

West Midlands Training Course in Clinical Biochemistry

Course Assessment – Spring 2005

Short Answer Questions. Answer all questions. Time allowed 1 hour.

1. The plot shown above was constructed during the development of an assay for serum rhubarb involving the use of the enzyme rhubarbase.

$$\text{Slope of line} = K_m / V_{\max}$$

Good Web site :-

<http://users.rcn.com/jkimball.ma.ultranet/BiologyPages/E/EnzymeKinetics.html>

- A) What is this type of plot called
Lineweaver-Burk plot
- B) Using the graph calculate the maximum velocity of the reaction (V_{\max}) in arbitrary units

$$\text{Intercept on Y axis} = 1/V_{\max}$$

Therefore: -

$$0.035 = 1/V_{\max}$$

$$V_{\max} = 1/0.035 = 28.6 \text{ Units}$$

- C) Using the graph calculate the Michaelis-Menten constant (K_m) for the reaction.

$$\text{Intercept on X axis} = 1/K_m$$

Therefore: -

$$-0.8 \times 10^{-4} = 1/K_m$$

$$K_m = 1/0.8 \times 10^{-4} = 1.25 \times 10^{-4} \text{ Molar}$$

D) Calculate the substrate concentration at which the velocity of the reaction is 91% of the maximum

At 91% of Vmax the velocity will be equal to $0.91 \times 28.6 = 26$

$$1/V \text{ or } 1/26 = 0.038$$

reading from graph

$$1/[S] = 0.08$$

therefore :-

$$S = 1/0.08 = 12.5 \times 10^{-4} = 1.25 \times 10^{-3} \text{ Molar}$$

Alternatively; -

$$1/V = (K_m / V_{max}) \cdot 1/S + 1/V_{max}$$

If $V_{max} = 100$ and $v = 91$

Then

$$1/91 = ((1.25/100) \cdot 1/S) + 1/100$$

$$1/S = 0.079 \text{ therefore } S = 1/0.079 = 12.65 \text{ units} = 1.265 \times 10^{-3} \text{ Molar.}$$

2. Briefly comment on the following set of data and suggest a further biochemical test that could be undertaken on the ward to indicate the nature of the ongoing pathology. The patient is a 74 year old man who has been receiving treatment for hyperlipidaemia. He was admitted complaining of having had muscle and chest pain for several days.

Serum	Ref Interval	Day 1	Day3	Day 5	Day 7
Sodium mmol/L	133-147	138	138	139	139
Potassium mmol/L	3.5 – 5.0	4.4	5.1	4.9	4.8
Urea mmol/L	2.5 – 7.5	13.4	18.5	21.6	17.9
Creatinine $\mu\text{mol/L}$	85 - 125	399	535	23.7	489

Creatine Kinase (CK)	<200	2510	5727	12240	15827
Aspartate Aminotransferase (AST)	<35	91		410	541
CK-MB%	<6	2.6	3.5		
Alkaline phosphatase U/L	50-200			116	
Bilirubin $\mu\text{mol/L}$	1 - 25			10	

Rhabdomyolysis with deteriorating renal function. Probably secondary to statins.

Ward tests would probably include dip sticking urine. A positive for blood would also occur in the presence of myoglobin. Combination of this with raised CK and clinical history would do to support the diagnosis in the absence of a test for myoglobin itself.

Few people commented on the error in creatinine concentration on day 5. Should have been chased through.

3. In healthy subjects the average within subject biological variation (CV_I) for creatinine in serum is 4.1% and the between subject (CV_G) 12.9%. Assume when answering the following that the analytical imprecision of creatinine assays at a value of 70 $\mu\text{mol/L}$ across the UK is 12.2% and that there is no assay bias between laboratories.

a. What is the analytical goal for imprecision based on biological variation?

b.
 $CV_I/2 = 4.1/2 = 2.05\%$

c. Calculate the index of individuality for creatinine.

$$\text{index of individuality} = (CV_I)/(CV_G) = 4.1/12.2 = 0.31$$

d. Does the index of individuality support the application of population based reference intervals?

