Diagnostic Engineering Part 1 and part 2 (weeks 1 and 2)

1. Emma Jane, who writes for *The* Australian, once described a Socially Unacceptable Illness X (also known as SUX). It is estimated that the prevalence of SUX in the Australian population is 0.1%. A diagnostic test has been developed for SUX with a sensitivity of 95% and a specificity of 90%.

   Remember from class that sensitivity is the probability that the test is positive given that the disease is present. This can be written in short hand as:

   Sensitivity = P(test is positive | disease is present)

   Similarly, Specificity = P(test is negative | disease is not present)

   Hamlet has applied for a graduate position at a world-leading Biomedical Engineering company that screen their job applicants for SUX using the diagnostic test. In this screening Hamlet tested positive for SUX. What is the probability that he actually has the disease?

   Use the table below as a starting point. SUX present means that a person actually has SUX and SUX not present means that the person does not have SUX. Fill in the table using the information given above and calculate the proportion of positive test results that are true positives.

	Test positive	**Test negative**	**Total**
SUX present			
SUX not present			
			1,000,000

2. The Zika virus outbreak in Brazil is causing concern for the Olympic games. The Zika virus belongs to the family of flaviviruses and was discovered more than 70 years ago. The current strain is new, different and more dangerous than the old strain. The new strain is associated with pediatric microcephaly and brain damage as well as adult conditions such as acute disseminated encephalomyelitis and Guillain-Barré syndrome. The incidence of zika virus in Rio de Janeiro is 157/100,000. You are a biomedical engineer working at the Centers for Disease Control and Prevention and are tasked with setting up an ELISA test at the Olympic village to test the athletes for zika virus. Your colleagues failed to send you the protocol for the ELISA test, but you have identified each of the reagents in the test kit: Zika antigen, 96-well ELISA plate, blocking agent, anti-IgM antibody, monoclonal anti-flavivirus antibody conjugated with horseradish peroxidase, colour substrate that is reduced by horseradish peroxidase, spectrophotometer, centrifuge, pipettes, buffer solution.

   a. Devise a protocol to detect the presence of anti-zika virus IgM antibodies in the serum of the athletes.
   b. The ELISA test used to detect Zika virus IgM antibodies in the blood has a sensitivity and specificity of approximately 90% and 98%. Of the athletes that test positive for the zika virus, what is the probability that an athlete has contracted the zika virus? Completion of the table below might help.

	Test positive	**Test negative**	**Total**
zika present			
zika not present			
			1,000,000

   c. What are the ramifications of the sensitivity and specificity of this diagnostic test? How can it be improved?

Ref: http://harvardpublichealthreview.org/off-the-podium-why-rios-2016-olympic-games-must-not-proceed/

3. You are trying to reproduce experimental data from the previous student in the lab. They reported that the enzyme under investigation had a Km of 25 µM and a V_{max} of 10 mM/s.

a) Calculate at which substrate concentrations in µM you should measure the initial rate v_0 to obtain the following values:

Substrate (µM)	V_o (mM/s)
	2.5
	4
	6
	9

b) Draw a predicted Michaelis-Menten plot for your enzyme.

c) Draw a predicted Lineweaver-Burke plot for your enzyme.

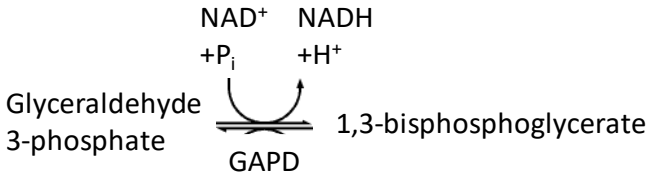
4. An enzyme is discovered to catalyse:

$$S + E \underset{k'_1}{\overset{k_1}{\rightleftharpoons}} ES \overset{k_2}{\rightarrow} E + P$$

We know that k_2 is 600 s^{-1} when [E] = 20 nM and [S] = 40 μM and v_o = 9.6 μMs^{-1}.

What is the K_m?

5. You are simulating glycolysis in the laboratory in order to develop a diagnostic test for metabolic disorders. Your reaction vessel is a cube (64L) which contains 60 g glucose and all the enzymes required for the reaction to go to completion, but your experiment is unable to oxidise NADH to NAD^{+}.
   a) How much NADH would you have in your reaction vessel? Express your answer in mol.
   b) What is the absorbance of the NADH in your reaction vessel? For NADH at 340 nm, ε = 6.22 ×10^3 $M^{-1}cm^{-1}$.
   c) The enzyme glyceraldehyde-3-phosphate dehydrogenase (GAPD) is involved in the following portion of the glycolysis reactions:



   If the reaction takes 2 h to go to completion, what is the maximum velocity of the enzyme assuming no end product inhibition? K_m for NAD^{+} for GAPD is 25μM.

