# West Midlands Training Course in Clinical Biochemistry

## Course Assessment – December 2005

### Model answers

1. If a radio-isotope has a half life of 12 hours. What percentage of the initial activity would remain after 24 and 72 hours.

The amount of activity remaining can be determined using the following equation: -

**$A = A_0 \cdot e^{-kt}$ …………………………………….. (1)**

*Where* $k$ = *the decay constant*

$A$ = *the amount of activity remaining at time t*

$A_0$= *the starting activity*

$t$ = *the time elapsed*

To solve the problem *k* must be determined. By rearranging equation (1) : -

**$\frac{A}{A_0} = e^{-kt}$ ………………………………………………..(2)**

k can be determined since t is given and we know that at one half life the concentration will fall from 100% to 50%. Therefore substituting into (2): -

$50/100 = e^{-k \times 12} = 0.5$

ln 2.0 = k x 12 =0.693

0.693/12 = k = 0.05775.

Alternatively remember that: -

$t_{½} = 0.693/k$

k= 0.693/12 = 0. 05775

having determined k the amount of isotope remaining at 24 and 72 hours can be simply determined by substitution into equation(2). Set $A_0$ as equal to 100%

For 24 hours:

$A = 100 \cdot e^{-(0.0057 \times 24)}$

$A/100 = e^{-(0.05775 \times 24)}$

ln A/100 = -1.386

A/100= 0.25

A = 25%

**25% remains alter 24 hours**

Even simpler in this case 24 hours = 2 half lives therefore  after 1 half life = 50% after 2 = 25%