If $II < 0.6$ population based reference intervals are of limited utility.

e. If the total error included only 1 analytical standard deviation what would be the expected range of creatinine concentrations seen across the UK in subjects having a mean creatinine concentration of 70 $\mu\text{mol/L}$.

$$\text{Total error} = CV_I + CV_G + CV_A = 4.1 + 12.9 + 12.2 = 29.2\%$$

Therefore range around 70 $\mu\text{mol/L}$ = 50 to 90 $\mu\text{mol/L}$

4. A test for a disease with a prevalence of 1 in 500 has a diagnostic sensitivity of 80% and an overall diagnostic of efficiency of 80%. If applied to a population of 100,000 individuals what would be the negative and positive predictive values for the test.

This problem can be approached very easily by construction of a contingency table using the available information.

The prevalence in the population is 1 in 500 therefore if applied to 100,000 individuals you would expect to find 200 diseased subjects.

*If the diagnostic sensitivity of the assay is 80% then 80% of the diseased population would be expected to give true positive results (TP = 160) and 20 % false negative results (FN = 40). Since Sensitivity = ((TP/(TP+FN))*100).*

The diagnostic efficiency is 80% therefore if 100,000 population are tested then 80,000 would be correctly classified as true positive and true negatives (TN). The number of true negatives must therefore be equal to (80,000 - 160 = 79,840).

With these figures the table can be populated as follows by use of simple arithmetic and the relevant predictive values calculated: -

	<i>Test Positive</i>		<i>Test Negative</i>		<i>Totals</i>
<i>Diseased</i>	160	TP	40	FN	200
<i>Non Diseased</i>	19960	FP	79840	TN	99800
<i>Totals</i>	20120		79880		100,000

$$\text{Positive Predictive value} = TP/TP+FP = 160/(160 + 19960) = 0.008$$

$$\text{Negative Predictive value} = TN/TN+FN = 79840/(79840+40) = 0.9994\%$$

5. What volume of 10% w/v magnesium chloride solution would need to be added to 1L of 5% dextrose solution in order to infuse 50 mmol? What would be the final molar concentration of dextrose in the dextrose/magnesium solution produced. (MW Mg = 24, Cl 35.5, glucose 180).

$$10\% \text{ w/v magnesium chloride} = 10\text{g}/100\text{mL} \text{ or } 100\text{g}/\text{L}$$

$$\text{the molecular weight of } \text{MgCl}_2 = 95 \text{ therefore the molarity of the solution} = 100/95 = 1.05 \text{ mol/L}$$

$$50 \text{ mmol Mg required therefore require } (50/1050) \times 1000 \text{ mL} = 47.6 \text{ mL}$$

The original dextrose solution was had a concentration of 5% or 50g/L however the final volume is no 1047.6 mL therefore the molar concentration: -

$$= (50/180)/1.0476$$

$$= (0.278)/ 1.0476 = 0.265 \text{ mol/L}$$

6. Using a diagram briefly illustrate the renin aldosterone axis.
7. Alanine transaminase is performed by measuring the rate of conversion of NADH to NAD in a linked enzymic reaction. The change in absorbance with time is directly proportional to the enzyme activity. Given the following information calculate the concentration of ALT activity in U/L of serum: -

Reaction time = 5 minutes
 Initial absorbance at 339 nm = 0.00
 Final Absorbance at 339 nm = 0.75
 Total volume in cuvette = 3.0 mL
 Volume of serum specimen = 0.2 mL
umolar absorptivity of NADH at 339 nm = 6.3×10^{-3}

