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	950	50	1,000
SUX not present	99,900	899,100	999,000
	100,850	899,150	1,000,000

Prevalence, 0.1% = 0.1% x 1000000 = 1000 (SUX present total)
Sensitivity 95%, test positive| disease present = 0.95 x 1000 = 950
Specificity 90%, test negative| disease not present = 0.9 x 999,000 = 899,150

Probability that Hamlet has the disease is 950/100,850, or 0.9%.

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.

Coat plate with Anti-IgM antibody
Block plate
Add patient serum (centrifuge blood to obtain serum) or zika antigen as a positive control, buffer for negative control.
Add monoclonal anti-flavivirus antibody conjugated with horseradish peroxidase
Add colour substrate
Read absorbance in spectrophotometer.

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	1,413	157	1,570
zika not present	19,969	978,461	998,430
	21,382	978,618	1,000,000

6.6%

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

Low sensitivity means many healthy people test positive when they do not have the zika virus. Needless worrying and follow up tests required.

Specificity is high for this test, but still some with the virus test negative and can pass it on to others in the community.

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.

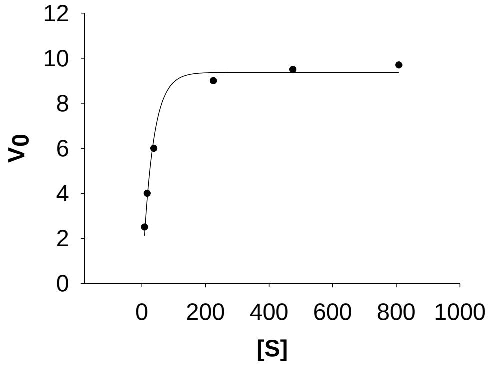
a) Calculate at which substrate concentrations in µM you should measure the initial rate v_0 to obtain the following values:

$$V = \frac{V_{max}[S]}{K_m + [S]} \; rearrange$$

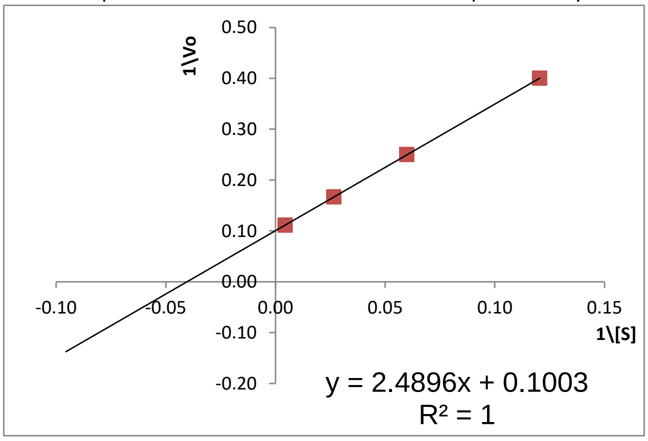
$$[S] = \frac{VK_m}{V_{max} - V}$$

Substrate (µM)	V_o (mM/s)
8.3	2.5
16.7	4
37.5	6
225	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:

$$\boldsymbol{S + E \;\frac{k_1}{k'_1}\; ES \overset{k_2}{\rightarrow} E + P}$$

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?

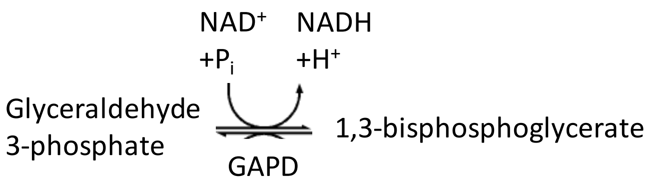
See equation 6 in the Michaelis-Menton kinetics derivation :

$$\boldsymbol{V_{max} = k_2[E]_0 = 600s^{-1} \times\ 20\ nM = 12\mu M/s}$$

$$\boldsymbol{v_0 = \frac{v_{max}[S]}{K_m + [S]}}$$

Rearranging, $\boldsymbol{K_m = \frac{v_{max}[S]}{v_o} - [S] = \frac{12\mu M/s \times 40\mu M}{9.6\ \mu M/s} - 40\mu M = 10\mu M}$