**For 72 hours**

$A = 100 \cdot e^{-(0.005775 \times 72)}$

$A/100 = e^{-(0.05775 \times 24)}$

$\ln A/100 = -4.158$

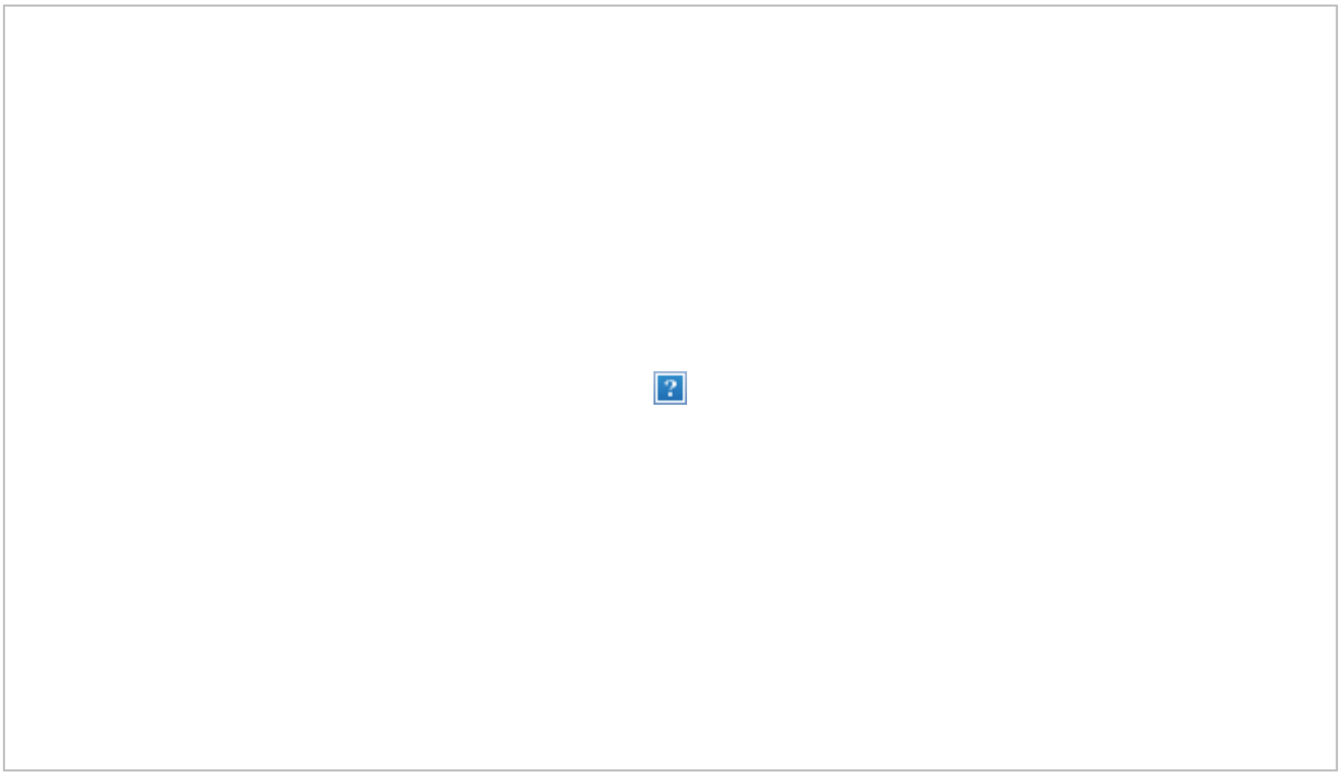
$A/100 = 0.0156$

A = 1.56%

**1.56% remains after 72 hours**

Even simpler in this case 72 hours = 6 half lives therefore  after 1 half life = 50% after 2 = 25% etc. 6 half lives = 72 hours.

2.



Study the biochemical pathway shown above and answer the following questions: -

2a. name of *Enzyme A*? ***MethylMalonyl CoA racemase***

2b. name of *Enzyme B?* ***MethylMalonyl CoA mutase***

2c name of *Compound 1**?*** ***D-methylmalonyl CoA***

2d name of *Compound 2?* ***homocystine***

2e **Briefly describe what is happening in *Process 1***

Remethylation of homocystine to methionine (Met) linked to B12 metabolism. , Remethylation of methionine (Met), in most tissues, is catalyzed by the ubiquitous methionine synthase (MS), which requires cobalamin (B-12) as cofactor and 5-methyltetrahydrofolate ($CH_3THF$) as substrate

3. Match the following diseases or syndromes with one choice from each of the subsequent sections: -

**A)** Wilson's disease **c) and i)**
B) Menkes' Syndrome **a) and iii)**
C) Phenylketonuria **d) and v)**
D) Tetrahydrobiopterin ($BH_4$) deficiency **b) and ii)**

a) Kinky or Steely hair
b) Patients may have a high frequency red hair within the population
c) Kayser-Fleischer rings
d) Fair colouring as a result of tyrosine deficiency
e) Most commonly present with megaloblastic anaemia and pallor

i) High liver copper concentrations
ii) Most commonly associated with 6-pyruvoyl-tetrahydropterin synthase deficiency
iii) Low activity of copper containing enzymes
iv) High tyrosine hydroxylase activity
v) Results from low phenylalanine hydroxylase activity

4. A screening test for a disease with a prevalence of 1 in 1500 is applied to a population of 150,000 subjects and is found to have a sensitivity of 90% producing 22485 false positive results. Calculate the following characteristics of the test: -

A. Negative predictive value = **(TN/(TN+FN)*100= (127415/(127415+10)*100= 99.99%**
B. Positive predictive value **=(TP/(TP + FP)) *100 = (90/(90+22485))100 = 0.4%**
C. Test specificity = **(TN/(TN+FP)) * 100 = (127415/149900) * 100 = 85%**
D. The efficiency (accuracy) of the test**= (TP + TN)/(TP + FP + TN + FN) = 127505/150000 = 0.85 = 85%**

Fill in a contingency table using the data given in the question, prevalence of 1:1500 means that there will be approximately 100 patients in the population tested. As the sensitivty of the assay is 90% then theoretically 90 true positive and 10 false negative results will be produced. The rest is all a matter of adding and subtraction to complete the table. See above for the answers to the rest of the question

| | TEST POSITIVE | | TEST NEGATIVE | | TOTALS |
|---|---|---|---|---|---|
| Disease Positive | 90 | TP | 10 | FN | 100 |
| Disease Negative | 22,485 | FP | 127,415 | TN | 149,900 |
| Totals | 22,575 | | 127,425 | | 150,000 |

5. The following results from a 16 day old term neonate with persistent jaundice and no clinical/biochemical evidence of liver dysfunction: -

Total serum bilirubin 65 μmol/L
Conjugated bilirubin 5 μmol/L

A) List the potential causes of hyper-bilirubinaemia in this type of scenario.

