00	23.00	2.00	=E3-E9	
Tumor cells 2	20.50	22.00	1.50		
Tumor cells 3	20.60	22.50	1.90		
Normal cells 1	20.00	26.00	6.00		
Normal cells 2	20.50	26.20	5.70		
Normal cells 3	20.30	26.40	6.10		
Avg delta Ct			5.93		

Step 4: Fold gene expression for each sample

Raise the negative ΔΔCt to power of two. Fold gene expression = 2^-(ΔΔCt)

Samples	Raw Ct		Delta Ct	Delta Delta ct	2^delta delta Ct
	GAPDH	p53			
Tumor cells 1	21.00	23.00	2.00	-3.93	15.27746566
Tumor cells 2	20.50	22.00	1.50	-4.43	21.60559914
Tumor cells 3	20.60	22.50	1.90	-4.03	16.37398227
Normal cells 1	20.00	26.00	6.00	0.07	0.954841604
Normal cells 2	20.50	26.20	5.70	-0.23	1.175547906
Normal cells 3	20.30	26.40	6.10	0.17	0.890898718
Avg delta Ct			5.93		
Average Tumor cells	17.7523				
Average Normal cells	1.0071				
Fold change Tumor/normal	=C11/C12				

p53 expression is upregulated by 17-fold

QUESTION 5: Biofuels **[15]**

5.1. Various feedstocks are used to produce biofuels. Using corn as a feedstock name and explain in detail the two processes employed for corn processing. (5)

Dry milling Dry milling simpler than wet milling
• Corn kernels milled with hammer mill or grinder
• Addition of water and heat to liquefy corn starch; thermostable α-amylase
Partially hydrolyzed starch maltodextrins & free Glc
• Cooling to 50-60ºC, pH 5; addition of glucoamylase
cleavage of single glucose units
• Critical parameter is maximizing Glc generation & minimizing reversion sugars
• Corn slurry-enzyme mix cooled to 40ºC & addition of S. cerevisiae
Fermentation over a few days; Glc to ethanol (9%).
While dry milling is less capital intensive, it also yields less ethanol per bushel of corn than wet milling.

Wet milling Steep corn with water & sulfur dioxide to swell corn
40-60 hrs with lactic-acid fermentation by *Lactobacillus*
• Separation of corn pericarp, starch, germ & soluble portion
• Series of separation processes to yield highly purified corn starch & corn oil
• Highly purified corn starch is largest product by weight
• Starch can be hydrolyzed (acid)
• Starch conversion at high T with α-amylase to high MW oligomers
• Maltodextrin coverted to >95% Glc, higher purity than dry mill process
• HFCS produced from xylose/glucose isomerase
Purified Glc is versatile & C source for many fermentative processes
Higher value products e.g. antibodies, amino acids, vitamins

5.2. Elaborate on the differences, advantaged and disadvantages of SHF and SSCF approaches to biofuel production. (6)

Biomass cellulose hydrolyzed with cellulases
Glc released is then fermented to ethanol
The process concept SHF involves a separation of the hydrolysis and fermentation by running
the reactions in separate units. Different factors influence the efficiency of the hydrolysis of
lignocellulosic material, including both pre-treatment conditions and process conditions. The
factors can be separated in two groups; substrate-related and enzyme-related. The process is cheap to rum, however produces less ethanol due to the above mention factors.

The enzymatic hydrolysis can be performed simultaneously with the co-fermentation of glucose and xylose in a process referred to as simultaneous saccharification and co-fermentation (SSCF). Besides reduced capital cost, SSCF process offers several advantages which include continuous removal of end-products of enzymatic hydrolysis that inhibit cellulases or β-glucosidases and higher ethanol productivity and yield than separate hydrolysis and fermentation.

5.3. Name and define the 2 categories of biofuels. (4)

First generation biofuels are also called conventional biofuels. They are made from things like sugar, starch, or vegetable oil. Note that these are all food products. Any biofuel made from a feedstock that can also be consumed as a human food is considered a first generation biofuel.

Second generation biofuels are produced from sustainable feedstock. No second generation biofuel is also a food crop, though certain food products can become second generation fuels when they are no longer useful for consumption. Second generation biofuels are often called “advanced biofuels.”