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.

a) Mol glucose = 60 g/180 g/mol = 0.33 mol

1 mol glucose → 2 mol NADH, therefore mole NADH = 0.66

d) A = εlc, V = l^3 = 64L = 64000 cm^3, l = 40 cm

A = 6.22 ×10^3 $M^{-1}cm^{-1}$ x 40 cm x 0.66 mol/64 L = 2592 OD units

$\boldsymbol{c)\ V = \frac{V_{max}[S]}{K_m+[S]} = \frac{0.66mol}{2h} \times \frac{h}{60min} \times \frac{min}{60s} \times \frac{1}{64L} = \frac{1.45 \times 10^{-6}M}{s}}$

$$\boldsymbol{[S] = \frac{0.66mol}{64L} = 1.03 \times 10^{-2}M}$$

$$\boldsymbol{V = 1.45 \times 10^{-6}\frac{M}{s} = V_{max}\frac{1.03\times10^{-2}M}{25\mu M + 1.03\times10^{-2}M} \approx V_{max} = 1.45\frac{\mu M}{s}}$$

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?

a) $V = \pi r^2 h = \pi*(4\mu m)^2*(2\ \mu m) = 1x10^{-10}\ cm^3$
number of RBCs = $0.45/1x10^{-10} = 4.5x10^9$/mL = $4.5x10^{12}$/L

b) sum of all blood cells = $4.9\ x\ 10^{12}$/L

c)

Cell	Time in blood (days)	#/ml	# in blood (6L)	# made in BM/day	# made/ml/day
Red blood cell	120	4.5×10^9	$4.5\times10^9*6000$ $=2.7\times10^{13}$	# in blood/time in blood $=2.7\times10^{13}/120$ $=2.25\times10^{11}$	#made in BM/day/ vol BM = $2.25\times10^{11}/2000$ $=1.13\times10^8$
Neutrophil	3	6×10^6	3.6×10^{10}	1.2×10^{10}	6×10^6
Lymphocytes	3	2×10^6	1.2×10^{10}	4×10^9	2×10^6
Monocytes	3	0.5×10^6	3×10^9	1×10^9	5×10^5
Eosinophils	0.5	0.1×10^6	6×10^8	1.2×10^9	6×10^5
Basophils	0.5	0.1×10^6	6×10^8	1.2×10^9	6×10^5
Platelets	9	400×10^6	2.4×10^{12}	2.7×10^{11}	6.7×10^6 * (divided by 20)
				Sum =	$1.3x10^8$

d) Yes

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?

a) Flow rate of RBCs = $\mathbf{0.45 \times 5 \frac{L}{min} \times 1.25 \times \frac{1000kg}{m^3} \times \frac{mol}{65kg} \times \frac{m^3}{1000\ L} = 0.043 \frac{mol}{min}}$

Oxygen flow rate = 4 × 0.043 = 0.173 mol/min/75kg = 2.3×10^{-3} mol/min/kg

b) Oxygen flow rate = 2.3×10^{-3} mol/min/kg = 3.8×10^{-5} mol/s/kg

Number of cells/kg = $\mathbf{\frac{500g}{10\times10^{-9}g}}$ = 5×10^{10}

Mol Oxygen/cell/s = $\mathbf{\frac{3.8\times10^{-5}}{5\times10^{10}}}$ = 7.33×10^{-16} mol/cell/s