ALT Activity in U/L ($\mu\text{mol/L/min}$) = $DA/\text{min} \times 1/\text{molar absorptivity NADH} \times \text{dilution}$

$$ALT = 0.75/5 \times 1/6.3 \times 10^{-3} \times 3.0/0.2 = 357$$

8. Very briefly define each of the following: -

f. CSF xanthochromia

Following haemorrhage into the CSF, red blood cells undergo lysis and phagocytosis; the liberated oxyhaemoglobin is converted in-vivo in a time-dependent manner into bilirubin , and sometimes methaemoglobin . The yellow colouration that results from the bilirubin is known as xanthochromia.. Bilirubin may be detected in CSF by spectrophotometry or by visual inspection for the yellow discoloration (xanthochromia) it imparts to CSF, however spectrophotometry is the method of choice for it's detection.. Detection of bilirubin is of particular value in the investigation of a CSF with an increased erythrocyte count as there is no other reliable way for distinguishing between SAH and a traumatic lumbar puncture. It is also of value in the investigation of CSF with a normal red cell count from a patient presenting several days after an event by which time the cells may no longer be present.

Bilirubin arises solely from in-vivo conversion. Oxyhaemoglobin and methaemoglobin may both be produced in-vitro as well as in-vivo.

See national guidelines at <http://www.immqas.org/CSFGuidelines.pdf>

g. Froin's syndrome

- *Also known as:*
Nonne-Froin syndrome
Nonne's syndrome I
Nonne's compression syndrome
Maladie du Froin

Synonyms:

Block syndrome, massive coagulation-xanthochromic syndrome, syndrome of compression of the spinal column.

Associated persons:

Georges Froin

Max Nonne

Description:

As commonly used the term «Froin's syndrome» refers to the increased amount of exudative products - coagulation of spinal fluid - found in the spinal fluid below the level of a partial or complete block of the spinal canal. The cerebrospinal fluid, obtained by lumbar puncture, is yellow and the protein content is raised.

As originally described by Froin in 1903 the syndrome included xanthochromia and marked coagulation that he attributed to meningeal irritation. Max Nonne in 1910 emphasized that an excessive amount of globulin was present. Raven of Nonne's clinic in 1912 attributed the high total protein to a spinal cord tumor.

h. Secondary hypertension

Hypertension is generally defined as a blood pressure reading greater than 140 over 90; pressures of 120–139 over 80–89 are now considered prehypertension. When the cause is unknown, the hypertension is called primary, or essential, hypertension. When a cause can be identified (e.g., a disorder of the adrenal glands, or kidneys), the condition is known as secondary hypertension.

i. Conn's syndrome

- *Aldosteronism - excess secretion of aldosterone - can be:*
- *Primary - due to primary pathology of the adrenal gland*
- *Secondary - due to reduced plasma volume and increased angiotensin production*
- *Secondary aldosteronism is due to cirrhosis, nephrotic syndrome or cardiac failure*

- **Conn's syndrome is primary hyperaldosteronism due to:**
 - **Aldosterone producing adenoma (50%)**
 - **Bilateral idiopathic hyperplasia - idiopathic hyperaldosteronism (40%)**
 - **Aldosterone secreting carcinoma**

Pathophysiology

- **Aldosterone is produced by the zona glomerulosa of the adrenal cortex**
- **Acts on distal convoluted tubule to increase sodium reabsorption**
- **Sodium reabsorption occurs at the expense of potassium and hydrogen ion loss**

Clinical presentation

- **Usually occurs between 30 and 60 years**
- **Conn's syndrome accounts for 1% of cases of hypertension**
- **Hypertension often responds poorly to treatment**
- **Biochemically there is usually a hypokalaemic alkalosis**
- **NB - serum potassium may be normal**

Investigation

- **Investigations need to:**
 - **Confirm primary hyperaldosteronism**
 - **Localise pathology**
 - **If there is an adrenal mass is it producing aldosterone ?**
- **Diagnosis depend on demonstration of**
 - **Reduced serum potassium:**
 - **Increased urinary potassium excretion**
 - **Increased plasma aldosterone**
- **CT is able to demonstrate 80% of adrenal adenomas**
- **MRI has a similar sensitivity**
- **Assessment of function may require isotope (NP59) scanning or renal vein sampling for aldosterone**

9. **If isotopic waste must be held until its activity falls to less than 1% of its original activity, how long would the following isotopes have to be stored?**