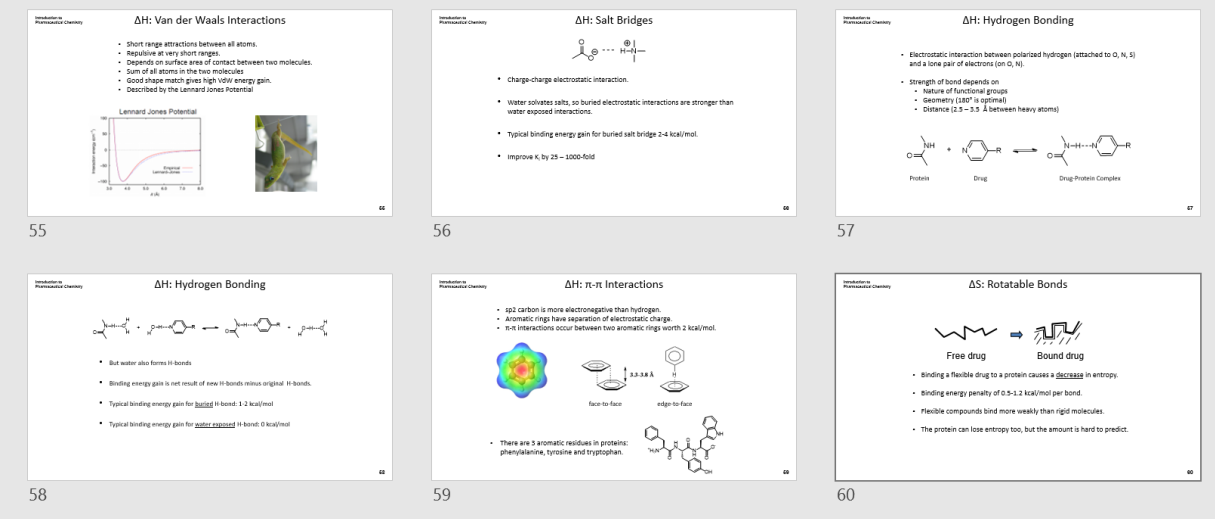
QUESTION 6: Biotechnology-based Drug Discovery [15]

6.1. The binding energy of a drug to a protein is given by the change in Gibbs Free Energy, which has an enthalpic and an entropic contribution:

$\Delta G = \Delta H - T\Delta S$

Name any 2 types of protein-drug interaction, and state whether they are enthalphic or entropic. (3)

Any two of the titles of slides 55-61, along with correct assignment of ΔH and ΔS.



6.2. For assessing a drug's ADME properties, logP measures the partition coefficient and logD the distribution constant, with logD = logP – | 7.4 - pK_a |

6.2.1 What behaviour of the drug does the partition coefficient quantify? (1)

How readily it enters either aqueous or hydrophobic (octanol) solvents

6.2.2 How it is measured? (1)

Add the drug to a water/octanol mixture, let them settle, then measure fraction of drug in each phase.

6.2.3 Given the equation above, which of the two quantities (logP or logD) is affected by the charge state of the molecule, and why? (1)

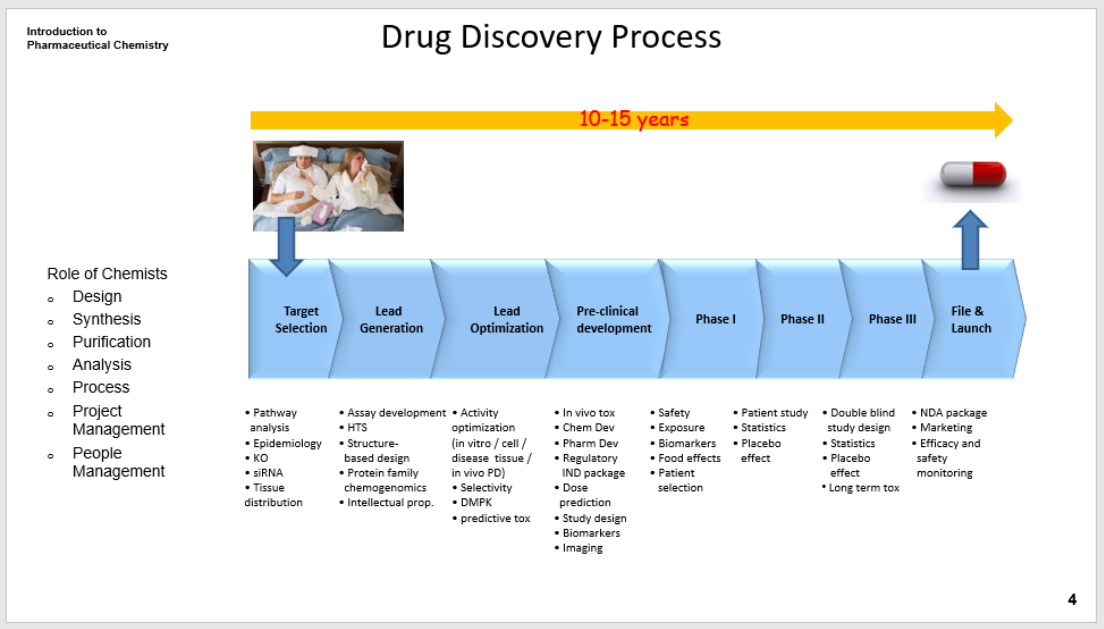
logD – it's affected by the pH, which changes the protonation state of the drug