c) Aerobic oxidation: $C_6H_{12}O_6 + 6O_2 \rightarrow 6CO_2 + 6H_2O$

Mole glucose/cell/s = mol oxygen/cell/s ÷ 6 = 1.22×10^{-16}

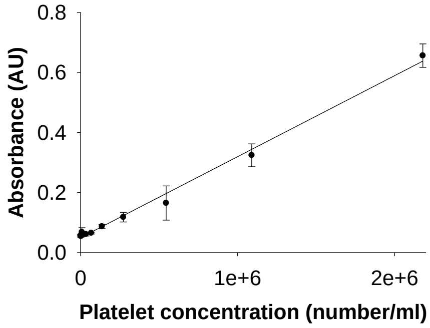
Mass glucose/cell/s = 180 × 1.22×10^{-16} = 2.2×10^{-14} g/cell/s

Number of mars bars = 2.2×10^{-14}/60 = 4×10^{-16}

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 0.627 0.664 0.707	0.304 0.320 0.379 0.292	0.201 0.141 0.222 0.098	0.095 0.128 0.130 0.119	0.076 0.090 0.090 0.091	0.063 0.068 0.062 0.065	0.057 0.070 0.061 0.058	0.059 0.060 0.060 0.063	0.072 0.056 0.087 0.057	0.058 0.055 0.054 0.055	0.054 0.054 0.058 0.051	0.053 0.054 0.054 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.



From graph, gradient = 3×10^{-7}
A = εlc, l=1cm, therefore ε is the gradient of the graph = 3×10^{-7}

(b) What are the units for this parameter?
ml/cm

(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.

Disadvantages: Limited concentration range given the concentration of platelets in blood, Requires platelets to be in a specific state to be detected which is difficult to achieve (would need to fix), Blood needs to be centrifuged to get plasma – a process which activates, or alters the state of platelets. ELISA assay is time consuming.
Advantages: may be a good marker of activation of platelets, may be used clinically for inhibit platelet activation, small blood volume, etc

Genetic testing (week 5)

1. Explain how DNA fingerprint analysis is performed.

   1. Obtain sample and extract DNA.
   2. Digest DNA with restriction enzymes and electrophorese on an agarose gel to separate on the basis of size.
   3. Visualise bands with dyes
   4. DNA fragments move through the gel at different rates, away from the negative and toward the positive end (small fragments migrate further into the gel than the larger fragments)
   5. The resulting "fingerprint" of fragments can be matched to a known sample**.**

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:

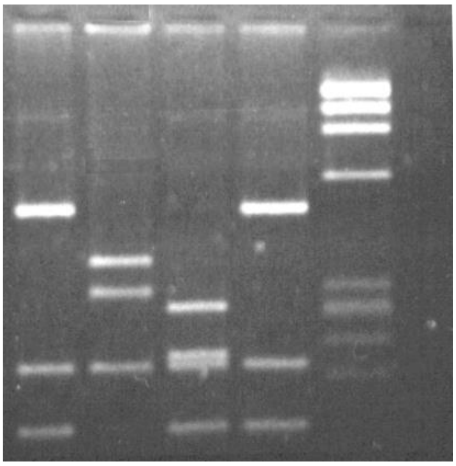
   i) Miss Jones the receptionist at the car dealer.
   ii) Mr Smith, a customer from earlier in the day who was seen at the crime scene close to closing time.
   iii) Dr Brown, the owner of the medical practice next door.

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

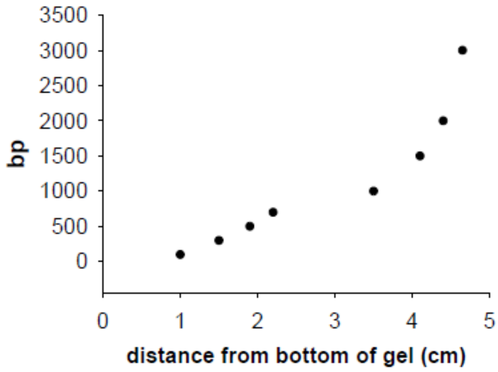
Lane 1: blood sample collected from the crime scene.
Lane 2: Miss Jones
Lane 3: Mr Smith
Lane 4: Dr Brown
Lane 5: DNA Ladder