Diagnostic Engineering Part 3 (Blood Diagnostics) (week 3)

1. The complete blood count (CBC) with differential is one of the most common laboratory tests performed. It gives information about the number of all blood cells and identifies the number of red blood cells as well as haematocrit. Jeff weighs 70 kg and has a blood volume of 6 L and a bone marrow of 2 L. A recent CBC indicated that he has the following levels of cells in his blood:

Haematocrit	0.45
Neutrophils	6×10^9/L
Lymphocytes	2×10^9/L
Monocytes	0.5×10^9/L
Eosinophils	0.1×10^9/L
Basophils	0.1×10^9/L
Platelets	400×10^9/L

   a) The report omitted the number of red blood cells present in Jeff's blood. If his red blood cells can be approximately by a disk 8 μm in diameter and 2 μm high and have a density of 1.25 g/mL, how many red cells does he have per L of blood?
   b) How many cells are circulating per L of Jeff's blood?
   c) The average time in the blood stream of a red blood cell is 120 days, neutrophils is 3 days, platelets is 9 days, lymphocytes and monocytes is 3 days and eosinophils and basophils is half a day. How many cells are made per day per ml of bone marrow if all cells survive to enter the blood is each megakaryocyte makes 20 platelets?
   d) Are each of Jeff's blood cell counts in the normal range?

2. The total volume of blood in an average adult (75kg) is about 6L. The heart pumps about 5 L/min and the haematocrit (proportion of red blood cell to total volume) is 45%. Assume that the red blood cell is all haemoglobin, haemoglobin has a specific density of 1.25 and the molecular weight of haemoglobin is 65000 Da. Assumptions: 100% oxygen saturation and each haemoglobin carries 4 O_2.
   a) How much O_2 is carried by blood per minute? Express you answer in moles O_2 per minute per kg of body tissue.
   b) How much oxygen is available per cell per second if 50% of your tissue consists of cells and each cell weighs 10 ng? Express you answer in moles per cell per second.
   c) How many mars bars (60g, assume all glucose) can a cell use per second?

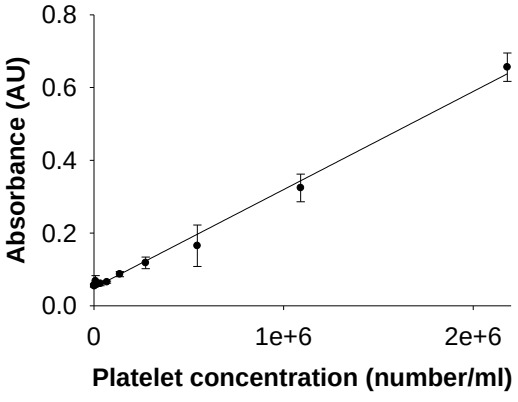
3. BIOM9420 students are preparing a new diagnostic to determine platelet counts in human plasma. They have collected preliminary data from their ELISA assay which detects a specific integrin on the surface of platelets. The data carefully recorded in their laboratory notebook and graph is as follows:

Number of Platelets /ml	2180000	1090000	545000	272500	136250	68125	34063	17031	8516	4258	2129	0
Absorbance (490nm)	0.625	0.304	0.201	0.095	0.076	0.063	0.057	0.059	0.072	0.058	0.054	0.053
	0.627	0.320	0.141	0.128	0.090	0.068	0.070	0.060	0.056	0.055	0.054	0.054
	0.664	0.379	0.222	0.130	0.090	0.062	0.061	0.060	0.087	0.054	0.058	0.054
	0.707	0.292	0.098	0.119	0.091	0.065	0.058	0.063	0.057	0.055	0.051	0.062
Average	0.656	0.324	0.165	0.118	0.087	0.065	0.061	0.061	0.068	0.056	0.054	0.056
Std Dev	0.039	0.038	0.057	0.016	0.007	0.003	0.006	0.002	0.015	0.002	0.003	0.004

(a) Calculate the extinction coefficient for this assay if the spectrophotometer that you are using has a path length of 1cm.

(b) What are the units for this parameter?

(c) The number of integrins on the surface of platelets varies with activation and the assay is only linear within the region graphed. Discuss the advantages and disadvantages of the diagnostic assay that you have developed.



Genetic testing (week 5)

1. Explain how DNA fingerprint analysis is performed.

2. A car dealer was broken into last night. The perpetrator appeared to cut themselves on the broken store window and blood was found on glass at the crime scene. The blood stained glass was taken to a forensics lab to extract DNA and analyse the sample. The suspects were also tested. The suspects were:

i) Miss Jones the receptionist at the car dealer.
ii) Mr Smith, a customer from earlier in the day who was seen at the crime scene close to closing time.
iii) Dr Brown, the owner of the medical practice next door.