The hyperbiliruinaemia is predominantly unconjugated. If the patient is not particularly sick then consider the following: -

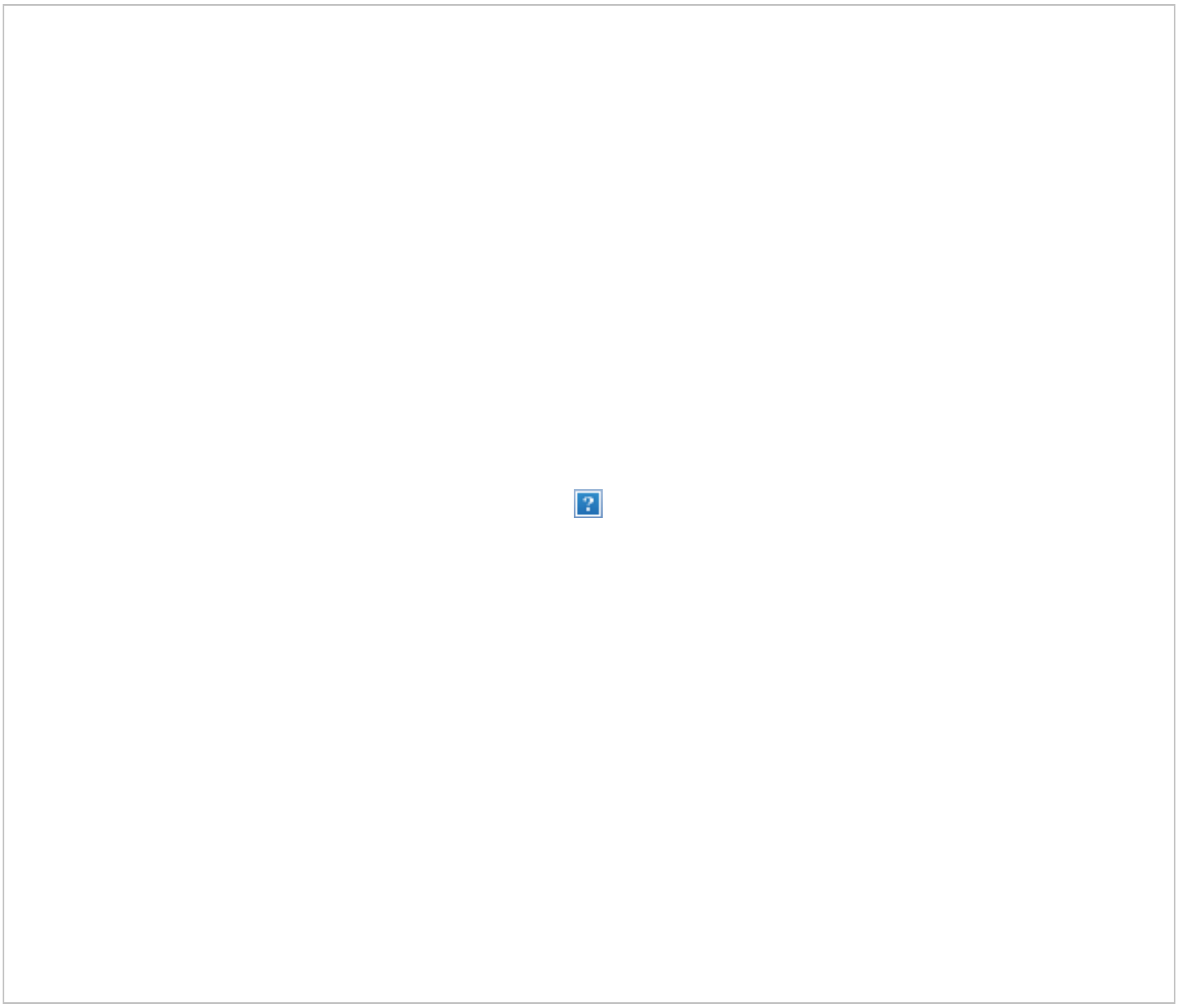
- Breast milk jaundice
- G6PD deficiency
- Hyopthyroidism
- Consider Crigler-Najjar Syndrome

Need to always consider the possibility of liver disease in early stages and other possible metabolic defects particularly if the patient is sick as well as jaundiced.

B) List further relevant initial investigations that should be requested.

- BloodFilm/FBC
- Group/direct antiglobulin test
- Urine bilirubin
- Reducing substances
- Stool coloour
- LFTs
- Infection screen
- RBC G6PD
- Thyroid function

6.