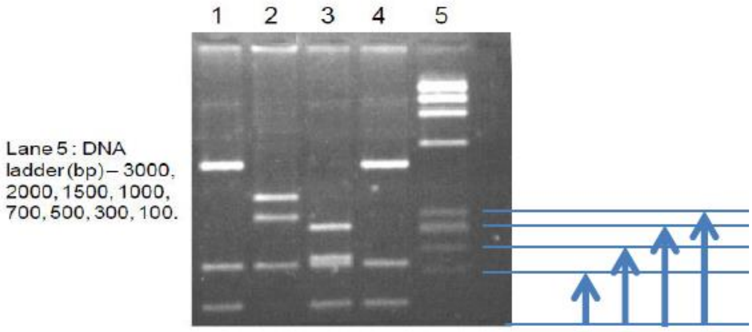
1 2 3 4 5

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.





Fit curve: $y = ae^{bx} + ce^{dx}$
DNA fragment lengths = 200 and 800 bp (smallest band is out of range of the standard curve).

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

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:

$$c_p = a_1e^{-b_1t} + a_2e^{-b_2t}$$

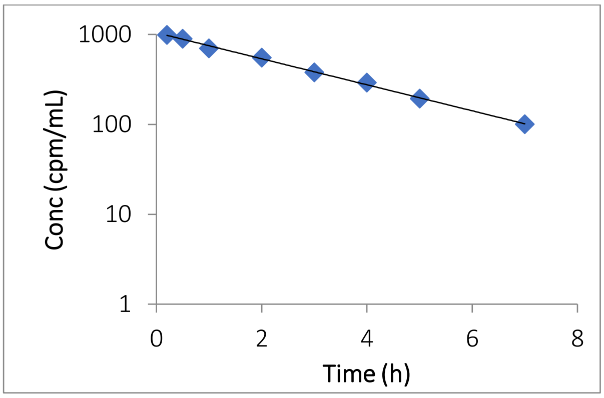
If values of a_1, b_1, a_2 and b_2 can be estimated, the area can be obtained from:

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

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.



a_1 = 1010 cpm/mL

b1 = gradient = $\frac{\ln(1000)-\ln(100)}{6.8-0.2} = 0.35/h$

$$\int_0^{\infty} c_{p(t)dt} = \frac{a_1}{b_1} = \frac{1010}{0.35} = 2886\ cpm/mL.h$$

Clearance, K = $\frac{d_o}{\int_0^{\infty} c_p(t)dt} = \frac{16\times10^6}{2886} = 5544\ ml/h$

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?

A mass balance around total blood flow is:
$Q_{b,in} = Q_{b,out} + Q_f$

Similarly, a mass balance around total plasma flow is:
$Q_{p,in} = Q_{p,out} + Q_f$

The flowrate of plasma into the kidneys,
$Q_{p,\,in}$ = (1-haematocrit) x Q_b = 0.58 x 1200 mL/min = 696 mL/min

$Q_{p,out} = Q_{p,in} - Q_f$ = 696 – 120 = 576 ml/min

$$C_{p,out} = \frac{\dot{m}_{p,out}}{Q_{p,out}} = \frac{\dot{m}_{p,in}}{Q_{p,out}} = \frac{\frac{80g}{L} \times 696 mL/min}{576\ mL/min} = \frac{97g}{L}$$

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%	Obstructive lung disease
50 year old male	65 pack/year smoker and a cough	1.9 L (62%)	1.1 L (42%)	56%	Obstructive lung disease
36 year old man	muscular dystrophy	1.2 L (31%)	1.0 L (33%)	85%	Restrictive lung disease
43 year old woman	with progressive dyspnea on exertion	1.7 L (51%)	1.4 L (49%)	78%	Obstructive lung disease