The results of the DNA fingerprinting are shown in the figure below. The samples were loaded as follows:

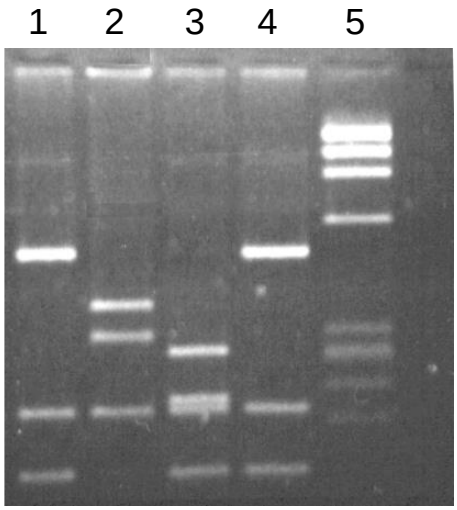
Lane 1: blood sample collected from the crime scene.

Lane 2: Miss Jones

Lane 3: Mr Smith

Lane 4: Dr Brown

Lane 5: DNA Ladder



Lane 5 : DNA ladder (bp) – 3000, 2000, 1500, 1000, 700, 500, 300, 100.

(a) Calculate the number of base pairs in the DNA fragments in the suspect's sample.

(b) Who is the most likely suspect after analysing the DNA gel?

Kidney function diagnostics (Week 8)

1. Glomerular filtration rate (GFR) can be measured using an indicator dilution technique in which a tracer is injected IV (amount = d_o) and the tracer concentration in plasma (c_p) is measured over an extended period. Renal clearance is calculated from the equation:

$$K = \frac{d_o}{AUC}$$

Where AUC stands for area under the curve and is the integral of the plasma concentration over time.

$$AUC = \int_0^\infty c_p(t)dt$$

The renal clearance of a solute is defined as its rate of elimination by the kidneys divided by its concentration in plasma:

$$K = \frac{\dot{E}}{c_p}$$

The total clearance, K, of an ideal tracer is GFR.

Let *q* represent the total amount of tracer in the body at any one time. A material balance and the definition of clearance lead to:

$$\frac{dq}{dt} = -Kc_p$$

Rearrange and integrate:

$$\int_{d_0}^{0} dq = -\int_0^\infty c_p(t)dt$$

Where at t=0, q=d_0 and q=0 at t=∞. Integration gives:

$$q|_{d_0}^{0} = -d_0 = -K\int_0^\infty c_p(t)\,dt$$

Rearranging:

$$K = \frac{d_o}{\int_0^\infty c_p(t)\,dt}$$

The integral can be estimated graphically or the data can be fitted by a mathematical function that can then be integrated. For ideal tracers, the data can usually be represented by the sum of two exponentials:

$$c_p = a_1 e^{-b_1 t} + a_2 e^{-b_2 t}$$

If values of a_1, b_1, a_2 and b_2 can be estimated, the area can be obtained from:

$$\int_0^\infty c_{p(t)dt} = a_1\int_0^\infty e^{-b_1 t} + a_2\int_0^\infty e^{-b_2 t} = \frac{a_1}{b_1} + \frac{a_2}{b_2}$$

A patient is undergoing a routine GFR test and has been injected with 16 $\times 10^6$ counts per minute (cpm) of ^{51}Cr-EDTA. The concentration of the tracer in the plasma is measured over time as follows:

Time (h)	[^{51}Cr-EDTA] (cpm/mL)
0.2	989.2
0.5	898.8
1	700.5
2	553.8
3	378.8
4	290.7
5	193.2
7	100.5

Plot the data with a semilog graph. If the data can be described by a single exponential, estimate the values of a_1 and b_1 and calculate the clearance.

2. George has a haematocrit of 0.42 and his blood flow to the kidneys is 1200 ml/min and the GFR is 120 ml/min. If his glomerulus is functioning properly, there will be no protein in the filtrate. If the concentration of protein in plasma entering the glomerulus is 80 g/L, what is the concentration of protein in plasma leaving George's glomerulus?

Lung function diagnostics (week 9)

1. Diagnose the type of lung disease suffered by each of the following patients.

Patient	History	FVC	FEV1	FEV1/FVC	Diagnosis
60 year old male	60 pack/year smoker and dysponea on exertion	3.1 L (73%)	0.7 L (23%)	25%	
50 year old male	65 pack/year smoker and a cough	1.9 L (62%)	1.1 L (42%)	56%	
36 year old man	muscular dystrophy	1.2 L (31%)	1.0 L (33%)	85%	
43 year old woman	with progressive dyspnea on exertion	1.7 L (51%)	1.4 L (49%)	78%	