6.3. Once a drug is absorbed in the small intestine, it is carried straight to the liver by the hepatic portal vein. What is the role of the liver in determining drug bioavailability, what are some of the general mechanisms it employs to affect this, and what other organ works in tandem with the liver to eliminate drugs? (3)

Liver has enzymes that modify molecules to make them far more soluble, so they can be eliminated from the body. The enzymes do this by adding specific chemical groups. The kidney will eliminate these solubilised compounds into the urine.

6.4. Given what you know about the process of drug discovery, discuss why it has been broadly assumed that it was not possible to develop a novel antiviral in time to affect the COVID-19 pandemic. (2)

(Assess whether the answer makes sense. Things they might invoke: the various stages on slide 4; that moving from each stage to the next takes time; legal barriers; paying for all of it; complexity of clinical trials. Don't hesitate to give full marks if the answer is knowledgeable.)



6.5. Discuss exactly why an effective antiviral drug would help quell the ongoing pandemic, and why it might be more effective than a vaccine. (4)

Deliberately open question – give points for well-argued answers, full marks if it's knowledgeable.

Some arguments: vaccines will struggle with the logistics of distribution, and politics of uptake; and the biological efficacy remains unknown, or how long immunity lasts. Whereas an antiviral need only be administered when needed, and additionally if it's safe enough, can be taken pre-emptively.

QUESTION 7: Vaccines [15]

7.1. Explain eradication and give an example of an eradicated disease. (5)

Permanent reduction to zero of the **worldwide** incidence of infection caused by a specific agent as a result of deliberate efforts; intervention measures are no longer needed. Example: smallpox.

7.2.1 Describe the two types of polysaccharide vaccines. (2)

- Pure Polysaccharide Vaccines : vaccine is composed of polysaccharide (sugar or carbohydrate) residues found on the surface of some bacteria
- Conjugate Polysaccharide Vaccines : vaccine is composed of polysaccharide residues chemically linked to an immunogenic protein.

7.2.2 Give their differences and explain why the conjugate polysaccharide vaccine was developed. (8)

Polysaccharide vaccines induce a T-Cell **independent** response, while the conjugated vaccine produces a T-cell dependent response. Polysaccharide vaccines produce antibodies that are mostly IgM, less effective, in lower quantities, and no memory response due to the absence of T cell help. Polysaccharide vaccines do not always produce a response in children less than 2 years of age, who need these vaccines the most. Conjugation to a protein allows for T- cell involvement, which produces differentiated antibodies that switch to IgG, are highly effective, produced in very high quantities, can produce memory cells and are effective in children <2 years.

QUESTION 8: Application of knowledge in project [5]

During your individual BICX00 research project, you made use of “omics” (genomics, proteomic, metabolomics, *etc.*) method(s) or aspects thereof (genetics using qPCR, *etc.*). Suggest an alternative approach (one not used in your project) to answer your scientific research question, and briefly give an outline of the experimental design you will follow.

Pending of student choice. Aspects to address would be:

Sample type and isolation

Method of analysis

Control *versus* treated

Interpretation of results