This question requires a simple bit of thinking. As the decay of all isotopes follow the same equation. The number of half lives for activity to fall to less than 1% is a constant number for all. If you determine that constant then it is simply a matter of multiplying the half life of the isotope by the constant to give the number of days storage required :-

$$A = A_0 \cdot e^{-kt} \dots\dots\dots (1)$$

Assuming starting conc = 100 units therefore final concentration = 1 unit

$$1 = 100 \cdot e^{-kt} \dots\dots\dots (2)$$

As $t_{1/2} = 0.693/k$ if we call 1 half life to be equal to 1 unit of time

Then $k = 0.693$

Therefore substituting into (2)

$$I = 100 \cdot e^{-0.6936 \times t}$$

$$1 \times e^{-0.6936 \times t} = 100 \cdot$$

$$-0.6936 \times t = \ln 1/100 = -4.605$$

$$t = -4.605 / -0.6936 = 6.65$$

As we stated that 1 half life is equal to 1 unit of time then 6.65 half lives must pass for activity to drop to 1% of original

- a. ^{125}I $t_{1/2} = 60 \text{ days} \times 6.65 = 399 \text{ days}$
- b. ^{60}Co $t_{1/2} = 5.26 \text{ years} \times 6.65 = 34.98 \text{ years}$
- c. ^{131}I $t_{1/2} = 8.07 \text{ days} \times 6.65 = 53.67 \text{ days}$
- d. ^3H $t_{1/2} = 12.26 \text{ years} \times 6.65 = 81.53 \text{ years}$
- e. ^{32}P $t_{1/2} = 14.28 \text{ days} \times 6.65 = 94.96 \text{ days}$

10. The following results were obtained on analysis of serum from a 30 year old male with a history of depression with psychotic episodes and self harm. He presented at casualty on day 1 distressed and incoherent. The patient's carer stated that apart from the patient's psychiatric problems he was known to be in good health with no known other medical problems.

		Ref Range	Day 1	Day 2	Day 3	Day 4
Sodium	mmol/L	133-147	147	144	143	146
Potassium	mmol/L	3.5-5.0	4.1	2.9	3.2	3.5
Urea	mmol/L	2.5-7.5	5.0	8.0	10	14.1
Creatinine	$\mu\text{mol/L}$	60-120	115	152	295	538
Alkaline Phosphatase	U/L	40-140	170	184	156	129
Aspartate aminotransferase (AST)	U/L	0-35	273	22712	10787	2827
Total Bilirubin	$\mu\text{mol/L}$	0-25	38	49	45	43
Albumin	g/L	35-48	55	51	40	35
Total Protein	g/L	60-85	83	80	63	53

Glucose	<i>mmol/L</i>	2.8-6.0		8.9		

The patient had taken an overdose of paracetamol: -

- a. *What is the most likely causes of this patients abnormal biochemistry?
Looking at the overall picture it looks toxic and paracetamol poisoning would be a good starting point*
- b. *What other biochemical investigations should have been undertaken on day1?
Paracetamol*
- c. *What other laboratory test should have been ordered?*

Should be aware of the possibility of poisoning with additional drugs such as salicylate which are easily measured in the lab at the same time as the paracetamol.

Compound Analgesic preparations contain a simple analgesic (usually Paracetamol) with an opioid component. Therefore an overdose carries the added complications of opiate poisoning. Examples of such preparations (of which there are many) include Coproxamol, which contains 325mg of Paracetamol and 32.5mg of Dextropropoxyphene hydrochloride per tablet, and Solpadeine, which contains 500mg of Paracetamol, 8mg of Codeine phosphate and 30mg of caffeine per tablet. Along with the signs and symptoms of paracetamol poisoning, the patient will also show signs of opiate poisoning:

- Respiratory depression*
- Pin-point pupils*
- Vomiting*
- Circulatory failure*
- Coma*

- d. *In view of your answer to question a. are there any indicators of poor prognosis for this patient in the tabulated data?*

In patients with paracetamol poisoning, any one of the following features is serious and influence the prognosis:

- 1. Prothrombin Time > 6.5*
- 2. Arterial pH < 7.3*
- 3. Serum Creatinine > 295 μ mol/L*