The figure above displays an interferogram illustrating the effect of haemoglobin on the analysis of a number of serum constituents on a new analyzer. If an average CV for all analytes measured is 2% then: -

A) At 0.2 g/100 mL haemoglobin which analytes demonstrate a clearly definable interference?

*If the CV is 2% the then to be confident that a value is different from the mean at a probability of 0.95 then it must deviate by more than 2 SDs. CV is the SD over the mean times 100. Therefore if a result is below 96% or greater than 104% then this may indicate interference in the assay. Therefore at 0.2g/100 mL haemoglobin serum phosphate appears to be the only analyte affected.*

B) At 0.6 g/100m L haemoglobin which analytes demonstrate a clearly definable negative interference?

*Serum phosphate again.*

C) What is the appropriate interpretation of the curves exhibited for creatinine kinase and uric acid?

*Creatine kinase assays are subject to intefrence at haemoglobin concetrations greater than 0.4 g/100 mL and urate at haemoglobin concentrations greater than 0.6 g/100mL. Conversely the assays are considered to be unaffected at concentrations of haemoglobin indicated.*

D) Which analytes are subject to positive interference by haemoglobin at concentrations above 0.6g/100 mL.

*Uric acid, bililirubin and creatine kinase*

7. A solution containing 1 x $10^{-5}$ M ATP has a transmission of 0.702 (70.2%) at 260 nm in a 1 cm cuvette. Calculate: -
   A) the transmission of a 5 fold dilution of the solution in a 3 cm cuvette.
   B) the absorbance of a 5 fold dilution in a 1 cm and in a 3 cm cuvette
   C) the absorbance and transmission of a 5 x $10^{-5}$ M solution in a 3 cm cuvette.
   A=εCL
   Where: -
   A = absorbance
   C = concentration in mol/L
   L = the path length in cm
   ε = molar absorptivity
   A = 2 – log% transmission
   A) Absorbance of 1x $10^{-5}$ M ATP = 2 – log 70.2 = 2 – 1.846 =0.153
   Absorbance of a 5 fold dilution = 0.154/5 = 0.0308
   Absorbance in a 3 cm cell = 0.0308 x 3 = 0.0924
   A=2 – log%T
   0.092 = 2- log%T
   1.908 = log%T
   **T = 80.9%**
   B) Absorbance of a five fold dilution : -
   1cm cell = 0.154/5 = 0.0308
   3 cm cell = 0.0308 x 3 = 0.0924

   C) Absorbance of 1x $10^{-5}$ M ATP in a 1 cm cell = 0.154 in a 3 cm cell = 0.462
   i) Absorbance of 5 x $10^{-5}$ M ATP in a 3 cm cell = 5/1 x 0.462= 2.31
   ii) log %T Transmission = 2-2.231 = -0.31
   %T = 0.49%

8. Calculate the molarity of pure water.
   *The density of water is 1 Kg/L and with molecular formula $H_2O$ has a molecular weight of 18.*
   *The molarity therefore mass in grams/molecular weight = 1000/18 = 55.5 molar.*

9. Calculate the pH of solutions containing: -
   A) 50 nmol/L of hydrogen ion
   pH is the negative log of the hydrogen ion concentration in mol/L.
   Therefore 50 nmol/L = 5.0 x $10^{-8}$ M
   Log = 5.0 x $10^{-8}$ M = -7.38
   **pH = 7.30**
   B) 27nmol/L of hydrogen ion.
   **pH = 7.57**

10. The serum seasonal turkey toxin was measured in 10,000 healthy male adults. Assuming a Gaussian distribution, the normal range was calculated to be 50 -150 nmol/L. How many results in the study population would you expect to find below 35 nmol/L?

    *Assuming the range is mean ± 2sd then (150 – 50)/4 = 1sd =25*

    *Then mean = ((150 – 50)÷2) + 50 = 100.*

*The difference between the mean and 35 = 100 – 35 = 65.*
*You can now calculate how many SDs away from the mean 35 is by dividing the difference (65) by the SD (25): -*

*65/25 = 2.6 SD*

*The data follow a Gausian distribution therefore 50% of the population have values below the mean. We know that the fro a Gausian distribution the mean ± 2.6 SD constitutes approximately 99% of the population therefore the number of values below 35 nmol/L must constitute 0.5% of the total.*
*10000 x 0.005 =* ***50***